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Superoxide Dismutase and Isozyme Analysis in Two Bread Wheat Genotypes Under Osmotic Stress

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ABSTRACT

Climate change, driven by human activity, has accelerated global warming, leading to heightened water scarcity and drought globally. In regions like Turkey and the Mediterranean basin, changing climate patterns threaten key sectors such as agriculture, where wheat is a staple crop. This study investigated the osmotic stress tolerance of two bread wheat genotypes, Bezostaya-1 and Karahan-99, by analyzing their superoxide dismutase (SOD) enzymes and isoenzymes under hydroponic conditions. Plants were exposed to osmotic stress at the vegetative and generative growth stages, alongside a control group. SOD enzyme activities and isozyme profiles were analyzed. The results showed that both genotypes produced different activities of Mn-SOD, Cu/Zn-SOD, and Fe-SOD isoenzymes under osmotic stress. Cu/Zn-SOD and Fe-SOD demonstrated peak activity, highlighting their vital role in mitigating oxidative damage. Total SOD activity increased significantly, especially during the generative stage, highlighting the importance of antioxidant defense mechanisms during critical growth phases under osmotic stress. Overall, the study highlights the importance of understanding how wheat genotypes respond biochemically to osmotic stress, particularly in the context of changing climate conditions. The results provide valuable information for the development of resistant wheat genotypes.

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Osmotik Stres Altındaki İki Ekmeklik Buğday Genotiplerinde Süperoksit Dismutaz ve İzozim Analizi

ÖZET

İnsan faaliyetlerinin yol açtığı iklim değişikliği, küresel ısınmayı hızlandırarak küresel ölçekte su kıtlığı ve kuraklığın artmasına neden olmuştur. Türkiye ve Akdeniz havzası gibi bölgelerde değişen iklim modelleri, buğdayın temel bir ürün olduğu tarım gibi kilit sektörleri tehdit etmektedir. Bu çalışmada, Bezostaya-1 ve Karahan-99 adlı iki ekmeklik buğday genotipinin ozmotik stres toleransı, hidroponik koşullar altında süperoksit dismutaz (SOD) enzimleri ve izoenzimleri analiz edilerek araştırılmıştır. Bitkiler, bir kontrol grubu ile birlikte vejetatif ve generatif büyüme aşamalarında ozmotik strese maruz bırakılmıştır. SOD enzim aktiviteleri ve izozim profilleri analiz edilmiştir. Sonuçlar, her iki genotipin ozmotik stres altında farklı Mn-SOD, Cu/Zn-SOD ve Fe-SOD izoenzim aktiviteleri ürettiğini göstermiştir. Cu/Zn-SOD ve Fe-SOD en yüksek aktiviteyi göstererek oksidatif hasarın azaltılmasındaki hayati rollerini vurgulamışlardır. Toplam SOD aktivitesi, özellikle generatif aşamada önemli ölçüde artarak, ozmotik stres altındaki kritik büyüme aşamalarında antioksidan savunma mekanizmalarının önemini vurgulamıştır. Genel olarak çalışma, özellikle değişen iklim koşulları bağlamında buğday genotiplerinin ozmotik strese biyokimyasal olarak nasıl tepki verdiğini anlamının önemini vurgulamaktadır. Sonuçlar, dayanıklı buğday genotiplerinin geliştirilmesi için değerli bilgiler sağlamaktadır.

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INTRODUCTION

Although the climate is a naturally variable system, today's climate change is unprecedented in its speed and the extent of human influence. Although the phenomenon, in which human activity plays a significant role, is commonly known as "global warming", its most significant impact is water scarcity and resulting drought. Drought, defined as a prolonged period of below-average precipitation (Schneider, 2011), limits crop production globally (Kutlu, 2010), accounting for 26% of stress factors in cultivated areas (Blum & Jordan, 1985). Due to global warming and climate change, Turkey and the Mediterranean basin are expected to experience significant drought. A major cause of this trend is the poleward shift of the 30° latitude high-pressure belt (Şahin & Kurnaz 2014). In Turkey, wheat (*Triticum* sp.) is a critical cereal, cultivated on 7.3 million hectares and producing ~20 million tons annually. A decline in wheat productivity due to drought could exacerbate socio-economic challenges linked to food security.

One of the climatic phenomena that occurs under growing conditions with global climate change is the change in precipitation regime rather than the amount of precipitation. The severity of osmotic stress effects on plants varies depending on the developmental stage, in addition to the effect of irregular rainfall regime (Gupta et al., 2001). Osmotic stress tolerance varies considerably among genotypes of the same species, and some osmotic stress-tolerant varieties are less affected by osmotic stress in terms of yield components (Majer, 2008). Recent studies emphasize that drought-tolerant wheat genotypes exhibit heightened antioxidant enzyme activity (e.g., SOD, CAT, APX) under water deficits, underscoring their role in stress mitigation (Aliyeva et al., 2023). Under osmotic stress, plants undergo a significant biochemical change, resulting in the formation of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, as well as non-radical ROS such as singlet oxygen and hydrogen peroxide (Bhargava& Sawant, 2013). The majority of the damage that occurs in plants under abiotic stress conditions, such as osmotic stress, is closely related to oxidative damage at the cellular level, and enhanced antioxidant protection is very important in terms of increasing osmotic stress tolerance. Specifically, the upregulation of mitochondrial Mn-SOD has been associated with improved drought tolerance in certain wheat genotypes, suggesting its pivotal role in ROS detoxification (Sheoran et al., 2015). Under optimal growth conditions, the plant's antioxidant defense system maintains a tight balance between the formation and consumption of reactive oxygen species (ROS). Primary antioxidant enzyme systems, such as SOD, react with existing radicals to convert them into less harmful forms or to limit the formation of new free radicals.

This study aims to evaluate the osmotic stress tolerance levels of two bread wheat genotypes, Bezostaya-1 and Karahan-99, under hydroponic conditions during different growth stages, specifically the vegetative and generative phases. The primary focus is on the activity and isoenzyme profiles of the superoxide dismutase (SOD) enzyme system, which plays a critical role in mitigating oxidative damage caused by osmotic stress in wheat leaves. By identifying and analyzing key SOD isoenzymes—Mn-SOD, Cu/Zn-SOD, and Fe-SOD—the research seeks to elucidate their distinct contributions and adaptive responses to osmotic stress conditions, offering insights into genotypic variations in biochemical responses. Previous research has identified multiple SOD isoenzymes with varying subcellular localizations in wheat, and their differential expression under stress conditions underscores the complexity of the antioxidant response (Miri-Hesar et al., 2019). Additionally, the study examines changes in SOD activity and isoenzyme dynamics during critical growth periods to better understand the antioxidant defense mechanisms employed by these genotypes. The findings are expected to advance the molecular and biochemical understanding of osmotic stress tolerance in wheat, particularly by highlighting the roles of specific SOD isoenzymes in stress adaptation. This study aims to address the growing challenges posed by water scarcity and climate change. It seeks to establish a foundation for targeted genetic improvement strategies and sustainable agricultural practices in water-limited environments. The findings will guide breeding strategies to enhance drought resistance. This is particularly relevant for Turkey and similar regions.

MATERIAL and METHODS

Materials

The bread wheat genotypes Bezostaja-1 and Karahan-99 were used as plant material in the study. Both bread wheat varieties were preferred because of their different responses to environmental stresses. While Bezostaja-1

is recommended for irrigated and rainy regions, Karahan-99 is highly drought-resistant and suitable for barren areas. In this context, Karahan is often used as a reference variety in international drought breeding programmes. These different physiological and morphological characteristics make it possible to compare the performance of varieties under different environmental conditions.

Bezostaya-1: The registered variety, recommended for irrigated areas with high rainfall and possessing a winter character, was developed by the Transitional Zone Agricultural Research Institute in 1968. It has an awnless and white spike, with red, hard, and coarse grains. The variety is sensitive to drought but resistant to cold. Depending on growing conditions, the grain yield ranges from 320-580 kg/da (Sakin et al., 2015)

Karahan-99: The registered variety, recommended for barren, semi-bottom, and bottom areas, has a winter character. It was registered by the Bahri Dağdaş International Agricultural Research Institute in 1999.

The recommended variety for cultivation in arid regions with rainfall-based agriculture has excellent drought tolerance capacity. Depending on growing conditions, the grain yield ranges from 200-500 kg/da (Mazid et al., 2009).

Methods

The research was conducted under hydroponic conditions. The materials were standardized by passing them through a 2.25 mm sieve, followed by surface sterilization in 5% sodium hypochlorite for 15 minutes, and then washed with sterile deionized water.

Seeds were sown in petri dishes and allowed to germinate under controlled conditions in a climate chamber set at $24\pm1^{\circ}\text{C}$ with a 16-hour light/8-hour dark cycle. Germinated seeds were transferred to 5-litre pots containing Hoagland's nutrient solution. The plants were subjected to a 16-hour light cycle with a light intensity of $350\text{ }\mu\text{mol m}^{-2}\text{s}^{-1}$, at a temperature of 25°C and 65% humidity, followed by an 8-hour dark cycle at a temperature of 24°C and 75% humidity. Each pot contained 20 plants, and the nutrient solution was changed every 5 days. During the following periods, an equal number of dilutions were made from each pot to ensure sufficient air circulation between the plants.

The study consisted of two treatments, vegetative period osmotic stress (D1) and generative period osmotic stress (D2), and a control group (C) for each treatment.

Vegetative Period Osmotic Stress (D1): Artificial drought conditions were created with PEG-6000 application between the beginning of emergence (Zadoks 30) and the beginning of spike (Zadoks 50) periods.

Generative Period Osmotic Stress (D2): Artificial drought conditions were created with PEG-6000 application between spike initiation (Zadoks 50) and milk maturity (Zadoks 70) periods.

Control: In order to determine the optimum performance for each of the genotypes in the experiment, osmotic stress was not applied. For the D1 and D2 treatments, an osmotic stress was induced using 15% PEG 6000 (-2.95 MPa) for 5 days at the specified times according to Zadoks (Ullah et al., 2010). To conduct SOD enzyme and isozyme analyses, leaf samples were collected from each group (D1, D2) on the same date as their respective control group.

Laboratory analyses

Enzyme extractions were conducted at $\pm 4^{\circ}\text{C}$. For both the treatment and control groups, 0.5 g of leaf samples were homogenized using liquid nitrogen in a chilled mortar with 50 mM Tris-HCl (pH 7.8) buffer containing 0.1 mM EDTA, 0.1% (v/v) Triton-X 100, 1 mM PMSF, and 2% (w/v) PVPP. The protein content and enzyme activities were determined using the supernatant obtained by centrifuging the homogenate at 4°C and 10.000 rpm for 20 minutes. Spectrophotometric measurements were carried out using the Shimadzu UV-1800 and Thermo Multiskan GO.

Electrophoretic separation of superoxide dismutase (SOD) isoenzymes was performed by staining with riboflavin and NBT (Beauchamp & Fridovich, 1971). Samples containing 40 μg of protein underwent non-denaturing polyacrylamide gel electrophoresis (PAGE) (Laemmli, 1970). SOD samples were run at 4°C under a constant current of 120 mA on a 5% stacking gel and 12% separating gel. Following electrophoresis, the SOD isozymes were identified by adding inhibitors to the dye solution. To inhibit Cu/Zn-SOD, potassium cyanide (KCN) was added to the dye solution, while H_2O_2 was used to inhibit Fe-SOD and Cu/Zn-SOD. Mn-SOD activity, on the other hand, remained resistant to both inhibitors. To determine SOD isozymes, we used 0.5 units of SOD standard with samples containing 60 μg protein for each genotype. After separating the proteins in non-denaturing PAGE (PAGE-polyacrylamide gel electrophoresis), we determined total SOD activities using densitometric method with Biorad Image Lab. Version 4.01 software programme.

Statistical Analyses

The study was conducted using a 'split plots in randomized blocks' experimental design with four replications. The varieties were the main plots, and the osmotic stress application period was the sub-plots. The study data underwent variance analysis, and the mean values of the data with significant differences, as determined by the 'F' test, were classified using the 'LSD' multiple comparison test.

The t-test was used to determine the differences and significance levels between the values of the treatment and control groups in our study (Mendes, 2012). The statistical analyses were performed using the JMP 11.2.1 statistical package programme to evaluate all the obtained data.

RESULTS and DISCUSSION

The results of the analysis of variance of total superoxide dismutase (SOD) enzyme activity (U mg protein⁻¹) with its isozymes obtained in the study carried out to determine the effects of vegetative and generative osmotic stress treatments on Bezostaya-1 and Karahan-99 bread wheat varieties are given in Table 1.

Table 1. Analysis of variance of total superoxide dismutase (SOD) enzyme activity (U mg protein⁻¹) values of Bezostaya-1 and Karahan-99 bread wheat total superoxide dismutase (SOD) enzyme activity values of vegetative and generative drought treatments

Çizelge 1. Bezostaya-1 ve Karahan-99 ekmeklik buğdaylarının vejetatif ve generatif kuraklık uygulamalarına ait toplam süperoksit dismutaz (SOD) enzim aktivitesi (U mg protein⁻¹) değerlerine ait varyans analizi

Source	DF	Sum of Squares	F
Replications	3	0.00022	0.3329
Variety	1	0.12426	2326.17**
Error ₁	3	0.00017	0.43
Drought App. Period	1	0.00801	149.96**
Variety* Drought App. Period	1	0.00731	136.85**
Error ₂	6	0.00032	
C.Total	15	0.14029	
CV (%)			3.88

** Significant difference at (P<0.01); DF: Degrees of Freedom; CV: Coefficient of Variations

The mean values of superoxide dismutase enzyme activity (U mg protein⁻¹) and multiple comparison test "LSD" groups of Bezostaya-1 and Karahan-99 bread wheat varieties at different osmotic stress application periods are given in Table 2.

For the Bezostaya-1 cultivar, the mean total SOD activity value (U mg protein⁻¹) was 0.100±0.00303. The values for D1 and D2 treatments were 0.099±0.006944 and 0.101±0.000943, respectively (Table 2). For the Karahan-99 variety, the mean value of total SOD activity (U mg protein⁻¹) was 0.276±0.017836, 0.233±0.002887 for the D1 treatment and 0.320±0.004714 for the D2 treatment (Table 2).

Table 2. Mean superoxide dismutase (SOD) enzyme total activity (U mg protein⁻¹) values and LSD groups of Bezostaya-1 and Karahan-99 bread wheat varieties at different drought treatment periods

Çizelge 2. Bezostaya-1 ve Karahan-99 ekmeklik buğday çeşitlerinin farklı kuraklık uygulama dönemlerindeki ortalama süperoksit dismutaz (SOD) enzimi toplam aktivite (U mg protein⁻¹) değerleri ve LSD grupları

	Karahan-99	Bezostaya-1	Mean
D1	0.233±0.002887 _b **	0.099±0.006944 ^c	0.166±0.027613 ^B **
D2	0.320±0.004714 ^a	0.101±0.000943 ^c	0.211±0.044293 ^A
Mean	0.276±0.017836 ^A **	0.100±0.00303 ^B	0.188±0.024971

** Significant difference at (P<0.01); LSD_{Var.}:0.009; LSD_{Drought App. Per.}: 0.009; LSD_{Var X Drought App. Per.}:0.013

Both varieties were found to contain Mn-SOD, Cu/Zn-SOD, and Fe-SOD isozymes, which are part of the SOD enzyme system and can be regulated during development and are highly reactive to stress conditions (Figure 1, Figure 2).

Regarding the average activity values of isozymes, it was found that Cu/Zn-SOD isozymes had the highest activity values during the D1 osmotic stress treatment period for the Bezostaya-1 variety, whereas Fe-SOD isozymes had the highest activity values during the D2 period (Table 3). For the Karahan-99 cultivar, Fe-SOD isozymes had the highest activity values during both osmotic stress treatment periods (D1, D2) (Table 3).

The Karahan-99 cultivar, Fe-SOD isozymes had the highest activity values during both osmotic stress treatment periods (D1, D2) (Table 3).

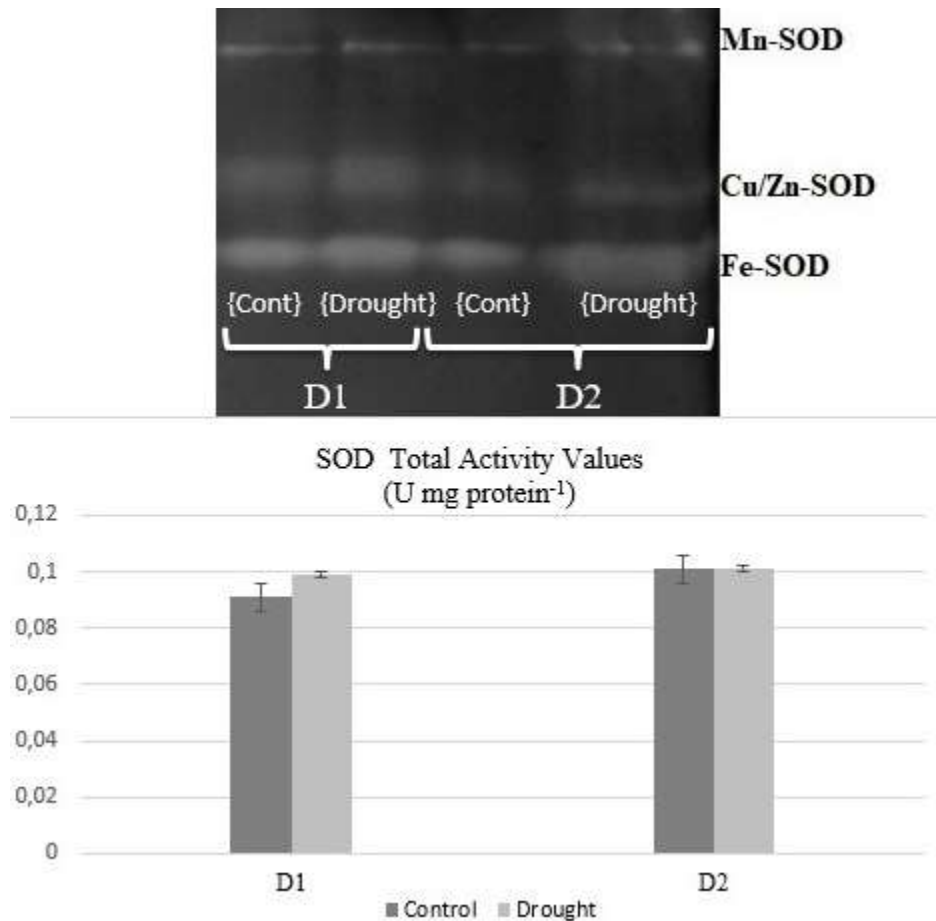


Figure 1. SOD isozymes and total activity (U mg protein⁻¹) of Bezostaya-1 bread wheat variety during vegetative and generative drought treatment periods with control groups

Şekil 1. Bezostaya-1 ekmeklik buğday çeşidinin kontrol grupları ile vejetatif ve generatif dönem kuraklık uygulaması SOD izozimleri ve toplam aktivitesi (U mg protein⁻¹)

Table 3. Mean activity (U mg protein⁻¹) values of different SOD isozymes of Bezostaya-1 and Karahan-99 cultivars at different drought application periods

Çizelge 3. Bezostaya-1 ve Karahan-99 çeşitlerinin farklı kuraklık uygulama dönemlerinde farklı SOD izozimlerinin ortalama aktivite (U mg protein⁻¹) değerleri

SOD izozim	Bezostaya-1		Karahan-99	
	D1	D2	D1	D2
Mn-SOD	0.013	0.057	0.043	0.080
Cu/Zn-SOD	0.045	0.274	0.081	0.055
Fe-SOD	0.041	0.388	0.110	0.186

The Bezostaya-1 cultivar exhibited an 8.08% increase in total SOD activity during the D1 treatment period compared to the control groups. However, there was no change observed during the D2 treatment period (Figure 1). Similarly, when comparing the total SOD activity values of the Karahan-99 cultivar with the control groups, significant increases of 13.6% and 78.3% were observed in both osmotic stress treatment periods (D1, D2) compared to the control groups. However, the increase in the D2 treatment period was particularly high (Figure 2). The t-test was conducted to determine the significance level of the difference between the SOD total activity values of both osmotic stress application periods (D1, D2) and the control group values. The results showed that the increase in the D1 osmotic stress application period and the stability in the D2 period were not statistically significant for the Bezostaya-1 variety (Table 4). For the Karahan-99 variety, there was a statistically significant difference ($P<0.01$) between the SOD total activity values of the osmotic stress application periods (D1, D2) and the control group values (Table 4).

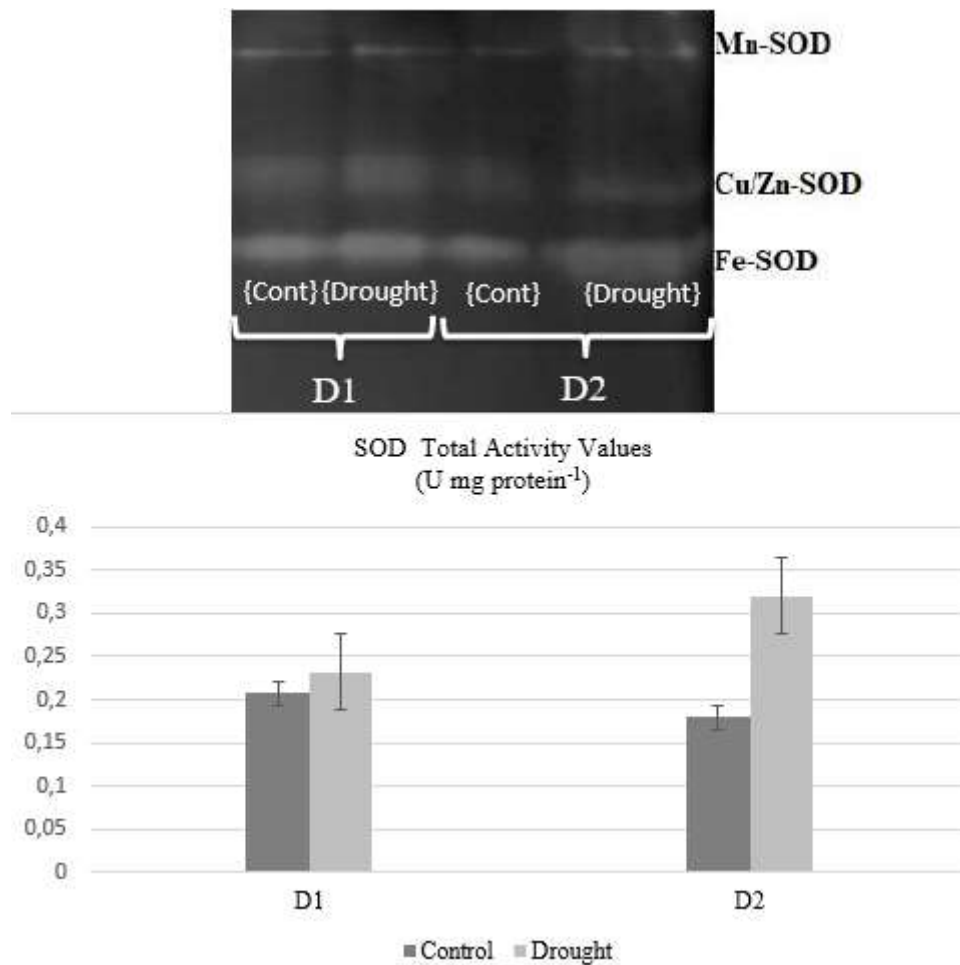


Figure 2. SOD isozymes and total activity (U mg protein⁻¹) of Karahan-99 bread wheat variety during vegetative and generative drought treatment periods with control groups

Şekil 2. Karahan-99 ekmeklik buğday çeşidinin kontrol grupları ile vejetatif ve generatif dönem kuraklık uygulaması SOD izozimleri ve toplam aktivitesi (U mg protein⁻¹)

Table 4. "t" test scores and significance levels of total SOD activity values (U mg protein⁻¹) between drought treatments and control groups

Çizelge 4. SOD toplam aktivitesi değerlerinin (U mg protein⁻¹) kuraklık uygulamaları ve kontrol grupları arasındaki "t" testi değerleri ve anlamlılık düzeyleri

	Bezostaya-1	Karahan-99
D1	1.25 ^{ns}	7.31 ^{**}
D2	0.00 ^{ns}	21.80 ^{**}

Superoxide dismutase (SOD; EC 1.15.1.1) catalyzes the dismutation reaction of superoxide (O₂^{•-}) radical anions to O₂ and H₂O₂ and is the first step in the key components of the cell's defense system against oxidative stress (Ren et al., 2016). Superoxide dismutase comprises three main isozymes: Mn-SOD, Cu/Zn-SOD, and Fe-SOD (Elshafei, 2020). These isozymes can actively dismutase superoxide radicals in various plant organelles. The same species, different genotypes within the same species, plant growth period, stress severity and duration can affect the expression rates of total SOD activity and isoenzymes separately for each at high levels and cause significant differences.

Furthermore, the total superoxide dismutase (SOD) activity values during the D2 osmotic stress treatment period were higher than those during the D1 osmotic stress treatment period for both cultivars. This is in agreement with Simova-Stoilova et al. (2008), who reported that good antioxidant protection during generative development is likely to play an important role in osmotic stress resistance.

The assessment of individual isoenzymes, which contribute differently and in varying proportions to the total activity of the SOD enzyme in wheat leaves during osmotic stress, is a crucial aspect (Huseynova et al., 2014). Furthermore, SOD isoenzymes also play an important role in maintaining redox homeostasis under abiotic stress

(Dumanović et al., 2021). SOD is a metalloprotein that acts as a catalyst in chloroplasts, mitochondria, stisols, peroxisomes and apoplasts with Cu, Zn, Mn and Fe cofactors (Miller, 2012). It has three isoenzymes that can be developmentally regulated according to their functional and structural roles (Bowler et al., 1994). Among these isozymes, Fe-SODs are found in plastids (Quartacci & Navari-Izzo, 1992), Mn-SODs in mitochondria (Kuźniak & Skłodowska, 2004) and peroxisomes (Palma et al., 1998), Cu/Zn-SODs in chloroplasts, stromal surface of thylakoid membranes (Ogawa et al., 1995), cytoplasm and apoplast (Ogawa et al., 1996; Del Río et al., 2002; Mittler, 2002). In addition, all of these isoenzymes can be targeted to organelles via NH₂-terminal targeting sequences (Bowler et al., 1994). Although Fe-SOD and Mn-SOD are structurally similar due to their electrical properties, Cu/Zn-SOD differs significantly from the other two isozymes because of its distinct electrical properties (Bannister et al., 1991).

The isoenzymes with high activity values in Bezostaya-1 and Karahan-99 cultivars were Cu/Zn-SOD and Fe-SOD isoenzymes, while Mn-SOD isoenzyme activity remained at low levels for both cultivars in both osmotic stress periods. The response of isozyme activity levels to oxidative stress may be related to genotypic variations and different subcellular locations of the isozymes. It is therefore important to consider the site of action of the different oxidative stresses encountered. The induction of widespread and high Cu/Zn-SOD and then Fe-SOD activities in subcellular structures for Bezostaya-1, Karahan-99 genotypes in the zone of influence of oxidative stress sources occurring depending on wheat growth periods, especially in the chloroplast, stisol, and apoplast, tends to increase to prevent damage due to ROS under osmotic stress.

In plants under various stress conditions, such as osmotic stress, free radicals tend to accumulate in association with the stress conditions (Smirnoff, 1993). Plant cells have the ability to detoxify ROS through various mechanisms that can be classified into three general categories (Jahnke et al., 1991): lipid-soluble and membrane-associated antioxidants (such as alpha-tocopherol), water-soluble reductants (such as glutathione and ascorbate), and enzymatic antioxidants like SOD. The ability of genotypes to utilise both enzymatic and non-enzymatic antioxidant systems is crucial in determining their tolerance levels (Molassiotis et al., 2006; Hasanuzzaman et al., 2021). SOD catalyzes the dismutation of the superoxide anion radical with high efficiency, resulting in the production of hydrogen peroxide and oxygen (Smirnoff, 1993). The activity of SOD enzyme varies depending on the severity and duration of osmotic stress, the genotype (Alscher et al., 2002; Seleiman et al., 2021), the rate of change of SOD activity (Menezes-Benavente et al., 2004), and the growth stage of the plant (Simova-Stoilova et al., 2009; Huseynova et al., 2016; In our study, the response of the SOD enzyme activity values to the control groups was quite different according to the varieties. This change was in the form of an increase of 13.6-78.3% in the D1 and D2 drought application periods for the Karahan-99 variety, and similarly, Kavuncu (2019) reported this rate as 70.0%. The Bezostaya-1 cultivar showed an increase in the D1 treatment, while no change was observed in the D2 treatment, which remained constant. This difference in response to stress conditions may be attributed to variations in the biochemical responses (Quitadamo et al. 2021) of the genotypes. In fact, it has been found that there are different trends of change in antioxidant enzyme activities depending on the osmotic stress loading pattern, duration, and severity of osmotic stress (Sairam & Srivastava, 2001). Many studies have reported that these trends are in the form of increased (Khaleghi et al., 2019), decreased (Huseynova et al., 2014) or unchanged (Bano et al., 2012) SOD activity under osmotic stress. Increases in SOD synthesis, as the primary O₂⁻ radical dismutation response step in the photosynthetic architecture, are associated with enhanced protective adaptation against oxidative damage, including lipid peroxidation associated with oxidative stress (Khaleghi et al., 2019). A decline in SOD activity may compromise the O₂ scavenging system of cells and promote its accumulation. Given that the measured enzyme activity is a result of both synthesis and degradation, a decrease in net SOD activity may be attributed to reduced enzyme synthesis or increased enzyme degradation intensity (Khayatnezhad & Gholamin, 2021). Similarly, excessive stimulation of H₂O₂ accumulation during osmotic stress can cause significant decreases in SOD activity by inhibiting the enzyme structure.

Another reason for the unchanged or decreased SOD activity may be due to the maintenance of stomatal aperture, which keeps ROS formation and associated SOD activity at the same or low level. This helps to maintain the excitation energy of the genotype at an optimum level during osmotic stress.

CONCLUSION

Osmotic stress response: Both genotypes exhibited Mn-SOD, Cu/Zn-SOD, and Fe-SOD isoenzymes, with Cu/Zn-SOD and Fe-SOD showing the highest activity levels under osmotic stress. This highlights the key role of these isoenzymes in scavenging reactive oxygen species (ROS) and mitigating oxidative damage.

Differential response to osmotic stress Although total SOD activity increased in response to osmotic stress in both genotypes, the extent of this response varied between developmental stages. Karahan-99 showed a

significant elevation in total SOD activity during both the vegetative and generative periods, indicating a stronger antioxidative defense. In contrast, Bezostaya-1 exhibited a moderate increase during the vegetative stage, but remained relatively unchanged during the generative stage, suggesting a less consistent response. Future studies: Transcriptomic and proteomic approaches may reveal the upstream signaling pathways involved in SOD regulation. Furthermore, integrating CRISPR-Cas9 mediated editing of candidate SOD genes with field trials under simulated Mediterranean drought conditions can validate their functional relevance and accelerate the development of climate resilient wheat varieties. Understanding the molecular mechanisms underlying SOD gene function, combined with well-designed field studies, will critically support breeding programs aiming at osmotic stress tolerance. These findings highlight the potential of genotype-specific antioxidant strategies and gene-editing technologies in improving drought and osmotic stress tolerance in wheat.

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Summary of researcher contribution rate declaration

This study was carried out within the scope of the PhD project titled 'Determination of Physiological and Biochemical Change Levels of Some Bread Wheat Genotypes Under Different Growth Period Drought Stress in Field and Laboratory Conditions' at Selçuk University, Institute of Science and Technology, Department of Biotechnology. This study was financially supported by Selçuk University Scientific Research Projects Coordination Office (Project No: 20211010).

Summary of researcher contribution rate declaration

As authors, we declare that we have each made an equal contribution to the article.

Conflict of interest

The authors of this manuscript declare that there are no conflicts of interest associated with this manuscript.

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Anti-biofilm, Anti-quorum sensing, Anti-swarming, and Antimicrobial Activity of *Rubus fruticosus* L.

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ABSTRACT

Over the past decade, antibiotic resistance has increased at an unprecedented rate, posing a serious challenge to healthcare systems worldwide. Research indicates that this resistance issue, which is projected to cause significant loss of life by the 2050s, is particularly alarming. Consequently, alternative methods to effective antibiotics are being explored to combat resistance. *Rubus fruticosus* L., commonly known as blackberry, is a shrub plant famous for its fruit. This fruit holds significant medicinal, cosmetic, and nutritional value. In our study, methanol, ethyl acetate, ethanol, and hexane extracts of *Rubus fruticosus* fruit from the Rize region were screened for their antimicrobial and quorum-sensing activities. Antimicrobial activity was investigated using the agar diffusion method against various Gram-negative and Gram-positive bacteria, as well as two *Candida* species. Anti-quorum sensing and antibiofilm activities were evaluated using *Chromobacterium violaceum* ATCC 12472 and *Pseudomonas aeruginosa* PAO1. The results showed that the methanol extract of *Rubus fruticosus* exhibited antimicrobial activity, while the ethanol extract demonstrated antibiofilm and anti-swarming activities. These findings suggest that *Rubus fruticosus* has the potential to be used as a natural agent in combating antimicrobial resistance.

Microbiology

Araştırma Makalesi

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Anahtar Kelimeler

Biofilm

Quorum sensing

Rubus

Swarming

Rubus fruticosus L. nin Anti-biyofilm, Anti-quorum sensing, Anti-swarming ve Antimikrobiyal Aktivitesi

ÖZET

Son on yılda dünya çapında benzeri görülmemiş bir oranda artan antibiyotik direnci sağlık sistemleri için ciddi bir zorluk oluşturmaktadır. Araştırmalar, bu direnç sorununun özellikle 2050'li yıllarda büyük can kayıplarına yol açacağını öngörmektedir. Dirençle mücadelede etkili antibiyotiklerin yerini alabilecek alternatif yöntemler araştırılmaktadır. *Rubus fruticosus* L., böğürtlen adıyla bilinen ve meyvesiyle ünlü bir çalı bitkisidir. Bu meyvenin tıbbi, kozmetik ve besin değeri oldukça yüksektir. Çalışmamızda, Rize iline ait *Rubus fruticosus* meyvesinin metanol, etil asetat, etanol ve hekzan ekstraktları antimikrobiyal ve anti-quorum sensing aktivite açısından incelenmiştir. Çeşitli Gram-negatif ve Gram-pozitif bakterilere ve iki *Candida* cinsine ait türe karşı antimikrobiyal aktivite, agar difüzyon yöntemi ile araştırılmıştır. Anti-quorum sensing ve antibiofilm aktiviteleri ise *Chromobacterium violaceum* ATCC 12472 ve *Pseudomonas aeruginosa* PAO1 kullanılarak değerlendirilmiştir. Sonuçlar, *Rubus fruticosus*'un metanol ekstraktının antimikrobiyal aktiviteye sahip olduğunu, etanol ekstraktının ise antibiofilm ve anti-swarming aktivitelerine sahip olduğunu göstermektedir. Bu bulgular, *Rubus fruticosus*'un antimikrobiyal dirençle mücadelede potansiyel bir doğal ajan olarak kullanılabileceğini düşündürmektedir.

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INTRODUCTION

Antimicrobial resistance (AMR) is a critical global health concern that has gained significant attention in recent years. The rise of multidrug-resistant bacterial strains, along with the limited development of new antibiotics, presents a complex challenge in combating infectious diseases (Terreni et al., 2021). Misuse and overuse of antimicrobials are recognized as key drivers in the development of drug-resistant pathogens (Zhou, 2023). This resistance poses a substantial threat to public health, emphasizing the need to explore innovative therapeutic approaches to address the issue (Kranjec et al., 2021).

Recent research has emphasized the importance of understanding the mechanisms of enhanced tolerance of biofilm-associated infections to antimicrobial therapy, given the escalation of drug resistance and the necessity for effective treatment strategies (Tsui et al., 2016). Moreover, exploring the incorporation of antimicrobial compounds beyond traditional antibiotics, particularly those with broader antimicrobial activity, is being considered to tackle emerging resistance (Bezuidenhout et al., 2015).

In response to the increasing antimicrobial resistance crisis, initiatives have been undertaken to promote alternative antimicrobial approaches like antimicrobial photodynamic therapy (aPDT), which has demonstrated potential as a light-based strategy to combat resistance (Cieplik et al., 2018). Additionally, strategies such as antimicrobial stewardship, surveillance, and leveraging artificial intelligence for the development of new antimicrobial medicines have been suggested to address the challenges posed by AMR (Ercan et al., 2023).

The global distribution of antimicrobial resistance in various settings, including in animals like swine, underscores the importance of monitoring antimicrobial use and resistance to protect public health (Hayer et al., 2022). The continuous evolution of research in antimicrobial properties, biofilm eradication, and the rational use of antibiotics reflects ongoing efforts to stay ahead of the increasing trend of antibiotic resistance (Permatananda et al., 2023). One of the critical mechanisms by which pathogenic microorganisms enhance their virulence is through quorum sensing. Quorum sensing is a bacterial communication system that regulates gene expression in response to population density via signaling molecules. This system controls the expression of virulence factors, secondary metabolite production, and stress adaptation mechanisms, allowing bacteria to become pathogenic (Merghni et al., 2022). Pathogens utilize quorum sensing to coordinate activities such as biofilm formation, violacein production, and swarming motility, which are crucial for their virulence. Inhibiting these quorum-sensing pathways is seen as a promising strategy to mitigate the impact of serious pathogenic bacteria and the infections they cause (Packiavathy et al., 2014).

Violacein is a natural bacterial pigment with significant biological activities. It has been extensively studied for its antimicrobial, antifungal, antiviral, anticancer, and antiparasitic properties (Kanade et al., 2022; Abedin, 2024). Recent research has focused on various aspects of violacein, including its biosynthetic pathway, production by different bacterial hosts, metabolic engineering for enhanced production, and its applications in synthetic biology, pharmaceuticals, and industry (Ahmed et al., 2021). Studies have shown that violacein induces cell death in tumor cells and inhibits tumor cell migration, suggesting potential therapeutic applications in cancer treatment (Mehta et al., 2015). Furthermore, the formulation of violacein in different carriers has been explored to improve its efficacy and overcome physiological barriers (Durán et al., 2007).

Historically, humans have relied on plants for both food and medicinal purposes. The World Health Organization (WHO, 2002) data shows that over 60% of the global population continues to use plant-based remedies due to the bioactivity of their phytochemicals (Acet, 2021). One such plant is *Rubus fruticosus* L., commonly known as blackberry, which belongs to the Rosaceae family. This plant is valued not only for its delicious taste and pleasant aroma but also for its nutritional and medicinal properties. *Rubus* is the largest genus within the Rosaceae family, encompassing around 700 species. The fruits of *Rubus* are consumed fresh and are also processed into various food products such as jams, wine, tea, ice cream, desserts, jellies, and pastries. In addition to their nutritional benefits, *Rubus* fruits have been used as natural antimicrobial agents. Studies have shown that *R. fruticosus* is effective against bronchitis and respiratory tract infections (Zia-Ul-Haq et al., 2014).

Rize province, located in northeastern Turkey on the eastern Black Sea coast, is a region rich in biodiversity and traditional knowledge of medicinal plants. Several studies highlight the significance of Rize province as a valuable source of medicinal plants. For instance, a study by Dalar (2018) emphasizes the importance of documenting traditional medicinal plants in Van province, Turkey, to preserve local medicinal knowledge threatened by

urbanization. This underlines the urgency of safeguarding the valuable traditional medicinal practices present in regions like Rize.

Furthermore, research by Yazıcı et al. (2023) focuses on the fruit quality parameters of mandarin genotypes from Rize province, indicating the diversity and richness of plant species in the region. Additionally, Abay et al. (2016) provide insights into the bryophyte checklist of Rize, highlighting the ecological importance of the province. Thus, *Rubus fruticosus* L., a species, was collected from Rize province based on its rich flora characteristics.

The selection of solvents is a critical factor in plant extraction processes, impacting both the extraction yield and the quality of the compounds obtained. Various studies emphasize the importance of choosing suitable solvents based on their polarity and efficiency in extracting specific bioactive compounds from plants. For example, ethanol and water are commonly utilized solvents due to their low toxicity, high extraction yield, and the ability to adjust solvent polarity by varying their ratios (Singh et al., 2022). Moreover, the use of aqueous mixtures of organic solvents such as ethanol, methanol, acetone, and others with water has been demonstrated to enhance the antioxidant efficiency of plant products compared to using water alone (Venkatesan et al., 2019).

To further explore the antimicrobial properties of *R. fruticosus*, researchers investigated the activity of different solvent extracts of the plant, including methanol, ethyl acetate, ethanol, and hexane, using the agar well diffusion method. This study focused on the effects of these extracts on quorum sensing in *Chromobacterium violaceum* strains and *Pseudomonas aeruginosa* PAO1. The quorum-sensing system in these bacteria plays a vital role in their pathogenicity, and disrupting this system could provide a novel approach to combating bacterial infections.

The ongoing research into plant-based antimicrobials is promising, particularly as antimicrobial resistance continues to rise. By understanding and harnessing the bioactive compounds in plants like *Rubus fruticosus*, scientists aim to develop new treatments that can work alongside existing drugs or as standalone therapies. The dual approach of targeting bacterial virulence factors and utilizing natural plant extracts offers a comprehensive strategy to address the growing challenge of antimicrobial resistance.

MATERIALS and METHODS

Collection of fruits and preparation of extracts

Methanol, ethyl acetate, ethanol, and hexane extracts of the *R. fruticosus* fruits (Figure 1) belong to Rize/İkizdere were prepared using the maceration method (Solanki & Nagori, 2012). 10-20 g of the fruits were powdered, and 10 times the relevant solvents were added to it. It was stirred at room temperature with a magnetic stirrer for 48 hours. Then, the extracts were filtered through filter paper, and the solvent was evaporated at 40 °C in the evaporator, and they were prepared in DMSO (Dimethyl sulfoxide) at concentrations of 50-200 mg mL.



Figure 1. *Rubus fruticosus* L.
Şekil 1. *Rubus fruticosus* L.

Antimicrobial Activity

Antimicrobial activity was tested using the agar well diffusion method. The plates were incubated at 18 h for bacteria, 48 h for *Candida* strains, and examined for any growth inhibition zones (Woods et al., 2003; Denev et al., 2014; Gür, 2016). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Yersinia pseudotuberculosis* ATCC, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumonia* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 10231, *Mycobacterium smegmatis* ATCC 607, *Chromobacterium violaceum* ATCC 12472 strains were used for the experiments.

Minimal Inhibitory Concentration (MIC) ($\mu\text{g/mL}$) was determined by the broth microdilution method (Murray et al., 2009). Tested derivatives were diluted with 100 μL of Mueller-Hinton broth-II in half increments starting from the first well of the microtiter plate. Brain heart infusion Broth (BHBI) was used for *M. smegmatis* (Woods et al., 2003). RPMI 1640 with 0.2 % glucose was used for *Candida* species (EUCAST, 2022). To determine the Minimum Bactericidal Concentration (MBC), samples from the MIC well and the previous three wells were inoculated into their respective media. The lowest concentration without growth in the media was determined as MBC (Saliha et al., 2020).

Antiquorum Sensing Activity

To detect the inhibition of violacein pigment, sub-MIC values of *R. fruticosus* against *C. violaceum* (ATCC 12472) were determined according to the strain. *C. violaceum* was cultured in 5 mL of Luria-Bertani (LB) medium in a shaking incubator for 24 hours. The prepared cultures were then poured onto LB agar plates and allowed to dry. Wells were created in the dried agar plates, and 50 microliters of the extracts at sub-MIC concentrations were added to each well. Wells that exhibited bacterial growth, but no pigment formation, were considered positive for violacein inhibition (McLean et al., 2004; Koh & Tham, 2009; Üreyen Esertaş et al., 2022).

Antiswarming activity

P. aeruginosa strain was prepared for the experiment. Then, 50 microliters of *R. fruticosus* extracts (100 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$) were added to LB soft agar medium. Prepared soft agars were poured onto LB plates and left to dry. A sample of *P. aeruginosa*, whose overnight culture was prepared with the help of a sterile toothpick, was dipped in the middle of the dried Petri dishes. After incubation, the result was interpreted according to the growth pattern of the bacteria in the petri dish (Rashid & Kornberg, 2000; Uğurlu 2013, Esertaş & Cora, 2024).

Antibiofilm activity

For the preparation of the experiments, a liquid culture of *Pseudomonas aeruginosa* was first established. The culture was grown overnight in LB broth to reach the appropriate density for the experiments. Once the culture was ready, the experimental setup began by adding specific volumes of the extract, bacterial culture, and medium to the wells of a microtiter plate. In each well designated for the experimental conditions, 40 μL of the extract was pipetted first. This was followed by the addition of 35 μL of the *Pseudomonas aeruginosa* PAO1 culture. Finally, 125 μL of LB broth was added to each well to achieve a final volume of 200 μL per well. This combination ensured that each well contained the precise ratios needed for the experiment. For the control wells, the setup was slightly different. No extract was added to these wells to serve as a baseline for comparison. However, the same volumes of bacterial culture (35 μL) and LB broth (125 μL) were added to maintain consistency in the total volume and conditions across all wells. Once all the wells were prepared, the microtiter plates were incubated under optimal conditions, usually at 37°C for a duration that allows for sufficient biofilm formation, typically 24 hours. After the incubation period, the medium was carefully removed from each well. The wells were then washed with sterile water to remove any non-adherent cells. To stain the biofilms, 200 μL of 0.3% crystal violet solution was added to each well. The plates were left to stand for 15 minutes, allowing the crystal violet to bind to the biofilm matrix. Following the staining step, the excess crystal violet was removed by washing the wells multiple times with sterile water. This step is crucial to ensure that only the biofilm-bound crystal violet remains in the wells. Next, 200 μL of 95% ethanol was added to each well to solubilize the bound crystal violet, and the plates were left for another 15 minutes to ensure complete dissolution. Finally, the optical density of the ethanol-crystal violet solution was measured at 570 nm using a spectrophotometer (Truchado et al., 2009; Esertaş et al., 2024).

Statistical Analysis

Statistical analysis was conducted using the SPSS 15.0 software. Mean $\pm$ standard deviation was employed to depict continuous variables conforming to a normal distribution. In cases where a normal distribution was not

evident, the median value was utilized. Qualitative variables were represented as percentages. Parametric variables underwent statistical assessment through the utilization of Student's T-test, applied to compare two distinct groups. A significance threshold of 0.05 was adopted to determine statistical significance.

RESULTS and DISCUSSION

Indigenous communities have historically utilized medicinal flora to address a variety of bacterial and fungal infections, primarily due to their affordability, accessibility, and the traditional belief in the antimicrobial properties attributed to these plants. Researchers worldwide are actively involved in scientifically validating the pharmacological activities that have been traditionally claimed for these plants. Moreover, the extensive use of antibiotics to treat microbial infections has led to increasing resistance among bacteria and fungi to the conventional antibiotics available commercially. (Abdova et al., 2018). Consequently, it is imperative to explore and identify novel natural resources, such as plants, for their potential to treat microbial infections. Our findings demonstrate that *Rubus fruticosus* different extracts exhibit considerable antimicrobial activity against common pathogens. In particular, the methanolic extract of *R. fruticosus* produced inhibition zones, showing notable efficacy against *C. albicans*, *A. haemolyticus*, *S. aureus*, and *C. violaceum*. The antibacterial activity observed may be attributed to the presence of tannins (Cavanagh et al., 2003) (Table 1).

Table 1...Antimicrobial activity of *Rubus fruticosus*
Çizelge. 1. *Rubus fruticosus*'ün antimikrobiyal aktivitesi

Microorganisms	Solvents			Controls
	MeOH	EtOAc	EtOH	
<i>S. aureus</i>	14.33 ±4.72	11.0 ±2.64	12.0 ±0	30.33 ±0.47
<i>B. subtilis</i>	16.0 ±1.73	0	0	22.33 ±0.47
<i>E. faecalis</i>	0	10.33 ±1.52	9.0 ±1.73	15.0 ±0
<i>E. coli</i>	0	12.0	8.33 ±2.30	25.0 ±0
<i>P. aeruginosa</i>	0	12.0	0	13.0 ±0.47
<i>A. haemolyticus</i>	14.0 ±1.73	12.33 ±2.08	16.33 ±1.52	23.0 ±0
<i>K. pneumoniae</i>	0	0	0	22.0 ±0
<i>E. aerogenes</i>	0	0	0	19.0 ±0.47
<i>S. thymurium</i>	12.0 ±4.58	0	0	17.0 ±0
<i>C. violaceum</i>	15.0 ±0	8.0 ±3.46	9.0 ±0	30.33 ±0.47
<i>C. albicans</i>	16.33 ±2.51	16.33 ±2.08	13.0 ±1.0	28.0 ±0
<i>C. parapsilosis</i>	0	0	0	25.0 ±0
<i>M. smegmatis</i>	0	0	0	24.33 ±0.47

Controls; Gram positive; Ampicilin, Gram negative; Gentamicin, *Candida*; Amphotericin B, *Mycobacterium*; Ciprofloxacin

The table was created by taking the average of the zone diameters obtained by repeating the agar well method 3 times. It was seen that the antimicrobial activity results of the extracts were similar, giving similar zone diameters. There is no significant difference between groups ($p < 0.05$).

MIC and MBC Results of Extracts

In this study, the MIC and MBK concentrations of bacteria with a zone diameter of 15 mm and above were determined, and when the results were compared within themselves, it was observed (Table 2) that the activity in *S. aureus* was better than the others.

Table.2...MIC and MBC ($\mu\text{g mL}$) results of *Rubus fruticosus*
Çizelge 2. *Rubus fruticosus*'ün MİK ve MBK ($\mu\text{g mL}$) sonuçları

<i>R. fruticosus</i>	MeOH		<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. violaceum</i>	<i>C. albicans</i>
			MIC	500	500	500
			MBC	500	1000	1000

Weli et al. (2020), in their study, stated that hexane, chloroform, and ethyl acetate extracts of *R. fruticosus* leaves showed antimicrobial activity, but no activity was detected in *S. aureus*. In study shows that ethyl acetate extracts are effective on *S. aureus*. Abachi et al. (2013) in their study, evaluated the activity of the ethanolic extract of *R. fruticosus* against nalidixic acid-resistant *Helicobacter pylori* and determined that it had a strong effect with an MIC value of 400-450 µg/mL. Researchers have determined a similar range of MICs in this study.

Violacein Inhibition Results

The different extracts were effective at inhibiting violacein production at all tested concentrations, as shown in Table 3. There are limited articles about violacein inhibition analysis of *R. fruticosus*. According to Oliveira et. al. 2016, it has been found that violacein inhibition by the phenolic extracts of *R. fruticosus*, especially at 118.60 mg GAE/L, inhibited 88.6%, a value higher than the positive control for this experiment (furanone), which inhibited 68.6%.

Table 3. Anti-violacein activity results of *Rubus fruticosus*

Çizelge 3. *Rubus fruticosus*'ün antiviolasin aktivite

	Solvent	<i>C.violaceum</i> 12472
<i>R. fruticosus</i>	MeOH	+
	EtOAc	-
	EtOH	+
	Hxn	-

Anti-Swarming Results

Antiswarming activity is crucial in inhibiting the collective movement of bacterial populations, with implications in agriculture, medicine, and waste management. Studies have investigated the use of natural compounds from fruit peel extracts and essential oils like *Origanum vulgare* to deter swarming behavior in bacteria such as *Salmonella enterica* and *Pseudomonas aeruginosa* (Mahadwar et al., 2015; Merghni et al., 2022). This antiswarming activity is vital for controlling the spread of pathogenic bacteria and biofilms, contributing to the development of novel antibacterial strategies.

Pseudomonas aeruginosa is a versatile bacterium known for its ability to exhibit various group behaviors, including swarming motility. Swarming motility is a coordinated movement of bacterial populations across surfaces facilitated by flagella. Studies have shown that the presence of sub-populations of *P. aeruginosa* deficient in flagellar activity can repress swarming in the entire population (Bru, 2023). Additionally, phage infection of *P. aeruginosa* can abolish swarming motility in infected subpopulations and induce the release of the *Pseudomonas* quinolone signaling molecule PQS, which repels uninfected subpopulations from approaching the infected area (Bru et al., 2019).

The swarming activity of microorganisms is one of the steps of the quorum sensing system and is an important virulence factor regulated by signal communication. *R. fruticosus* EtOH extract was found to have high swarming properties. It was seen that this effect is dose-dependent, and the swarming activity disappears as the dose decreases (Table 4, Figure 2).

Several studies have reported that lower MICs of essential oils from various plants, such as tea tree, eucalyptus, and clove, inhibit swarming activity in *P. aeruginosa* PAO1 in a concentration-dependent manner (Noumi et al., 2018). Merghni et al. (2022) revealed that their results showed a differential reduction in the swarming activity of PAO1 by the tested agents, similar to this study.

Table 4. Swarming suppression results with *Rubus fruticosus* EtOH extract (100/50 µg/ml) in *P.aeruginosa* PAO1 strain

Çizelge 4. *Rubus fruticosus* EtOH (100/50 µg/ml) özütünün *P. aeruginosa* PAO1 suşunda swarming baskılama aktivitesi

	Zone Diameter (mm)
PAO1 control	29
PAO1+100 µg/mL EtOH extract	7
PAO1+50 µg/mL EtOH extract	19
PAO1 + 3% DMSO (solvent)	27

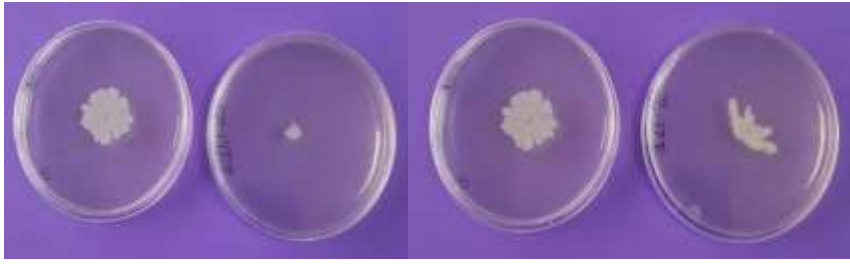


Figure 2. Swarming suppression results of *R. fruticosus* EtOH at 100 and 50 µg/mL
Şekil 2. *R. fruticosus* EtOH 100 ve 50 µg/mL'de swarming baskılama sonuçları

Biofilm Inhibition Results

Biofilm formation in Quorum sensing steps can provide protection against the immune system defense of the host and plays an important role in gaining resistance against various antimicrobials. As a result of biofilm experiments, it is seen that *R. fruticosus* EtOH extract inhibited biofilm formation intensively at 100 µg/mL. It is observed that biofilm suppression activity is lost at low doses, depending on the dose.

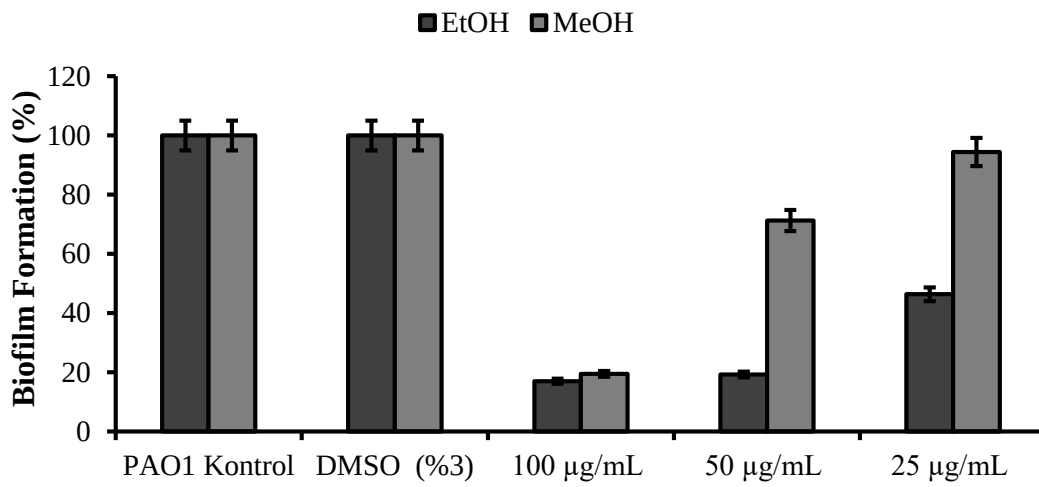


Figure 3. *R. fruticosus* antibiofilm activity
Şekil 3. *R. fruticosus* antibiyofilm aktivitesi

In recent years, the increase in multi-antibiotic-resistant and biofilm-forming bacteria has led to the ineffectiveness of conventional antibiotic therapy, and this problem has become a global threat (Cepas et al., 2019). Quorum sensing is a successful cell-cell communication network that manages numerous mechanisms, such as antibiotic production, biofilm formation, and virulence factor production, especially in pathogenic microorganisms. Current research focuses on the identification of new antimicrobials of natural origin and their effects on virulence factors in order to control the spread of dangerous pathogens.

The search for new antimicrobial agents has focused on understanding the protective mechanisms created by plants that can live with high bacterial density for infections. In this direction, herbal products such as plant extracts and herbal polyphenolic compounds present a new strategy as non-toxic inhibitors in the inhibition of the QS system. In a study to show the effects of plant extracts and similar ones used as traditional medicine on the quorum sensing mechanism (Chu et al., 2013). *Fructus gardeniae* and *Ancdrographis paniculata* plants showed anti-QS activity using *C. violacaum* 12472 and *P. aeruginosa* PAO1. The results of the study on the antibiofilm activity of *Rubus ulmifolius* extracts show that plant-derived sugars exhibit different effects on microbial biofilm formation. Phenolic compounds are known to have growth inhibitory activity as well as antibiofilm activity. This suggests that interactions between sugar and biological targets are important (Fontaine et al., 2017).

Vattem et al. reported that raspberry (*Rubus idaeus*), blueberry (*Vaccinium angustifolium*), and grape (*Vitis* sp.) plants inhibited violacin production due to AHL mechanism by 60%, 42%, and 20% (Vattem et al., 2007). Literature data includes *R. rosaefolius* and *R. idaeus* species in quorum sensing studies with *Rubus* species. In this respect, in study is the first quorum sensing screening with *R. fruticosus*.

CONCLUSION

The results of the study demonstrate that *R. fruticosus* extracts possess significant antibacterial, quorum-sensing, antibiofilm, and anti-swarming properties. These findings suggest that *R. fruticosus* could be effectively evaluated as a potent antimicrobial agent for the treatment of bacterial infections. The antibacterial properties of the extracts indicate their potential to inhibit the growth of various bacterial strains, thereby reducing the prevalence and severity of bacterial infections. The quorum-sensing activity highlights the ability of *R. fruticosus* extracts to disrupt bacterial communication systems, which play a crucial role in the regulation of virulence factors and biofilm formation. This disruption can prevent bacteria from coordinating their pathogenic activities, making them less effective in establishing infections.

Furthermore, the antibiofilm activity of the extracts suggests that *R. fruticosus* can inhibit the formation of biofilms, which are protective matrices that bacteria produce to shield themselves from environmental stresses and antibiotic treatments. By preventing biofilm formation, *R. fruticosus* extracts can enhance the susceptibility of bacteria to antimicrobial agents, thereby improving treatment outcomes. Additionally, the anti-swarming activity indicates the potential of the extracts to inhibit bacterial motility, further limiting the spread and colonization of pathogenic bacteria.

In future studies, it is planned to isolate and purify the active compounds present in *R. fruticosus* extracts by thoroughly investigating their chemical properties. This purification process will enable researchers to identify the specific bioactive molecules responsible for the observed antimicrobial effects. By understanding the chemical nature of these compounds, it will be possible to optimize their efficacy and develop targeted antimicrobial therapies. Additionally, further research will involve assessing the safety and toxicity profiles of the purified compounds to ensure their suitability for clinical applications. Overall, the promising results of this study pave the way for the development of novel antimicrobial agents derived from *R. fruticosus*, offering a natural and effective solution to combat bacterial infections and address the growing challenge of antibiotic resistance.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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Morphology and Molecular Phylogeny of *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Isolated from Soil

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ABSTRACT

In this study, two successive populations of *Blepharisma* were observed in cultures prepared from soil samples collected from Tekirdağ (Türkiye). The cells and cysts belonging to these populations were examined morphologically and morphometrically, and their phylogenetic positions were determined based on SSU rDNA gene sequences. Both populations were morphometrically very similar to each other, but population 1, the initial population observed in soil cultures, had a pyriform body that was inflexible and rigid while population 2, observed subsequent to the disappearance of population 1, exhibited a flexible, fragile, lanceolate body. In addition, the cell width/length ratios of both populations were found to be statistically different from each other. However, since the SSU rDNA nucleotide sequences of both populations are very similar to each other and to other *B. hyalinum* populations described previously, it is concluded that the observed *Blepharisma* populations represent different morphotypes of *B. hyalinum*. It has been hypothesised that the observed morphological differences may result from diet, environmental factors, and variations in the life stages of the cultured cells. Comparison of SSU rDNA nucleotide sequences of *Blepharisma* populations and phylogenetic analyses showed that the nucleotide sequences of some different species are identical or very similar. To clarify this chaotic situation, extensive molecular data based on detailed morphological studies on *Blepharisma* populations are necessary.

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Topraktan İzole Edilen *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Türünün Morfolojisi ve Moleküler Filogenisi

ÖZET

Bu çalışmada Tekirdağ (Türkiye) topraklarından hazırlanan kültürlerde birbiri ardınca ortaya çıkan iki *Blepharisma* popülasyonu gözlemlendi. Bu popülasyonlara ait hücre ve kistler morfolojik ve morfometrik olarak incelenmiş ve SSU rDNA gen dizisine dayanarak filogenetik konumları belirlenmiştir. Her iki popülasyon da morfometrik olarak birbirine çok benzerdi, ancak toprak kültürlerinde gözlenen ilk popülasyon olan popülasyon 1, esnek olmayan ve sert olan piriform biçime sahipken, popülasyon 1'in kaybolmasının ardından gözlenen popülasyon 2 ise, esnek, kırılabilir, mızrak biçimindedir. Ayrıca, her iki popülasyonun hücre genişliği/uzunluğu oranlarının istatistiksel olarak birbirinden farklı olduğu bulunmuştur. Bununla birlikte, her iki popülasyonun SSU rDNA nükleotid dizileri birbirlerine ve daha önce tanımlanan diğer *B. hyalinum* popülasyonlarına çok benzediğinden, bu çalışmada gözlemlenen *Blepharisma* popülasyonlarının *B. hyalinum*'un morfotipleri olduğu sonucuna varıldı. Gözlenen morfolojik farkların çevresel faktörlere ve kültürlerdeki hücrelerin farklı yaşam evrelerinden kaynaklanabileceği varsayılmıştır. *Blepharisma* popülasyonlarının SSU rDNA nükleotid dizilerinin karşılaştırılması

Mikrobiyoloji

Araştırma Makalesi

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Blepharisma hyalinum

Morfoloji

Dinlenme kisti

Filogeni

Türkiye

ve filogenetik analizler bazı farklı türlere ait nükleotid dizilerinin aynı ya da çok benzer olduklarını göstermiştir. Bu kaotik durumun açıklığa kavuşturulması için *Blepharisma* popülasyonları üzerinde ayrıntılı morfolojik incelemelere dayalı çok sayıda moleküler veriye ihtiyaç duyulmaktadır.

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INTRODUCTION

Ciliates (Ciliophora) are heterokaryotic protists. Within one cell, there are macro- and micronuclei of different sizes and functions (both somatic and germline). By regulating specific metabolic pathways, soil ciliates increase nutrient flow for the benefit of other microorganisms, plants, and animals and contribute to soil fertility (Kuikman et al., 1989; Foissner, 1999; Li et al., 2010; Ceja-Navarro et al., 2021). They also have higher susceptibility, reproduction rate, and trophic niche diversity (bacterivore, carnivore, omnivore) and thus respond faster to environmental contamination (Madoni, 2005; Li et al., 2010; Lara & Acosta-Mercado, 2012).

Ciliate species belonging to the genus *Blepharisma* (class Heterotrichea, order Heterotrichida) are cosmopolitan organisms. In many parts of the world, they are found in many habitats, including soil, lichen, freshwater (lakes, ponds, streams and wastewater treatment ponds), brackish and seawater (Kahl, 1932; Bhandary, 1962; Isquith et al., 1965; Dragesco, 1970; Giese, 1973; Dragesco & Dragesco-Kernéis, 1986; Aesch & Foissner, 1998; Lee & Shin, 2009; Fernandes et al., 2013; Yan et al., 2016; Hao et al., 2022) and even in extreme habitats (Post et al., 1983; Al-Rasheid et al., 2001; Pan & Stoeck, 2017). Most, but not all, *Blepharisma* spp. have a reddish or pink colouration due to granules of the light-sensitive pigment "blepharismisin" (formerly zoopurpurin) located just below the plasma membrane (Giese, 1973; Lee & Shin, 2009; Gupta et al., 2015; Pan & Stoeck, 2017). Colourful blepharismids show a photophobic response, avoiding bright light and tending to accumulate in shady or dark areas (Giese, 1973; Fabczak et al., 2001).

Blepharisma was first established by Perty (1849), and its diagnostic properties were described. There are many confusions, renaming and reorganisations in the taxonomic history of the genus (Suzuki, 1954; Bhandary, 1962; Hirshfield et al., 1965; Aesch & Foissner, 1998). The most important reason for this situation is that the morphological and morphometric characteristics of *Blepharisma* species are related to environmental factors such as nutrients, temperature, and pH (Hirshfield et al., 1965; Giese, 1973; Lee & Shin, 2009; Pan & Stoeck, 2017). In feeding experiments, extreme changes in macronuclear configuration and cell size were observed in some species depending on the nutrient (Hirshfield et al., 1965). Nevertheless, in early studies on the taxonomy of the genus, the macronuclear configuration was widely used as the main criterion for species distinction (Stein, 1867; Suzuki, 1954; Bhandary, 1962; Hirshfield et al., 1965; Isquith et al., 1965). Furthermore, the available ontogenetic data do not support the identification of subspecies and subgenera based on nuclear configuration (Aesch & Foissner, 1998).

As a result of the development and application of modern taxonomic techniques (e.g. silver staining, electron microscopy, molecular biology), some less-known *Blepharisma* species and new species have been described (Yan et al., 2016; Hao et al., 2022), while some species are reported to be synonyms (Fernandes et al., 2013). About 75 nominal *Blepharisma* species have been reported so far (Hao et al., 2022). Phylogenetic relationships among ciliates are based primarily on structural features of the cortex, infraciliature, extrusomes and information from ontogeny (Greenwood et al., 1991). The fine structure of the somatic and oral ciliature is an important feature for phylogeny because they are not variable. However, the importance of integrative approaches that combine morphology, morphogenesis, and molecular phylogeny to understand ciliate systematics and ecosystem function is also clear (Liu et al., 2017).

Some molecular studies on *Blepharisma* species show that they do not form a monophyletic clade, and it is noteworthy that different populations of the same species cluster far from each other, while populations belonging to different species cluster closely. Since there is very little molecular data on *Blepharisma* species, more generally on ciliates, the phylogenetic relationships of these organisms have not been fully clarified and contain contradictory situations. In order to resolve this problem, a large amount of molecular data from different geographical regions combined with detailed morphological methods is needed. Apart from the record of Çapar (2007) and the oral presentation produced from a PhD thesis in progress (Ural et al., 2023), no taxonomic study on the species belonging to the genus *Blepharisma* in Tekirdağ province or even in Türkiye. In the present study, two populations

of ciliates belonging to the genus *Blepharisma* isolated from soil in Tekirdağ were examined morphologically and morphometrically using live and silver-staining methods (Vďačný & Foissner, 2012; Foissner, 2014), and their phylogenetic positions were evaluated by comparing their SSU rDNA gene sequences with the gene sequences of the genus *Blepharisma* obtained from NCBI (<https://www.ncbi.nlm.nih.gov/nucleotide>). This study is the first comprehensive study of *Blepharisma* isolated from the soils of Türkiye. Some notes on the cyst and division morphology of *Blepharisma* populations are also presented in this study.

MATERIAL and METHOD

Study area, sampling, preparation of cultures (Adobe Inc, 2014)

Soil samples were taken from pine and meadow areas (40°59'58.0" N, 27°34'53.9" E) on the campus of Tekirdağ Namık Kemal University between 2019 and 2022. The samples were taken from near the surface (to a depth of 10 cm), together with vegetative debris, and dried in the shade for one month in order to stimulate encystment. Soil analyses were carried out at the soil analysis laboratory of Tekirdağ Namık Kemal University. Physicochemical analysis of the soil samples indicated a clay loam texture, slightly acidic reaction (pH 6.82), moderate calcareous content (lime content 6.84%), organic matter 7.23%, electrical conductivity 520 μ S/cm, phosphorus 6.1 ppm, total nitrogen 0.36%, and potassium 93.17 ppm. The soil is a slightly acidic reaction, salt-free class, with very high organic matter content, and moderately calcareous. Potassium and phosphorus contents are low or very low, nitrogen content is high.

Non-flooding Petri dish cultures were prepared to reactivate resting cysts of ciliates found in dried soil samples and incubated at room temperature out of direct sunlight (Foissner et al., 2002; Foissner, 2014). *Blepharisma* populations were observed in the cultures from day 5–9 until the end of one month. At the end of this period, the cultures were renewed due to microbios (Foissner, 1987; 1997). For resting cyst morphology, cells isolated from cultures were kept in sterilized ambient water for a week. To observe the morphology of resting cysts, vegetative individuals were kept in sterile, nutrient-free media for one week.

Morphological methods

About 2-5 mL of culture liquid taken from the Petri cultures was transferred to a watch glass and examined under the stereo microscope (Euromex), at 20X-40X magnification, blepharismid ciliates were observed live, and their movement and morphological characteristics were recorded. For more detailed live observations, ciliate cells collected with capillary micropipettes from a watch glass were examined at 100-1000X magnification with the CX41 Olympus light research microscope, and images of morphological features of cells were recorded. Morphometric data were obtained based on intact naturally structured specimens during the live observation process. Various silver staining methods (protargol, silver nitrate, silver carbonate) were used to determine diagnostic features such as nuclear pattern, infraciliature (Foissner et al., 2002; Foissner, 2014). Resting cysts were examined either directly or by supravital staining (methyl green-pyronin). Measurements, counts, and photomicrographs were done with the SC30 Olympus digital camera, compatible with the CX41 light microscope, and the Cell Software micro imaging and measurement system. Illustrations of live cells were based on freehand sketches, micrographs, and video recordings; those of silver-impregnated cells were drawn from micrographs and preparations. The processing of the photographs was carried out on the computer (Adobe Inc, 2014), taking into account the original dimensions and proportions. Taxonomy and terminology were according to Lynn & Small (2000), Lynn (2008).

Biostatistical analyses

The data obtained from measurements and counting of various morphological characters of the *Blepharisma hyalinum* cells were analyzed using SPSS 15.0 (SPSS Inc, 2006) and Minitab 16 (Minitab LLC, 2016) statistical programs, and the results of descriptive statistics were summarized in tables.

Molecular phylogenetic methods

Cells isolated from soil culture were first transferred to sterile (filtered with a 0.22 μ m syringe filter and autoclaved) ambient water and washed three times with sterile distilled water to prevent possible contamination. Usually 1-3, rarely more, cells were transferred to 0.2 mL microcentrifuge tubes with 1-2 μ L of purified water and stored in a deep freezer (-20°C) for PCR (polymerase chain reaction).

Frozen samples were subjected to 5-10 freeze-thaw cycles to disintegrate the cell pellicle and nuclear membrane. After that, cells were directly subjected to PCR without further genomic DNA extraction, and 18S rDNA gene regions were amplified using universal eukaryotic primers (Medlin et al., 1988; Ural et al., 2020; Yıldız, 2021). The presence and size of the PCR product were checked by running 1.5% agarose gel electrophoresis in the presence of

marker DNA.

Sequencing was performed in the NABILTEM laboratory of Tekirdag Namık Kemal University using a Beckman Coulter GenomeLab sequencer. PCR products were sequenced with PCR primers and the internal primer (reverse BR2-5'-AAG AAC GGC CAT GCA CCA-3'). The sequences obtained in SCF format were checked in CodonCode Aligner ver. 9.0.1 (www.codoncode.com). Consensus sequences were obtained by aligning good-quality and reliable sequence regions. The nucleotide sequences of the present populations were aligned with the gene sequences of *Blepharisma* species obtained from GenBank (NCBI) using the Clustal W algorithm in the Mega 11 software (Tamura et al., 2021). Nucleotide similarity and dissimilarity matrices between *Blepharisma* species were derived with BioEdit 7.2 software (Hall, 1999).

The best substitution model (TIM2 I+G) for Bayesian phylogenetic analyses was selected according to AIC with JModelTest 2.1.10.2 software (Darriba et al., 2012). Bayesian analysis (BI) was performed using MrBayes v.3.2.7a (Ronquist et al., 2012), sampling 3,000,000 generations and one tree every 100 generations (Ayres et al., 2012; Ronquist et al., 2012). The course of the analyses was monitored with the Tracer software (Rambaut et al., 2018). It was checked whether the sample size was sufficient (ESS>800) and whether the sample size was saturated. The first 25% of the phylogenetic trees were burned, and the consensus tree was constructed from the remaining trees. Maximum likelihood (ML) analysis was done with the Iqtree2 for Mac (Minh et al., 2020). Model selection for ML analysis (TIM+F+I+R2) was performed using the same software. The ML analysis was carried out as 1000 bootstrap runs, and the phylogenetic tree was constructed. Both phylogenetic trees rooted and edited the position of the taxa in FigTree v. 1.4.4 software (<http://tree.bio.ed.ac.uk/software/figtree>).

RESULTS

In this study in soil cultures, two *Blepharisma* populations (population 1 and population 2) differing in some morphological characteristics were observed. Morphological and morphometrical data were collected from both populations based on live observations and silver impregnated preparations. Although there are some differences in the shape and movement of the living cells of the two populations, morphometry and DNA sequence comparisons indicate that the two populations belong to *Blepharisma hyalinum*. The morphological characteristics of both populations are given below.

Blepharisma hyalinum Perty, 1849 (Syn: *B. lateritium* f. *minima* Roux, 1902)

Morphological descriptions

Population 1 (P1). Since it was the first blepharismid ciliate population observed in soil cultures in this study, it was named population 1. Body shape is elongated pyriform, anterior end distinctly pointed, posterior broadly rounded; ventral side slightly depressed (concave) along the adoral region (peristome), dorsal side slightly bulbous (convex) (Figs. 1a, 2a). Cells are rigid, not flexible, and move slowly between soil particles, mainly at the bottom. Cell size was approximately 66–126 µm in length and 18–36 µm in width in vivo, with a mean value of 96×28 µm, whereas the cells were shrunken to 75×21 µm in silver-impregnated preparations. The width is approximately 28% of the length, and this value is 30% in living cells (Table 1, 2). The macronucleus is usually in the center of the cell, closer to the anterior body end; elongate, egg-shaped (oblong); contains many small spherical nucleoli (Figs. 1a, 2d). Size is variable, approximately 10–24 µm in length and 3–7 µm in width. Its distance to the anterior tip is approximately 25 µm in silver impregnated specimens and about 30 µm in live cells (Table 1, 2). In some individuals, more macronuclear fragments of smaller size (17×6 µm, 14×5 µm, 17×9 µm, 13×6 µm, 13×7 µm) were observed, which are thought to be associated with the various life cycle stages. Micronucleus numbers vary from 1 to 7; individual micronuclei are globular in shape, 0.90–1.45 µm across (Table 1), adjacent to or close to the macronucleus at various positions (Figs. 1a, c, 2d). Single contractile vacuole globular and conspicuous, located at the posterior body end, 6.5–9.0 µm across in vivo (Figs. 1a, 2a). The cortex is distinctly corrugated along the ciliary rows (kineties), contains innumerable colorless granules. Cortical granules are arranged in a band of 5–6 granules rows between the ciliary rows (Fig. 2c). The Cytoplasm is colorless and contains many food vacuoles, mainly in the posterior body part.

Somatic and oral ciliature as in congeners. Somatic ciliature entirely composed of dikinetids; number of ciliary rows fairly variable, 14–19 somatic kineties (including 2 postoral kineties) run longitudinally along the cortical ridges (Figs. 1c, 3a, b; Table 1). Adoral zone membranelles (AZM), conspicuous, J-shaped, starting about 4 µm ($2-4$ µm; N=20) behind the anterior end, proximal part distinctly bent into a semicircle, ends at cytostome (Figs. 1a, c, 2a). AZM extends almost to the middle of the cell, up to about 46% of the cell in protargol-impregnated samples (Table 1) and up to 48% in live samples (Table 2), composed of 26–32 membranelles, each adoral membranelle consisting of two long and one short row of kinetosomes (Fig. 1e), base of longest adoral membranelle length up to 7 µm (Table 1). The paroral membrane is located to the right of the peristome, ends posteriorly at the cytostome; length approximately 29 µm ($22-35$ µm) (Table 1), differentiated into two distinct parts (PMA, PMp); anterior part

(PMa) 26-29% of paroral membranal length, composed of monokinetids in a straight line, while the posterior consists of dikinetids arranged in a “zig zag” pattern (Figs. 1e, 4a-d).

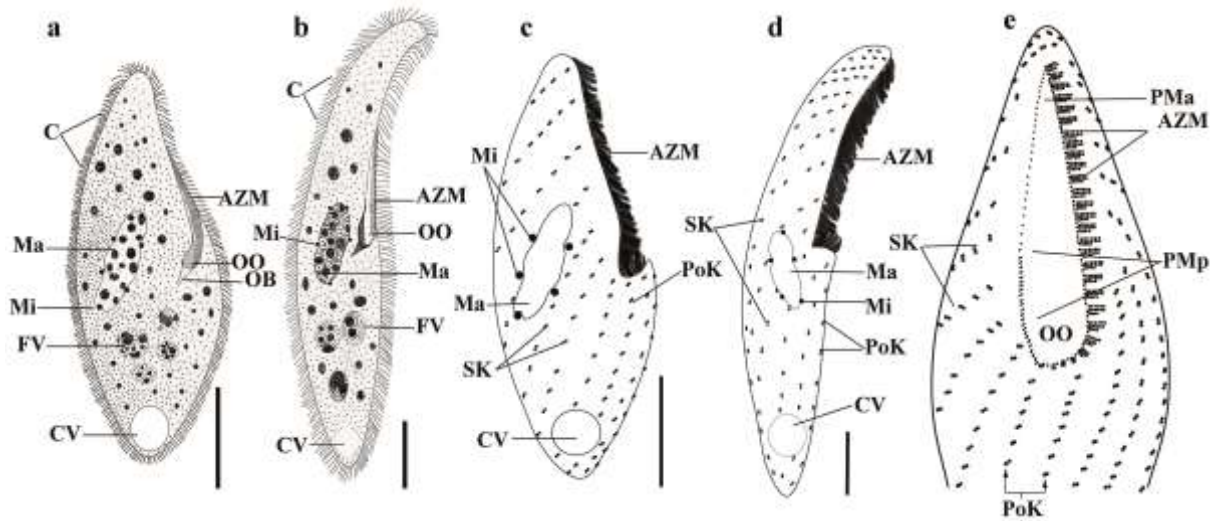


Figure 1. Morphology and ciliary pattern of *B. hyalinum* from life (a,b) and after silver impregnation (c-e). Right lateral views of representative individuals from population 1 (a) and population 2 (b). Infraciliature of the right lateral of population 1(c) and population 2 (d). e: Ventral infraciliature and details of oral region. AZM: adoral zone of membranelles, C: cilia, CV: contractile vacuole, FV: food vacuoles, Ma: macronucleus, Mi: micronucleus, OO: oral opening, PMa: anterior portion of paroral membrane, PMp: posterior portion of paroral membrane, PoK: postoral kineties, SK: somatic kineties. Scale bars 20 µm (a, c), 50 µm (d, e).

Şekil 1. *B. hyalinum*'un canlı (a, b) ve gümüş boyanmış örneklerinin (c-e) morfolojisi ve siliyatürü.

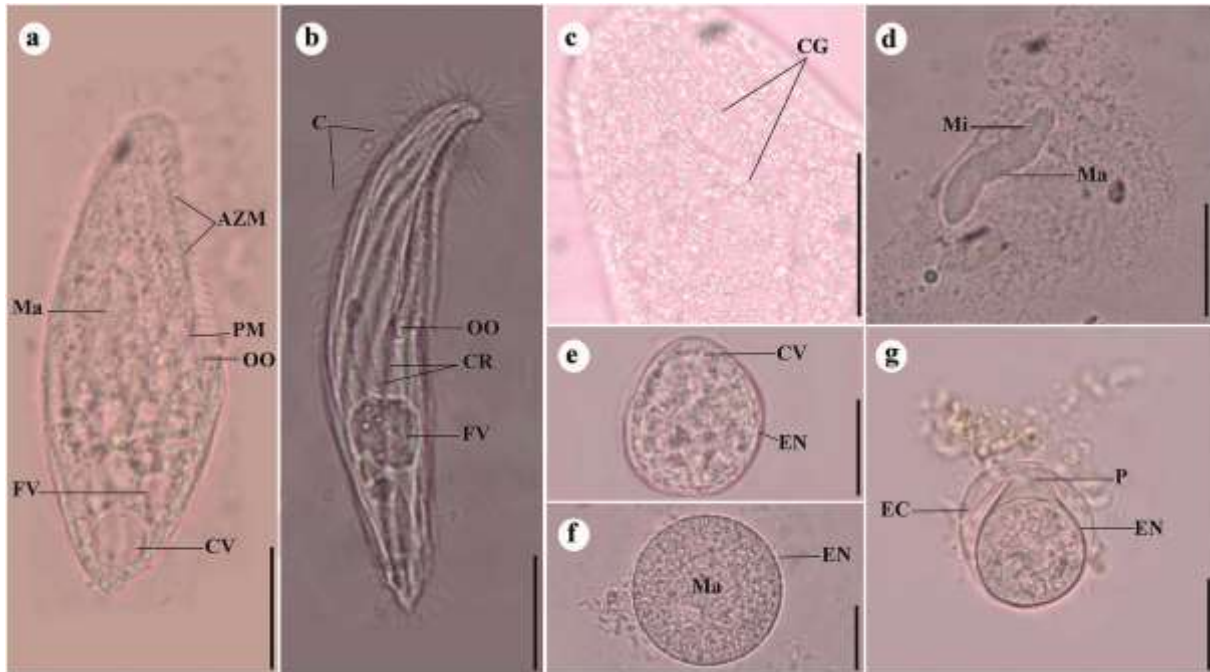


Figure 2. Photomicrographs of *B. hyalinum* from life. a, b: General views of typical individuals from population 1 (a) and population 2 (b). c: Cortical granules between somatic kineties. d: A squashed individual, showing the nuclear apparatus. e, f, g: Some stages of resting cyst in vivo. In the early phase of encystment, the contractile vacuole and endocyst membrane are observed (e, f). Mature resting cyst with an endocyst, an ectocyst, and a conical plug (g). AZM: adoral zone of membranelles, C: cilia, CV: contractile vacuole, CR: cortical ridges, CG: cortical granules, EN: endocyst, EC: ectocyst, FV: food vacuole, Ma: macronucleus, Mi: micronucleus, OO: oral opening, P: plug, PM: paroral membrane. Scale bars 20 µm.

Şekil 2. *B. hyalinum*'un canlı fotomikrografları.

Table 1. Morphometric characteristics of fixed specimens of *B. hyalinum* P1 (upper line) and P2 (lower line). Measurements are in μm^* .
Çizelge 1. *B. hyalinum* P1 (üst satır) ve P2 (alt satır) tespitli örneklerinin morfolometrik özellikleri. Ölçümler μm olarak verildi*.

Characteristics	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length (P)	74.68	75.40	8.60	1.92	11.52	60.00	87.47	20
	74.51	74.76	13.79	3.08	18.51	48.50	93.30	20
Body, maximum width (P)	20.67	20.02	3.53	0.79	17.08	15.80	28.30	20
	16.31	16.55	2.80	0.63	17.17	10.80	22.90	20
Body width/length, ratio (%)	27.74	27.09	3.88	0.87	13.99	23.10	36.85	20
	22.09	22.32	2.45	0.55	11.09	18.49	26.24	20
AZM, total length (P)	31.83	31.90	3.88	0.87	12.19	24.15	38.80	20
	30.93	31.10	4.42	0.99	14.29	18.60	39.30	20
Anterior body end to proximal point of AZM, distance (P)	33.82	33.54	4.07	0.91	12.03	25.61	40.35	20
	33.37	33.75	4.14	0.93	12.41	22.00	39.78	20
Distance from the proximal of the AZM to the anterior body end/body length, ratio (%)	45.47	45.06	4.42	0.99	9.72	38.59	53.52	20
	45.80	44.10	7.83	1.75	17.10	33.59	64.74	20
Adoral membranelles, number (SC)	29.37	29.50	1.59	0.29	5.41	26.00	32.00	30
	29.00	29.00	1.93	0.35	6.66	24.00	32.00	30
Base of longest adoral membranelle, length (SC)	4.58	4.50	0.74	0.14	16.16	3.10	6.60	30
	4.87	4.90	0.69	0.13	14.17	3.50	6.40	30
Paroral membrane, length (P)	28.72	28.50	3.72	0.83	12.95	22.24	35.20	20
	27.92	28.28	3.87	0.86	13.86	17.90	33.50	20
Macronucleus, number (P)	1.00	1.00	0.00	0.00	0.00	1.00	1.00	20
	1.00	1.00	0.00	0.00	0.00	1.00	1.00	20
Anterior body end to macronucleus, distance (P)	25.03	24.22	4.52	1.01	18.06	16.40	36.60	20
	24.03	24.05	4.11	0.92	17.10	16.60	33.10	20
Macronucleus, length (P)	16.39	16.30	3.97	0.89	24.22	9.60	23.89	20
	15.27	15.05	2.51	0.56	16.44	11.20	19.30	20
Macronucleus width (P)	5.21	5.46	1.01	0.23	19.39	3.10	6.90	20
	5.05	4.60	1.35	0.30	26.73	3.10	8.20	20
Micronucleus, number (P)	3.35	3.00	1.79	0.40	53.43	1.00	7.00	20
	3.22	3.00	1.31	0.31	40.68	1.00	6.00	18
Micronucleus, largest diameter (P)	1.20	1.20	0.15	0.03	12.50	0.90	1.45	20
	1.27	1.22	0.25	0.06	19.69	0.93	2.00	18
Somatic kineties, postoral number (SC)	16.63	16.00	1.38	0.25	8.30	14.00	19.00	30
	16.63	16.00	1.71	0.31	10.28	14.00	20.00	30
Postoral kineties, number (SC)	2.00	2.00	0.00	0.00	0.00	2.00	2.00	30
	2.00	2.00	0.00	0.00	0.00	2.00	2.00	30

*Data based on randomly selected protargol-impregnated (P) and silver carbonate-impregnated (SC) specimens from soil cultures. AZM: adoral zone of membranelles, CV: coefficient of variation in %, M: median, Max: maximum, Min: minimum, N: number of individuals investigated, SD: standard deviation, SE: standard error of the mean, $\bar{x}$: arithmetic mean.

Table 2. Morphometric characteristics of living specimens of *B. hyalinum* P1 (upper line) and P2 (lower line). Measurements are in μm^* .

Çizelge 2. *B. hyalinum* P1 (üst satır) ve P2 (alt satır) canlı örneklerinin morfolometrik özellikleri. Ölçümler μm olarak verildi*.

Characteristics	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	95.82	91.80	17.10	3.42	17.85	65.50	126.60	25
	100.78	98.40	11.37	2.27	11.28	81.80	128.80	25
Body, width	27.85	27.70	4.55	0.91	16.34	17.80	35.50	25
	21.56	20.40	3.72	0.74	17.25	16.10	30.00	25
Body width/length, ratio (%)	21.48	22.37	3.29	0.66	15.32	15.16	27.34	25
	43.40	42.90	7.47	1.59	17.21	28.90	55.60	22
AZM, total length	43.40	42.90	7.47	1.59	17.21	28.90	55.60	22
	41.80	40.93	3.92	0.88	9.38	36.60	50.00	20
Anterior body end to proximal point of AZM to apical, distance	49.42	49.15	7.36	2.13	14.89	35.80	60.02	12
	46.92	45.95	3.68	0.92	7.84	40.60	53.00	16
Distance between the proximal point of AZM and anterior end of the body /body length, ratio (%)	48.44	47.39	6.30	1.82	13.01	40.31	65.47	25
	46.08	45.80	3.99	1.00	8.66	39.83	52.90	16
Anterior body end to macronucleus, distance	30.28	30.68	6.78	2.77	22.39	20.00	38.80	6
	38.57	40.80	8.37	3.16	21.70	23.26	49.50	7
Macronucleus, length	20.81	21.49	4.75	1.37	22.83	11.70	29.30	12
	16.82	15.20	4.06	1.23	24.14	12.00	24.00	11
Macronucleus, width	9.37	9.15	2.84	0.82	30.31	4.53	13.70	12
	6.51	7.00	1.21	0.37	18.59	4.40	8.00	11

*Data based on randomly selected specimens from soil cultures. AZM: adoral zone of membranelles, CV: coefficient of variation in %, M: median, Max: maximum, Min: minimum, N: number of individuals investigated, SD: standard deviation, SE: standard error of the mean, $\bar{x}$: arithmetic mean.

Population 2 (P2). This population was named population 2 because it appeared after population 1 in Petri cultures. The overall body shape is spindle-shaped and different from the previous population (Figs. 1b, 2b). Slender with tapered posterior end, anterior pole curved ventrally; flexible, not contractile. In the liquid phase, it glides slowly by swinging its rear around the long axis. Except for body width, morphometry is similar to the previous population (Table 1). In vivo length approximately 101 μm (82-129 μm), width 22 μm (16.10 -30.00 μm). The body width is approximately 22% of the length (15.16-27.34 μm) (Table 2). Cortical ridges are more prominent than in the previous population, giving the cell a wavy appearance (Fig. 2b). The AZM runs to midbody, 46% of body length (Table 2). The cytoplasmic appearance, nuclear apparatus, nutrient vacuoles, and contractile vacuole are similar to the previous population (Figs. 1b, 2b). The macronucleus is compact, ellipsoidal (Fig. 2b), with numerous nucleoli (Fig. 3g). The somatic and oral ciliature is identical in general organization to the previous population (Figs. 1d, e, 3c-h). The posterior part of the paroral membrane is composed of tightly packed dikinetids. Near the front, the distance between the kinetids is greater (Fig. 1e).

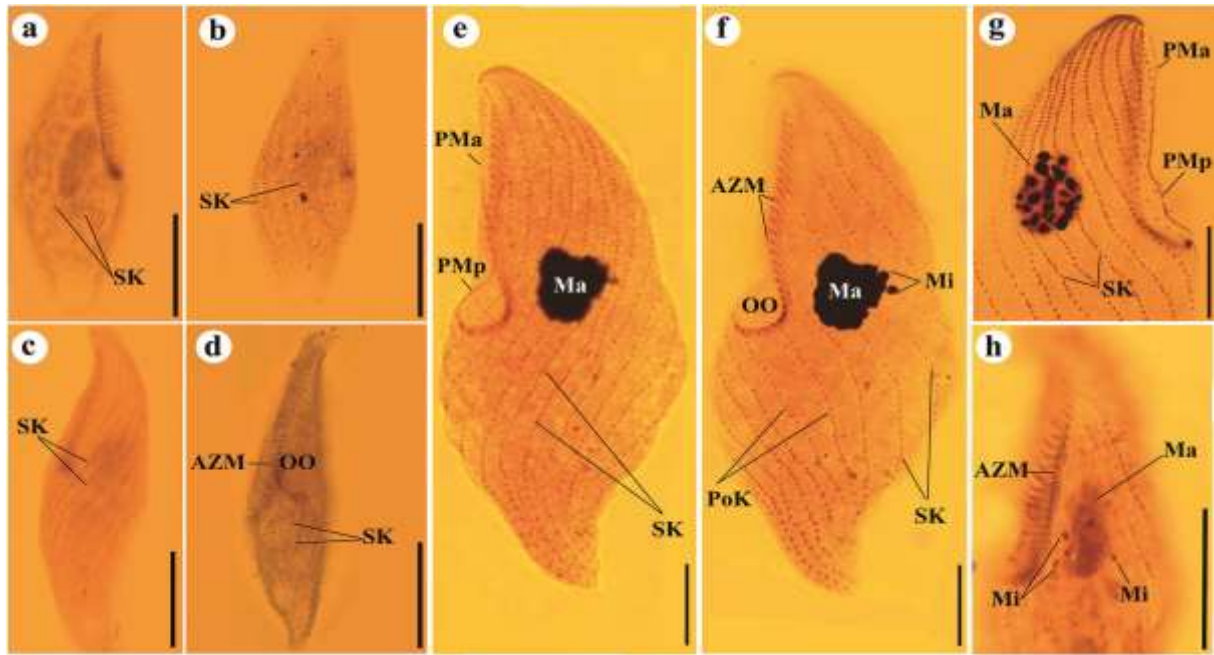


Figure 3. Photomicrographs of *B. hyalinum* after staining with protargol (a-d, h) and silver carbonate (e, f, g), showing somatic and oral infraciliature. AZM: adoral zone of membranelles, Ma: macronucleus, Mi: micronuclei, OO: oral opening, PMa: anterior portion of paroral membrane, PMp: posterior portion of paroral membrane, PoK: postoral kineties, SK: somatic kineties. Scale bars 20 μm .

Şekil 3. *B. hyalinum*'ün protargol (a-d, h) ve gümüş karbonat (e-g) ile boyanmış somatik ve oral infrasiliyatürü gösteren fotomikroagrafları.

Notes on the divisional morphogenesis. Stomatogenesis begins with kinetosomes proliferating within and to the left of the middle portion of a postoral ciliary row (Fig. 4a).

The irregular groups of kinetosomes formed at the end of replication clump together to form a single oral anlage. With the continuous proliferation of kinetosomes, the oral anlage gradually enlarges (Figs. 4b, 4c). The oral primordium is unequally longitudinally divided, and two kinetosomal areas are formed. The narrow kinetosomal area on the right side forms the paroral membrane anlage and the wide kinetosomal area on the left side forms the adoral membrane anlage (Fig. 4d). Until this stage, the oral anlage of the opisth (posterior cell) is formed, whereas no reorganization of the oral structure in the proter (anterior cell, parental) is observed. In the late stage of stomatogenesis, the oral structures (AZM and PM) of the opisthine become fully differentiated. Following stomatogenesis, transverse division (cytogenesis) occurs (Figs. 1e, 2f). The division of the macronucleus is simple: the elongated macronucleus divides into two parts before the two daughter cells separate from each other. The behavior of the micronuclei in the division process is not observed. The daughter cells are almost the same size as each other.

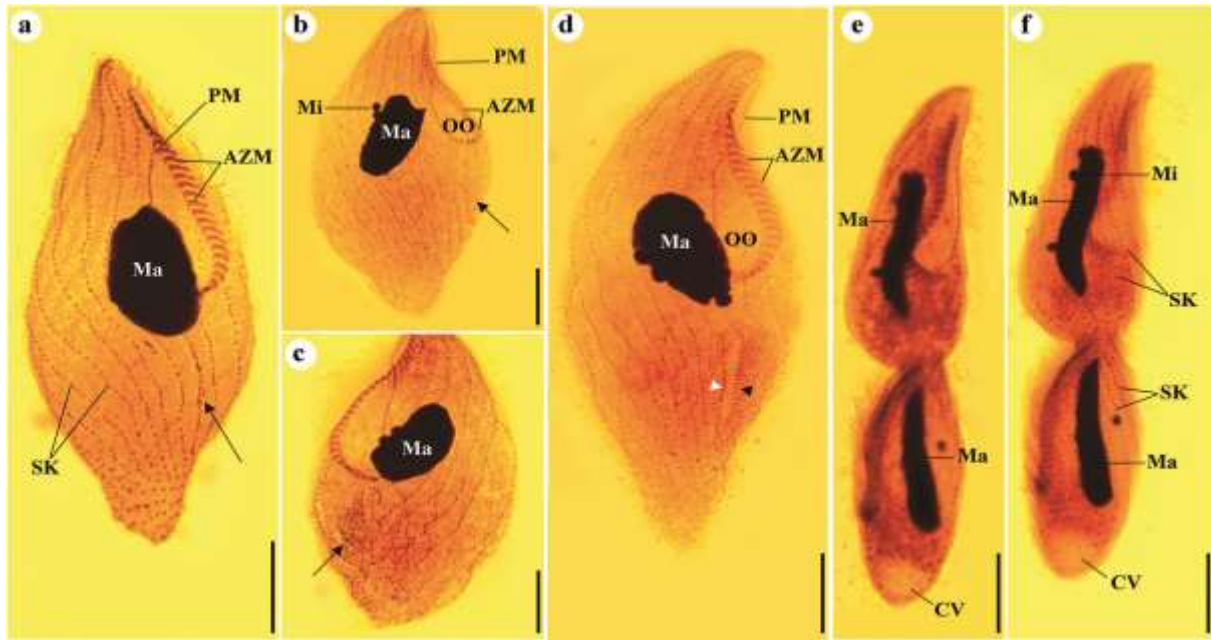


Figure 4. Morphogenesis in *B. hyalinum* after silver carbonate. Formation of the oral primordium of the opisthe (a-d). a: Ventral view of an early divider, showing proliferation of kinetosomes (arrow). b, c: The oral primordium (arrow) has enlarged by continued proliferation of kinetosomes. d: Ventral view of a middle divider, showing paroral membrane (white arrowhead) and adoral membranelles anlage (black arrowhead). e, f: Late divisional stage, showing ventro-lateral and dorso-lateral views of a cell before cytokinesis. Proter and opisthe are about to separate. AZM: adoral zone of membranelles, CV: contractile vacuole, Ma: macronucleus, Mi: micronuclei, OO: oral opening, PM: paroral membrane, SK: somatic kineties. Scale bars 20 μ m.

Şekil 4. *B. hyalinum*'da gümüş karbonat sonrası morfogenez.

Notes on the resting cyst morphology. It was possible to obtain a small number of cysts in this study. Therefore, detailed analysis could not be performed in the encystment and excystment process. The formation of cysts in this ciliate is a long process that takes 4-5 days. The morphology of the cysts is characteristic of the genus *Blepharisma*, it has three morphological structures: an inner wall (endocyst), an outer wall (ectocyst), and an emergency pore (plug).

At the end of the 4th or 5th day, the general body shape of starved vegetative individuals begins to change, with cell volume decreasing, followed by the formation of cyst walls. Individuals that will form cysts will begin to rotate rapidly in a clockwise direction around themselves, gradually becoming smaller and rounder. Cyst is surrounded by a cyst wall (endocyst) with a width of 0.93 μ m (n=4) (early stage of cyst formation). At the first stage, contractile vacuoles are observed, and there is motility in the cytoplasm (Fig. 2e). Cysts are spherical, 40-50 μ m in diameter, in the early stage. After a few hours, the contractile vacuole disappears, and the cytoplasm becomes homogeneous (Fig. 2f). At 7-9. days, an outer wall (ectocyst) (6.21 μ m, n= 4) and a conical plug (emergence pore) (length: 8.07 μ m, n= 4) independent of the ectocyst are recognized around the cyst (Fig 2g). The mature cysts measure approximately 36.29 μ m in length and 31.74 μ m in width (n=4).

Inter-population variation. One-way analysis of variance (ANOVA) showed no significant difference between the two populations in most morphometric traits (Table 3). Although there was no significant difference between cell lengths ($p > 0.05$), the difference between cell widths was significant ($p < 0.001$), and the difference between the ratio of body width to body length (%) was very significant ($p < 0.001$).

Molecular results

The 18s rDNA gene region, with a length of 1289 base pairs (bp) for the P1 population and 1658 bp for the P2 population, was sequenced. The SSU rDNA nucleotide sequences of *Blepharisma hyalinum* (P1) and *Blepharisma hyalinum* (P2) were deposited in GenBank with accession numbers PV489986 and PV489987, and G+C ratios were calculated to be 45.7% and 46.8%, respectively.

Table 3. Nucleotide similarity and dissimilarity matrix based on alignment of base positions of *Blepharisma* species.

Çizelge 3. *Blepharisma* türlerinin baz pozisyonlarının hizalanmasına dayalı nükleotid benzerlik ve farklılık matrisi.

Difference (cont.)		Similarity (%)																											
		1. <i>B. hyalinum</i> (P1)	2. <i>B. hyalinum</i> (P2)	3. <i>B. hyalinum</i> (KP970225)	4. <i>B. hyalinum</i> (AM713184)	5. <i>B. elongatum</i> (AM713186)	6. <i>B. americanum</i> (MF004000)	7. <i>B. americanum</i> (AM713182)	8. <i>B. americanum</i> (MF002366)	9. <i>B. americanum</i> (MF002365)	10. <i>B. americanum</i> (MF002364)	11. <i>B. americanum</i> (MT175503)	12. <i>B. lamniconchoidum</i> (KX116022)	13. <i>B. undulans</i> (KP970231)	14. <i>B. undulans</i> (KY855549)	15. <i>B. undulans</i> (KP970230)	16. <i>B. undulans</i> (AM713183)	17. <i>B. sinosum</i> (MK354013)	18. <i>B. sinosum</i> (KP970229)	19. <i>B. sinosum</i> (JN27438)	20. <i>B. steini</i> (AM713187)	21. <i>B. japonicum</i> (KP970228)	22. <i>B. japonicum</i> (KP970228)	23. <i>B. halophilum</i> (MF437020)	24. <i>B. musculus</i> (KJ651813)	25. <i>B. parvum</i> (MT175501)	26. <i>Blepharisma</i> sp. (KF204427)		
1	1	—	0.982	0.982	0.982	0.879	0.756	0.791	0.747	0.747	0.721	0.791	0.807	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791
2	2	2	—	0.982	0.879	0.756	0.791	0.747	0.747	0.721	0.791	0.807	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791
3	2	2	2	—	1.000	0.896	0.773	0.808	0.765	0.765	0.739	0.808	0.824	0.808	0.808	0.808	0.808	0.808	0.808	0.808	0.808	0.782	0.754	0.779	0.800	0.282	0.808	0.833	0.815
4	2	2	0	—	0.896	0.773	0.808	0.765	0.765	0.765	0.739	0.808	0.824	0.808	0.808	0.808	0.808	0.808	0.808	0.808	0.782	0.754	0.779	0.800	0.282	0.808	0.833	0.815	0.815
5	14	14	12	12	—	0.794	0.829	0.786	0.786	0.786	0.760	0.829	0.827	0.829	0.829	0.829	0.829	0.829	0.829	0.829	0.803	0.706	0.800	0.820	0.277	0.829	0.818	0.801	0.801
6	28	28	26	26	24	—	0.965	0.961	0.961	0.991	0.913	0.965	0.900	0.965	0.965	0.965	0.965	0.965	0.965	0.965	0.939	0.721	0.949	0.956	0.305	0.965	0.826	0.808	0.808
7	24	24	22	22	20	4	—	0.968	0.956	0.956	0.930	1.000	0.834	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
8	29	29	27	27	25	1	5	—	1.000	0.904	0.956	0.791	0.956	0.956	0.956	0.956	0.956	0.956	0.956	0.956	0.930	0.713	0.940	0.947	0.296	0.956	0.817	0.800	0.800
9	29	29	27	27	25	1	5	0	—	0.904	0.956	0.791	0.956	0.956	0.956	0.956	0.956	0.956	0.956	0.956	0.930	0.713	0.940	0.947	0.296	0.956	0.817	0.800	0.800
10	32	32	30	30	28	10	8	11	11	—	0.930	0.785	0.930	0.930	0.930	0.930	0.930	0.930	0.930	0.930	0.904	0.678	0.915	0.921	0.254	0.930	0.791	0.773	0.773
11	24	24	22	22	20	4	0	5	5	8	—	0.834	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
12	22	22	20	20	20	23	19	24	24	27	19	—	0.834	0.834	0.834	0.834	0.834	0.834	0.834	0.834	0.808	0.824	0.805	0.826	0.350	0.834	0.956	0.938	0.938
13	24	24	22	22	20	4	0	5	5	8	0	19	—	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
14	24	24	22	22	20	4	0	5	5	8	0	19	0	—	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
15	24	24	22	22	20	4	0	5	5	8	0	19	0	0	—	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
16	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	—	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
17	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	—	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
18	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	0	—	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843	0.843
19	27	27	25	25	23	7	3	8	8	11	3	22	3	3	3	3	3	3	—	0.721	0.940	0.965	0.288	0.973	0.834	0.817	0.801	0.801	
20	30	30	28	28	24	32	29	33	33	37	29	20	29	29	29	29	29	29	32	—	0.720	0.739	0.299	0.747	0.833	0.615	0.615	0.615	0.615
21	28	28	26	26	24	6	4	7	7	10	4	23	4	4	4	4	4	4	4	7	33	—	0.957	0.305	0.966	0.830	0.813	0.813	0.813
22	25	25	23	23	21	5	1	6	6	9	1	20	1	1	1	1	1	1	1	4	30	5	—	0.305	0.991	0.852	0.834	0.834	0.834
23	86	86	84	84	86	82	81	83	83	86	81	76	81	81	81	81	81	81	81	84	82	84	82	—	0.313	0.341	0.324	0.324	0.324
24	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	0	0	0	3	29	4	1	81	—	0.860	0.843	0.843	0.843
25	21	21	19	19	19	21	20	16	21	21	24	16	5	16	16	16	16	16	16	19	19	20	17	77	16	—	0.962	0.962	0.962
26	23	23	21	21	23	22	18	23	23	26	18	7	18	18	18	18	18	18	18	21	21	22	19	79	18	2	—	0.962	0.962

The nucleotide sequences of the present populations were aligned with the gene sequences of other *Blepharisma* species obtained from the GenBank using the ClustalW multiple alignment algorithm, and 123 of the 1289 bp were found to differ by at least one base (Fig. 5). Similarity and dissimilarity matrices obtained using aligned nucleotide sequences constructed only from sites showing polymorphism showed that the current populations (P1 and P2) differed from the other populations by only two nucleotide sites, showing a similarity of 98.2% (Table 3).

It is noteworthy that there are no nucleotide differences between populations belonging to the same or different species such as *B. americanum* (AM713182, MT175503), *B. undulans* (KP970231, KY855549, KKP790233, AM131183) *B. sinosum* (MK354013, KP970229) *B. musculus* KJ651813 and the similarity is complete (100%) (Table 3).

Since the phylogenetic trees produced by Bayesian inference (BI) and maximum likelihood (ML) are topologically very similar, the results of the phylogenetic analysis are presented by showing the BI posterior probability values (pp>0.50) on the ML tree (Fig. 6).

Although the vast majority of *Blepharisma* populations form a monophyletic clade derived from a common ancestor, *B. halophilum* populations are more closely related to Folliculinidae than their congeners (Fig. 6).

The distinction between the major clade Blepharismidae and the clade containing Folliculinidae, Fabreidae, and Maristentoridae, in which *B. halophilum* is clustered, was strongly supported by Bayesian phylogenetic analysis (BI=0.96), while maximum likelihood analysis moderately supported this distinction (ML=54). In both phylogenetic analyses, this populations (PV489986, PV489987) were placed together as sister taxa with strong support (BI/ML=0.99/85). Other populations of *B. hyalinum*, AM713184 and KP970225, were positioned at the base of this populations by both algorithms with full support. At the basal position of the *B. hyalinum* cluster, *B. elongatum* (AM713186) appears as a closely related population.

DISCUSSION

Studies on the species belonging to the genus *Blepharisma* go back a long way. *B. hyalinum* was first isolated and named by Perty (1849) from freshwater containing duckweed (*Lemna*) and filamentous green algae. However, the description was inadequate as the type of population consisted of only a small number of individuals. In the present study, 2 blepharismid populations, which were morphologically and mode of movement distinguishable from each other, were successively observed in soil cultures. As a result of one-way ANOVA, cell width and the ratio of cell width to length (%) of both populations were found to be significantly different ($P<0.001$). However, when the 18S

rDNA gene region was compared, the nucleotide sequences were very similar, and it was concluded that these two populations were two different morphotypes of *B. hyalinum*. It is widely believed that intra-population variations in species belonging to the genus *Blepharisma* are to a large extent influenced by environmental factors, such as diet and stage of the life cycle (Giese, 1973; Larsen & Nilsson, 1983). Such morphological variations therefore make no taxonomic sense (Yan et al., 2016), but tend to confuse those trying to identify the species (Gupta et al., 2015). These variations also cause many morphological features to overlap between *Blepharisma* species, as shown in previous studies (Stein, 1867; Kahl, 1932; Larsen, 1982; Al-Rasheid et al., 2001; Lee & Shin, 2009; Fernandes et al., 2013; Yan et al., 2016; Pan & Stoeck, 2017; Hao et al., 2022). Among its congeners, *B. hyalinum* is the only one that closely resembles *B. elongatum*. *B. hyalinum*, as well as *B. elongatum*, has colourless cortical granules and a single compact macronucleus (Larsen & Nilsson, 1983; Larsen & Nilsson, 1988).

	10	20	30	40	50	60	70	80
<i>B. hyalinum</i> (P1)	AATCTACAAA	TCG--TAGAT	ATT-GGCCGC	GATACTGGTT	GGTCT-TTGT	TTCG-ACGAT	CCACCATTTCG	CGGC-GGG-T
<i>B. hyalinum</i> (P2)	AATCTACAAA	TCG--TAGAT	ATT-GGCCGC	GATACTGGTT	GGTCT-TTGT	TTCG-ACGAT	CCACCATTTCG	CGGC-GGG-T
<i>B. hyalinum</i> (KP970225)	AATCTACAAA	TCG--TAGAT	ATT-GGCCGC	GATACTGGTT	GGTCT-TTGT	TTCG-ACGAT	CCACCATTTCG	CGGC-GGG-T
<i>B. hyalinum</i> (AM713184)	AATCTACAAA	TCG--TAGAT	ATT-GGCCGC	GATACTGGTT	GGTCT-TTGT	TTCG-ACGAT	CCACCATTTCG	CGGC-GGG-T
<i>B. elongatum</i> (AM713186)	AATCTACAAA	TCG--TAGAT	ATTGGCCGCG	GATTCCGGCT	GGTCT-TGAT	TTCGCACGAT	CCACCATTTCG	CGGC-GGG-T
<i>B. americanum</i> (MF002400)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. americanum</i> (AM713182)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. americanum</i> (MF002385)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. americanum</i> (MF002395)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. americanum</i> (M97909)	AATCTGAAAA	TCG--TAGAT	AT---GGCCG	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. americanum</i> (MT175503)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. bimicrocneleum</i> (KX119522)	ATTCTAAGAA	TCG--TAGAC	ATT-GGCCGC	GGCCCGGGCT	GGTCT-CAGC	TTCG-ATAGT	CCACCGCTCG	CGGC-GGG-T
<i>B. undulans</i> (KP970231)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. undulans</i> (KY855549)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. undulans</i> (KP970233)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. undulans</i> (AM713183)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. sinuosum</i> (MK354013)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. sinuosum</i> (KP970229)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. sinuosum</i> (JN627438)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. steini</i> (AM713187)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. japonicum</i> (KP970226)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. japonicum</i> (KP970228)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. halophilum</i> (MF437020)	TGTGTTAAAGT	CTCTTAGCGC	GCT-GATTAG	ACAGCTCGAA	TAGCTACTAG	CCTA-GTGA	CTCGGTCGGT	TCCA-CTA-C
<i>B. musculus</i> (KJ651813)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-TGAC	TTCG-ATAGT	CCACCTCTCG	TGGC-GGG-T
<i>B. penardi</i> (MT175501)	AATCTGAAAA	TCG--TAGAT	ATT-GGCCGC	GGTCTGGCT	GGTCT-CAGC	TTCG-ATAGT	CCACCGCTCG	CGGC-GGG-T
<i>Blepharisma</i> sp. (KF206427)	AATCCGAAAA	TCG--TAGAC	ATT-GGCCGC	GGCCCTGACT	GGTCT-CAGC	TTCG-ATGGT	CCACCGCTCG	CGGC-GGG-T
	90	100	110	120				
<i>B. hyalinum</i> (P1)	ATCATCGT-A	TTCAG-TCAG	CCTGAGTCTT	CTCTCAGAGT	CAC			
<i>B. hyalinum</i> (P2)	ATCATCGT-A	TTCAG-TCAG	CCTGAATATT	CTCTCAGAGT	CAC			
<i>B. hyalinum</i> (KP970225)	ATCATCGT-A	TTCAG-TCAG	CCTGAATCTT	CTCTCAGAGT	CAC			
<i>B. hyalinum</i> (AM713184)	ATCATCGT-A	TTCAG-TCAG	CCTGAATCTT	CTCTCAGAGT	CAC			
<i>B. elongatum</i> (AM713186)	ATCATCGT-A	CCAAG-TCAG	CCTGAATCTT	CTTTCAAAGT	CAC			
<i>B. americanum</i> (MF002400)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTCCAAAGT	CGC			
<i>B. americanum</i> (AM713182)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. americanum</i> (MF002385)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTCCAAAGT	CGC			
<i>B. americanum</i> (MF002395)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTCCAAAGT	CGC			
<i>B. americanum</i> (M97909)	ATCATCGT-C	TAAACAGTCAG	TG-GAATCCT	CTTCCAAAGT	CGC			
<i>B. americanum</i> (MT175503)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. bimicrocneleum</i> (KX119522)	ATCATCGT-A	CCAAG-TCAG	CCTGAATCCT	CTCTCAGAGT	CGC			
<i>B. undulans</i> (KP970231)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. undulans</i> (KY855549)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. undulans</i> (KP970233)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. undulans</i> (AM713183)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. sinuosum</i> (MK354013)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. sinuosum</i> (KP970229)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. sinuosum</i> (JN627438)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. steini</i> (AM713187)	ATCATCGT-A	C-----CAG	CTG-AGCCT	CTCTCAGAGT	CGC			
<i>B. japonicum</i> (KP970226)	ATCATCGTTC	TAAACAGTCAG	CCTGAATCCT	CTTCCAAAGT	CGC			
<i>B. japonicum</i> (KP970228)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. halophilum</i> (MF437020)	GCCGCCGT-A	CCAAA-TGAG	CCTAAATCCC	AGCAAGGCTG	TGC			
<i>B. musculus</i> (KJ651813)	ATCATCGT-C	TAAACAGTCAG	CCTGAATCCT	CTTTCAAAGT	CGC			
<i>B. penardi</i> (MT175501)	ATCATCGT-A	CCAAG-TCAG	CCTGAATCCT	CTCTCAGAGT	CGC			
<i>Blepharisma</i> sp. (KF206427)	ATCATCGT-A	CCAAG-TCAG	CCTGAATCCT	CTCTCAGAGT	CGC			

Figure 5. Nucleotide divergence sequence of *Blepharisma* species based on alignment of 18S rDNA base positions.
Şekil 5. 18S rDNA baz pozisyonlarının hizalanmasına dayalı *Blepharisma* türlerinin nükleotid farklılık dizisi.

The geographical distribution of *B. hyalinum* is very wide; it has been reported at low densities but in all geographical regions (Holarctic, Neotropical, Palaetropical, Austral, Antarctic). It has been isolated from the eutrophic pond on the island of Funen in Denmark (Larsen & Nilsson, 1983), the Ivory Coast in West Africa (Dragesco & Dragesco-Kernéis, 1986), Austrian soil samples (Foissner, 1989), the Jubail Marine Wildlife Sanctuary on the Persian Gulf coast of Saudi Arabia (Al-Rasheid, 1999), the Anzali Wetland of the Caspian Sea (Asadullayeva & Alekperov, 2007), and terrestrial Antarctica (Thompson et al., 2019). Despite being one of the most common species within the genus *Blepharisma*, detailed morphometric data on *B. hyalinum* remain scarce, except for the studies by Larsen & Nilsson (1983), Foissner (1989), and Aescht & Foissner (1998). The populations under study here are consistent with the original and other previous definitions in terms of general morphology and infraciliature. However, morphometrics differ slightly (Table 3). Compared to previous descriptions of *B. hyalinum*, this populations have a larger body size (population 1: about 96 x 28 µm; population 2: about 101 x 22 µm). The cell size of this populations is closer to aquatic populations (100-130 µm, 100 µm, 80-160 µm) (Kahl, 1932; Larsen & Nilsson, 1983; Dragesco & Dragesco-Kernéis, 1986) than to soil isolated populations (about 70 µm) (Foissner, 1989; Aescht & Foissner, 1998), contrary to what Foissner (1989) previously stated. Foissner (1989) noted that populations in freshwater tend to be larger than those in terrestrial environments.

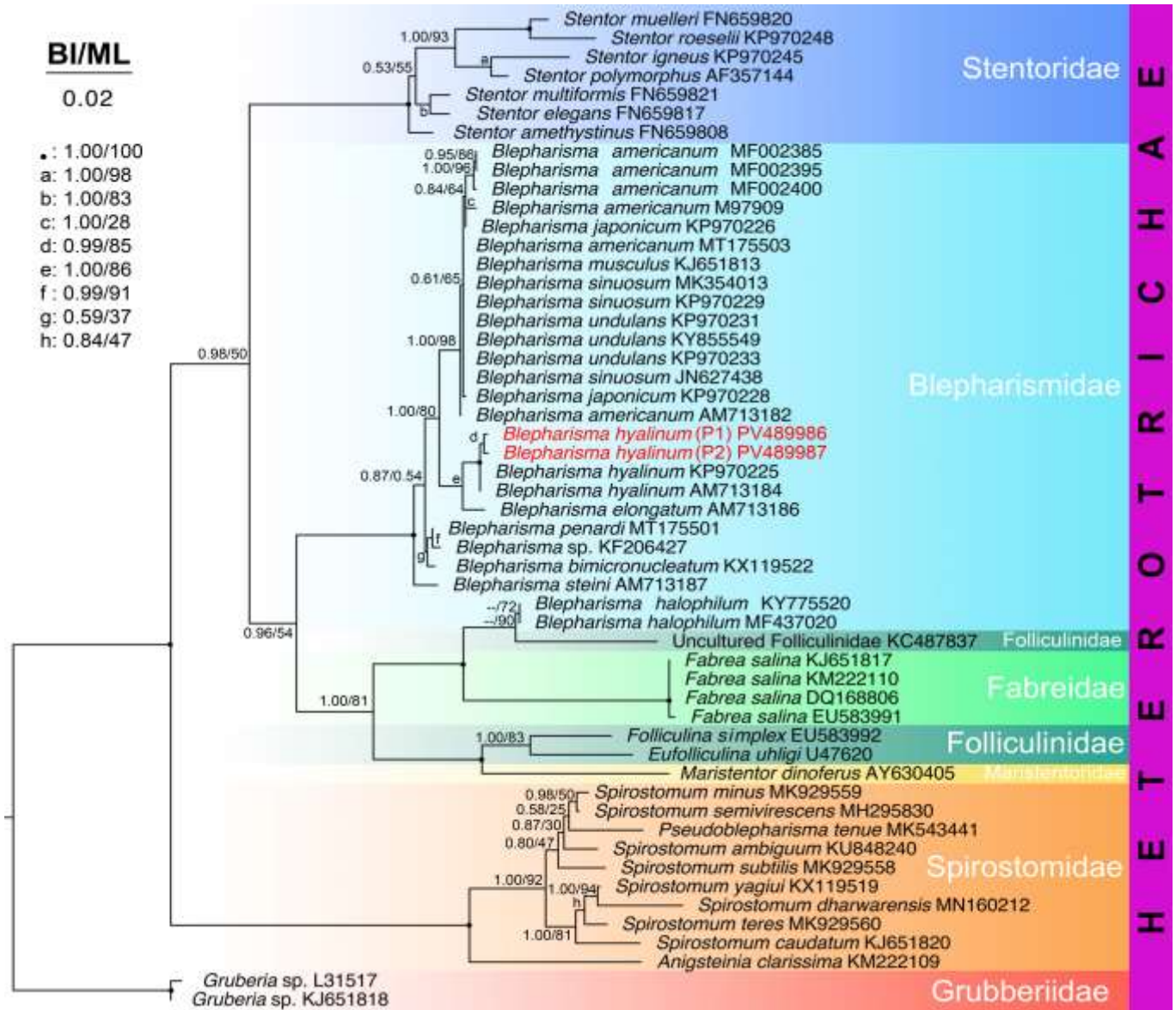


Figure 6. Phylogenetic tree of BI (Bayesian inference) and ML (maximum likelihood) based on the sequence of the 18S rDNA gene. The populations shown in red on the tree are those isolated in this study. Numbers at node indicate Bayesian posterior probability (BI) and maximum likelihood bootstrap (ML) values. Solid circles on the nodes indicate full support by both algorithms. The scale bar corresponds to 2 substitutions per 100 nucleotide positions.

Şekil 6. 18S rDNA gen dizisine dayalı BI (Bayesian çıkarılması) ve ML (maksimum olabilirlik) filogenetik ağacı.

This observations agree well with Aescht & Foissner (1998) on stomatogenesis. The divisional morphogenesis of *B. hyalinum* is parakinetal stomatogenesis, typical of heterotrich ciliates, in which the oral primordia are derived from postoral somatic kineties (Lynn, 2008). In contrast to some other *Blepharisma* species (e.g., *B. americanum*, *B. undulans*; Aescht & Foissner, 1998), the proliferation of the kinetosomes for the oral primordium of the opisthe occurs only one post-oral ciliary row (monoparakinetal) and no reorganization of the ancestral oral apparatus in the proter is seen.

The formation of cysts is a survival strategy of the ciliates in unfavorable conditions, and it also plays an important role in their geographical dispersal (Cavaleiro et al., 2018). The cyst morphology of *B. hyalinum* is the same as that of its congeners (Isquith et al., 1965; Repak, 1968; Larsen, 1982; Larsen & Nilsson, 1988; Cavaleiro et al., 2018; Li et al., 2022). Within the genus *Blepharisma*, the frequency of cyst formation varies between species (Isquith et al., 1965; Larsen, 1982). *Blepharisma hyalinum*, which is widely distributed, is expected to have a high encystment capacity and consequently a high incidence of cyst formation. However, despite repeated attempts under laboratory

conditions, cyst formation was observed less frequently than anticipated. The process of cyst formation also takes a longer time (in 4–5 days).

The positions of *Blepharisma* populations on the phylogenetic tree (Fig. 6) and the similarities and differences of nucleotide sequences (Table 4) are compatible with each other. The two populations obtained in the present study were concluded to represent two distinct morphotypes of the same species, as they showed high similarity to previously reported *B. hyalinum* populations (AM713184 and KP970225) (Schmidt et al., 2007; Fernandes et al., 2016), in terms of nucleotide sequences. This populations differ from others by only two nucleotides.

The first molecular phylogenetic studies on *Blepharisma* spp. have shown that this genus is a monophyletic taxon (Miao et al., 2009; Thamm et al., 2010; Fernandes et al., 2013; Shazib et al., 2014; Fernandes et al., 2016; Yan et al., 2016). Recent studies, including gene sequences of *B. halophilum* reveal that the genus *Blepharisma* is paraphyletic (Pan & Stoeck, 2017; Jin et al., 2021; Ye et al., 2021; Hao et al., 2022). In this study, populations of *B. halophilum* (KY775520 and MF437020) were found more closely related to members of the families Folliculinidae, Fabreidae, and Maristentoridae than their other congeners, supporting that the genus is a paraphyletic taxon. In previous studies (Pan & Stoeck, 2017; Hao et al., 2022), it was stated that *B. halophilum* was included in the genus *Blepharisma* due to its morphological features, such as a contractile body, unfragmented and two-part paroral membrane, but should be considered in a separate genus because of its extremely saline habitat, as in the karyorelicted ciliates *Loxodes* and *Remanella*. The nucleotide similarities and differences determined in this study and the location of *B. halophilum* populations (KY775520 and MF437020) on phylogenetic trees support the suggestion of Hao et al. (2022).

Table 4. Comparison of the main characteristics of *B. hyalinum* populations (P1: population 1, P2: population 2).
Çizelge 4. *B. hyalinum* popülasyonlarındaki ana karakteristiklerin karşılaştırılması (P1: popülasyon 1, P2: popülasyon 2).

Characteristics	Denmark, Funen (Larsen & Nilsson, 1983)	Austria, Pfennigberg (Foissner, 1989)	Austria, Pfennigberg (Aescht & Foissner, 1998)	P1	P2
				Present work	
Body length, µm	80-160	47-78	61-79	66-127	82-129
Body width, µm	24-48	12-25	16-24	18-36	16-30
Macronucleus length, µm	25	9-20	11-19	12-29	12-24
Macronucleus width, µm	12	4-7	5-8	5-14	4-8
Number of micronuclei	4-7	2-5	1-2	1-7	1-6
Diameter of micronucleus, µm	2	1.2-1.7	1-2	1-1.45	1-2
Number of somatic kineties	18	11-14	12-14	14-19	14-20
AZM length, µm	-	-	32-43	26-40	22-40
Number of adoral membranelles	30	22-30	26-30	26-32	24-32
Habitat	eutrophic lake	forest soil	forest soil	pine and meadow soil	

In the similarity and dissimilarity matrices, *B. americanum* (MT175503), *B. musculus* (KJ651813), *B. sinuosum* (JN627438, MK354013, KP970229), *B. undulans* (KP970231, KY855549, KP970233), and *B. japonicum* (KP970228) populations have no or very low nucleotide differences, while nucleotide similarity is complete or quite high. This similarity is clearly reflected in the phylogenetic trees obtained with both algorithms (BI and ML), where populations from morphologically distinct species are closely positioned and exhibit polyatomic relationships within the same cluster. Similar results were found in the phylogenetic trees reported in previous studies (Luo et al., 2019; Chi et al., 2021; Hao et al., 2022). We think that this taxonomic uncertainty may be caused by nucleotide sequences obtained from morphologically misidentified populations. In morphological studies in soil cultures, variations in cell shape and size, macronucleus shape, and number were observed in populations in different culture dishes or different periods of culture.

In conclusion, it is clear that the genus *Blepharisma* needs to be revised using modern taxonomic techniques, as previously suggested by (Foissner et al., 2002). Therefore, study provides valuable morphological and molecular data that may serve as a basis for future taxonomic revisions within the genus *Blepharisma*.

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Contribution Rate Statement Summary of ResearchersContribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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Assessment of the Antimicrobial Effect of Honey on *Listeria monocytogenes* Strains

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ABSTRACT

This study aimed to determine the antimicrobial effects of different honey types against *Listeria monocytogenes* strains. The antimicrobial activity of 40 honey samples, collected from different provinces of Türkiye and categorized as filtered floral honey, comb floral honey, acacia honey, and pine honey, was evaluated against 29 *L. monocytogenes* strains using the well diffusion method. The inhibition zone diameters formed by honey samples at 100% and 50% concentrations against *L. monocytogenes* strains ranged from 0.0 to 4.8 cm. The average inhibition zone diameter of the 100% honey samples was found to be 3.54 cm, while the average inhibition zone diameter of the 50% concentration was 3.30 cm. The 100% concentration of honey samples has higher antimicrobial activity, and when the honey samples are diluted, the antimicrobial activity decreases. Pine honey exhibited the highest antimicrobial activity in this study. Following pine honey, the antimicrobial effects decreased in the following order: filtered flower honey, comb flower honey, and acacia honey.

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Balın *Listeria monocytogenes* Suşları Üzerindeki Antimikrobiyal Etkisinin Değerlendirilmesi

ÖZET

Bu çalışma, farklı bal türlerinin *Listeria monocytogenes* suşlarına karşı antimikrobiyal etkilerini belirlemeyi amaçlamıştır. Türkiye'nin çeşitli illerinden toplanan ve süzme çiçek balı, petek çiçek balı, akasya balı ve çam balı olarak sınıflandırılan 40 bal örneğinin antimikrobiyal aktivitesi, 29 *L. monocytogenes* suşuna karşı kuyucuk difüzyon yöntemi kullanılarak değerlendirilmiştir. *L. monocytogenes* suşlarına karşı %100 ve %50 konsantrasyonlardaki bal örneklerinin oluşturduğu inhibisyon zon çapları 0,0 ile 4,8 cm arasında değişmiştir. %100 bal örneklerinin ortalama inhibisyon zon çapı 3,54 cm, %50 konsantrasyonda ise 3,30 cm olarak bulunmuştur. %100 konsantrasyondaki bal örneklerinin daha yüksek antimikrobiyal aktivite gösterdiği ve bal örnekleri seyreltilince bu etkinin azaldığı belirlenmiştir. Bu çalışmada en yüksek antimikrobiyal aktivite çam balında gözlemlenmiştir. Çam balını sırasıyla süzme çiçek balı, petek çiçek balı ve akasya balı takip etmiştir.

Mikrobiyoloji

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INTRODUCTION

Each year, approximately 600 million people worldwide fall ill due to the consumption of contaminated food, with approximately 420,000 of these cases resulting in death. Similarly, around 125,000 children under the age of five die annually as a consequence of consuming contaminated food (Anonymous, 2024). Foodborne diseases caused by various pathogens pose a significant threat to public health and food safety in contemporary society. Pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Clostridium botulinum*, *Campylobacter jejuni*, and *Vibrio parahaemolyticus* are frequently isolated from food sources and are known to

adversely affect human health (Istanbullugil et al., 2023). Among these, *L. monocytogenes* holds particular importance as it is responsible for listeriosis infections. Gastrointestinal symptoms such as headache, abdominal pain, fever, and vomiting are commonly observed in listeriosis cases. However, in individuals with compromised or suppressed immune systems, such as pregnant women, the elderly, infants, and patients with AIDS or cancer, *L. monocytogenes* can lead to severe complications, including meningitis, miscarriage, neonatal death, and septicemia, which may ultimately result in mortality (Grigore-Gurgu et al., 2024).

L. monocytogenes is commonly found in nature and, consequently, is frequently encountered in food and food processing environments. It can be isolated from soil, animal feed, water, feces of mammals and birds, raw foods, meat and meat products, poultry and their products, raw milk, and dairy products. This widespread presence enables it to cause contamination at various stages of food production, storage, and distribution (Zamuz et al., 2021). *L. monocytogenes* is characterized as a bacterium resistant to adverse environmental conditions. It can survive and proliferate even under conditions designed to control the microbial load of food, such as drying, cold storage, freezing, and increasing acidity. This pathogen is capable of growth within a temperature range of 0–45°C and thrives across a broad pH spectrum (4.4–9.4). Furthermore, it can survive and multiply in environments with high water activity (above 0.92) and in saline conditions (Orsi et al., 2011; Aktop et al., 2020; Taylor & Zhu, 2021). Various methods are employed in food processing and preservation to control microbial growth, while also maintaining the nutritional properties and sensory quality of food. Commonly used techniques include thermal processing, reduction of water activity, the use of antimicrobial substances, application of antioxidants, cold storage, freezing, irradiation, and modified atmosphere packaging (Dweh et al., 2024). However, contemporary consumer preferences have shifted toward natural, healthy, and minimally processed foods. Consequently, the food industry has begun exploring alternative methods to prevent contamination by pathogenic microorganisms (Ariyamuthu et al., 2022). For centuries, honey has been valued not only as a food but also as a natural medicine and food preservative. Recent research has further validated its beneficial bioactive effects on human health (Tsadila et al., 2021).

Honey plays a significant role in human health due to its antimicrobial, antioxidant, and anti-inflammatory properties, attributed to its rich bioactive components. Extensive research has highlighted honey's broad-spectrum antimicrobial effects, including antibacterial, antifungal, antimycobacterial, and antiviral activities (Çınar, 2020; Ali et al., 2023). These properties have been confirmed through numerous in vitro studies and a limited number of clinical trials (Istanbullugil et al., 2023). Honey, containing over 200 components, has been used since ancient times in the treatment of various diseases and exhibits antimicrobial activity against many pathogenic microorganisms. Honey is primarily composed of carbohydrates. Additionally, it contains small quantities of proteins, amino acids, phytochemicals, and enzymes derived from plants and bees, such as glucose oxidase and catalase (Tsadila et al., 2021). Honey's antibacterial activity can be attributed to several factors, including the production of hydrogen peroxide, its high sugar concentration, low pH, low water activity, phenolic compounds, and flavonoids. (Bayrak, 2005; Brudzynski et al., 2012; Doğan, 2014). Poltorak et al. (2018) demonstrated that incorporating honey into sausage production reduces microbial load. Similarly, Çınar (2020) reported that multifloral honeys exhibit greater antimicrobial activity than monofloral honeys. Among monofloral honeys, lemon blossom honey was found to have the highest antimicrobial activity, followed by lavender and thyme honeys. Yalazi & Zorba (2020) investigated honeydew honeys from the Kazdağları region and found that they exhibited very low or low antimicrobial activity against *E. coli*, *S. aureus*, and *S. Typhimurium*. However, these honeys showed no antimicrobial effect against *C. albicans*, *S. cerevisiae* yeast strains, or *B. cereus* bacteria.

Different honey samples exhibit varying levels of antimicrobial activity against *L. monocytogenes* strains (Çam, 2006). Ertürk et al. (2009) found that "mad honey" obtained from *Rhododendron* (rosebay) demonstrated antimicrobial effects against *L. monocytogenes*. Similarly, Silici et al. (2010) reported that 50 honey samples at 50% and 75% concentrations showed moderate inhibition against *L. monocytogenes* strains. Polat (2011) observed that pine, davulga, and heath honeys produced in the Southern Marmara region exhibited antimicrobial activity against *L. monocytogenes* at concentrations of 40% or higher (100%, 80%, 60%, 40%, and 20%). Sousa et al. (2016) noted that 24 honey samples showed low antimicrobial activity against *L. monocytogenes* strains isolated from food products. According to Çınar (2020), multifloral honeys exhibited higher antimicrobial activity compared to monofloral honeys, such as lavender, lemon blossom, and thyme honeys. Among monofloral types, lemon blossom honey was identified as the most effective, demonstrating moderate antimicrobial activity against *L. monocytogenes*.

The aim of this study is to evaluate the antimicrobial effects of solutions prepared at different concentrations from honey samples, obtained directly from primary producers and available in the market, against *L. monocytogenes* strains that were previously isolated from ready-to-eat foods and identified using molecular methods.

MATERYAL and METOD

Honey Samples

In this study, 40 honey samples were categorized into four groups: filtered floral honey (FFH), comb floral honey (CFH), acacia honey (AH), and pine honey (PH). These samples were obtained from primary producers in the provinces of Ankara, Adana, Artvin, and Mersin in Türkiye (Table 1).

Table 1. Honey samples used in the study and their provinces of origin

Çizelge 1. Çalışmada kullanılan bal örnekleri ve menşei illeri

Honey code numbed	Honey type	Province of Origin	Honey code numbed	Honey type	Province of Origin	Honey code numbed	Honey type	Province of Origin
1	FFH	Ankara	15	FFH	Ankara	29	FFH	Adana
2	CFH	Ankara	16	AH	Ankara	30	FFH	Adana
3	FFH	Ankara	17	PH	Ankara	31	FFH	Adana
4	FFH	Ankara	18	FFH	Ankara	32	CFH	Adana
5	CFH	Ankara	19	FFH	Ankara	33	CFH	Adana
6	FFH	Ankara	20	FFH	Ankara	34	CFH	Adana
7	FFH	Ankara	21	FFH	Artvin	35	CFH	Mersin
8	FFH	Ankara	22	FFH	Artvin	36	CFH	Mersin
9	FFH	Ankara	23	FFH	Artvin	37	CFH	Mersin
10	AH	Ankara	24	FFH	Artvin	38	PH	Mersin
11	PH	Ankara	25	PH	Adana	39	PH	Mersin
12	FFH	Ankara	26	FFH	Adana	40	FFH	Mersin
13	FFH	Ankara	27	PH	Adana			
14	AH	Ankara	28	PH	Adana			

FFH: Filtered Floral Honey; CFH: Comb Floral Honey; AH: Acacia Honey; PH: Pine Honey

Listeria monocytogenes and Reference Strains

In the study, 29 *L. monocytogenes* strains, which were previously isolated from ready-to-eat foods and molecularly identified, were used as bacterial cultures. All *L. monocytogenes* strains and the *L. monocytogenes* ATCC 7644 reference strain were obtained from the culture collection of the Department of Food Engineering, Faculty of Engineering, Ankara University.

Preparation of Stock Cultures

The reference bacterium and *L. monocytogenes* strains were prepared as stock cultures in Brain Heart Infusion (BHI) broth and Tryptic Soy Broth (TSB) medium containing 20% sterile glycerol. The stock cultures were stored at -20°C. Throughout the study, the cultures were developed in TSB and/or BHI broth at 37°C for 18 hours before being used as working materials.

Preparation of Bacterial Cultures

In this study, a single colony of *L. monocytogenes* was first obtained by streaking on Tryptic Soy Agar (TSA) plates to isolate a pure culture. After 24 hours of incubation at 37°C, single colonies were selected using a sterile loop and transferred into 5 mL of TSB, then incubated for 24 hours at 37°C. After incubation, 100 µL of the culture was transferred to 10 mL of TSB, followed by another 24 hours of incubation at 37°C. After the final enrichment step, 100 µL of the culture was again transferred to 10 mL of TSB and incubated for 24 hours at 37°C. The microorganisms were then adjusted to a turbidity value of 0.5 McFarland (approximately 10⁸ CFU/mL).

Comparison of Honey Samples and Pathogens Cultured Under *In Vitro* Conditions

The well diffusion method, modified from Bauer et al. (1966), was used to determine the antimicrobial efficacy of the selected honey samples against the *L. monocytogenes* strains. *L. monocytogenes* cultures containing the culture at 1×10⁸ CFU/mL cell density were transferred from 10 mL to 90 mL of TSA when the optimal temperature for plating was reached (45°C). The medium was thoroughly mixed, and approximately 25 mL of the mixture was distributed into four Petri dishes. The Petri dishes were placed on a flat surface, and the agar was allowed to solidify. Wells of 6 mm in diameter were created on the agar surface using a cork borer. After each use, the cork borer was dipped in alcohol and sterilized by flaming. Once the agar solidified (Aytar et al. 2019). The 100% and

50% concentrations of honey samples were tested against *L. monocytogenes* strains. To prepare the 50% (v/v) concentrations of the honey samples, sterile distilled water was used. 100 µL of the 100% and 50% concentrations of honey were aseptically added to the wells created on the agar surface, and the Petri dishes were incubated at 37°C for 24 hours. After the incubation period, the inhibition zone diameters were measured using a ruler. The antimicrobial susceptibility of *L. monocytogenes* strains against honey samples was evaluated by measuring the inhibition zone diameter. Sterile distilled water was used as a negative control, and erythromycin (15 µg/disc) was used as a positive control. The honey samples were sterilized through 0.45 µm pore size sterile membrane filters (Sartorius, Germany).

Statistical Analysis

The average and standard deviation values of the measurements obtained from the analyses were determined. The differences in the average inhibition zone diameters were analyzed using a T-test. To evaluate the average inhibition zone diameters of honey samples at both 100% and 50% concentrations, based on the honey types classified into four groups, an analysis of variance (ANOVA) method was applied. The study was conducted according to the randomized plot design with two replications. Significant differences between the means were compared using Duncan's Multiple Comparison Test at a $p<0.05$ level. SPSS (Version 23) software was used for the analysis.

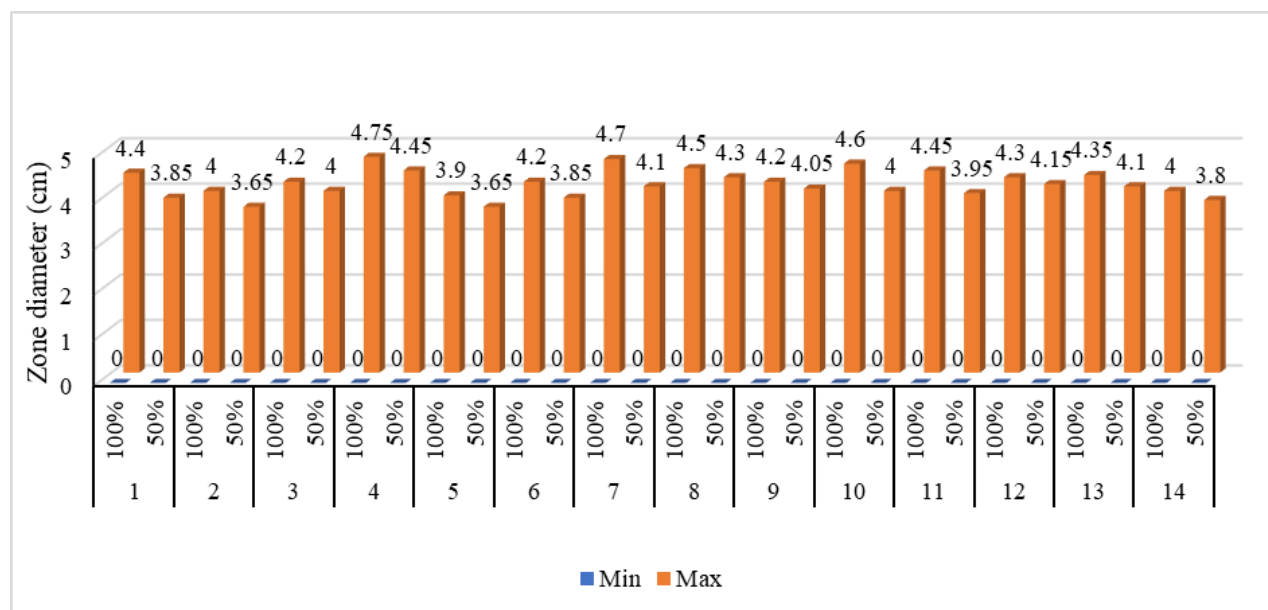
RESULTS and DISCUSSION

In the study, the antimicrobial activity values of different honey types against *L. monocytogenes* strains were evaluated by measuring the inhibition zone diameters. Accordingly, the maximum zone diameters formed by the different honey types against *L. monocytogenes* varied at different concentrations. All honey samples exhibited varying levels of antimicrobial activity against *L. monocytogenes* strains. For the 100% concentration, the zone diameters ranged from 4.25 to 4.8 cm, while for the 50% concentration, they ranged from 4.1 to 4.45 cm (Table 2, Figure 1, Figure 2).

Table 2. Minimum and maximum inhibition zone diameters of honey types against *L. monocytogenes* strains

Çizelge 2. Bal türlerinin *L. monocytogenes* suşlarına karşı minimum ve maksimum inhibisyon zon çapları

Honey type	Concentration			
	100%		50%	
	Min	Max	Min	Max
FFH	0	4.80	0	4.45
CFH	0	4.25	0	4.25
AH	0	4.6	0	4.1
PH	0	4.6	0	4.2



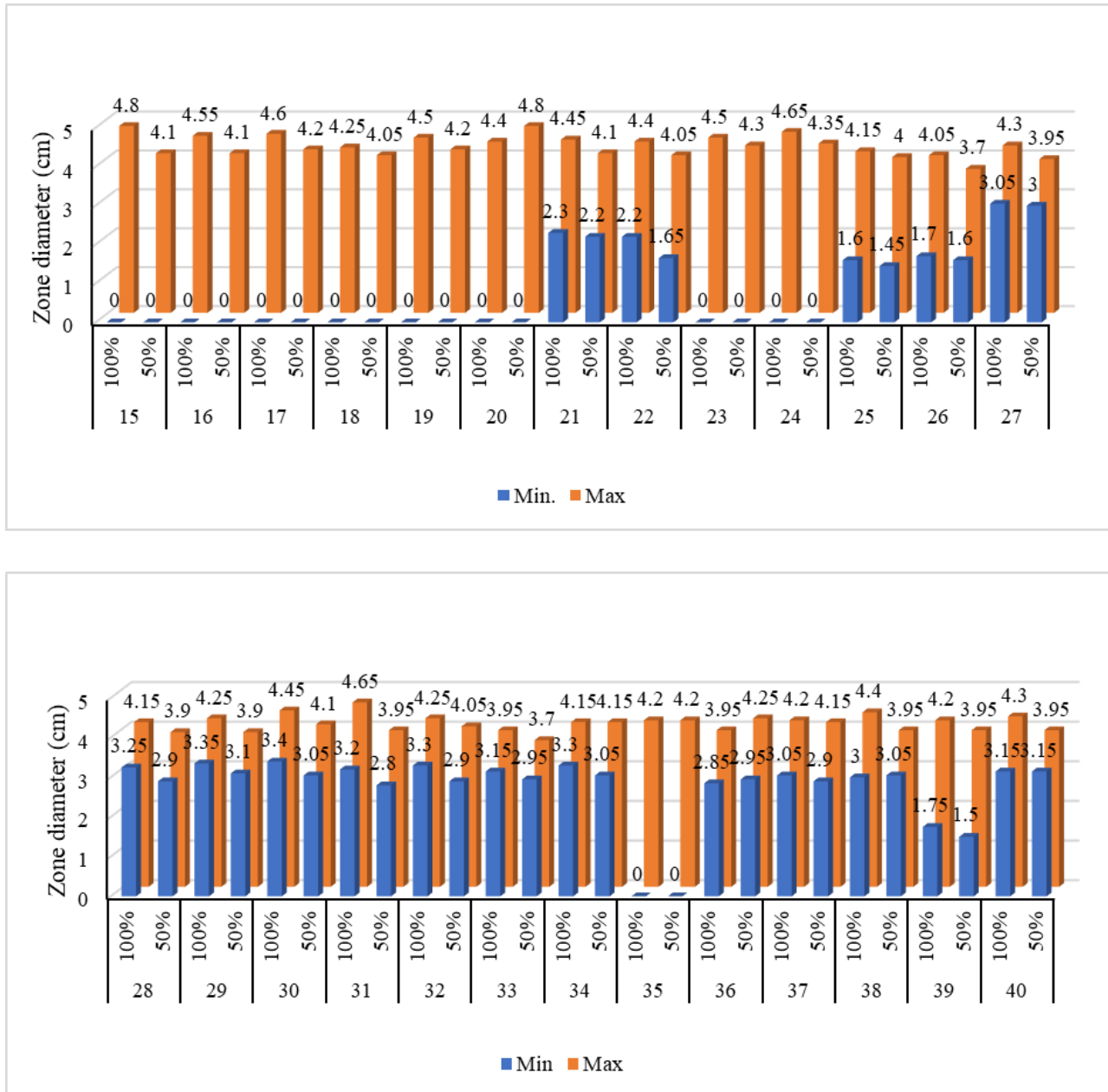


Figure 1. Minimum and maximum inhibition zone diameters (cm) of honey types against *L. monocytogenes*
Şekil 1. Bal türlerinin *L. monocytogenes*'e karşı minimum ve maksimum inhibisyon zon çapları (cm)

The highest antimicrobial effects were observed at 100% concentration, with inhibition zone diameters of 4.75 cm, 4.70 cm, and 4.8 cm in the filtered flower honey samples obtained from Ankara, numbered 4, 7, and 15, respectively (Figure 1). The average values of inhibition zone diameters and the changes according to concentration for honey samples at 100% and 50% concentrations against *L. monocytogenes* strains are shown in Figure 2. The highest antimicrobial effects were measured at 4.8 cm, 4.7 cm, and 4.65 cm inhibition zone diameters for the strains 142-1, 107-2, ATCC7644, and 151-1P. The difference between the antimicrobial activity measured by the average inhibition zone diameters of the honey samples at 100% concentration and the inhibition zone diameters at 50% concentration was statistically analyzed. The average inhibition zone diameter of the 100% honey samples was found to be 3.54 cm, while the average inhibition zone diameter of the 50% concentration was 3.30 cm (Table 3). The antimicrobial activity of honey samples, categorized by type (filtered flower honey, comb flower honey, acacia honey, and pine honey), against *L. monocytogenes* strains was also investigated. Statistically significant differences were observed in the average inhibition zone diameters formed by these honey samples against *L. monocytogenes* strains (Table 4).

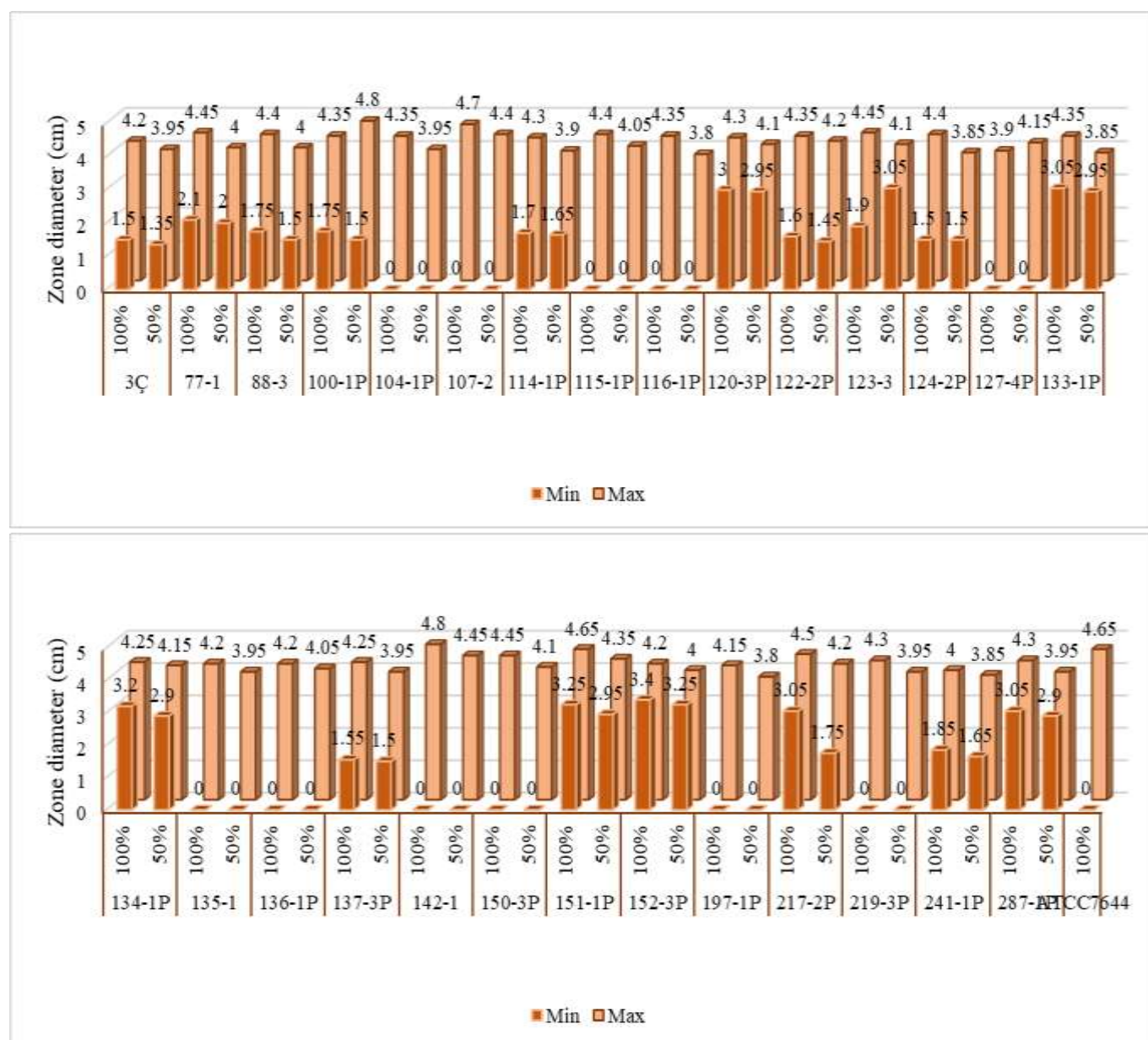


Figure 2. Minimum and maximum inhibition zone diameters (cm) observed in *L. monocytogenes* strains
Şekil 2. *L. monocytogenes* suşlarında gözlenen minimum ve maksimum inhibisyon zon çapları (cm)

When evaluating the average inhibition zone diameters formed by different honey types against *L. monocytogenes*, it was found that pine honey exhibited the highest antimicrobial activity in this study. Following pine honey, the antimicrobial effects decreased in the following order: filtered flower honey, comb flower honey, and acacia honey. However, based on the 95% confidence interval, the differences in the average zone diameters formed by filtered flower honey, comb flower honey, acacia honey, and pine honey against *L. monocytogenes* were found to be statistically insignificant ($p>0.05$) (Table 4). Therefore, it can be statistically concluded that the antimicrobial effects of the different honey types on *L. monocytogenes* strains did not differ significantly from one another.

In the T-Test analysis, it was determined that this difference in zone diameters was statistically significant at a 95% confidence level ($p<0.05$). Based on this result, it was concluded that the 100% concentration of honey samples has higher antimicrobial activity, and that when the honey samples are diluted, the antimicrobial activity decreases (Table 5).

In Table 6, the honey types were compared individually with each other, and the differences in the average inhibition zone diameters were found to be statistically insignificant based on the 95% confidence interval analysis ($p>0.05$). In this context, no superiority could be measured between the honey types based on the pairwise comparisons.

Table 3. Average inhibition zone diameters of 100% and 50% honey concentrations against *Listeria monocytogenes*
Çizelge 3. *Listeria monocytogenes*'e karşı %100 ve %50 bal konsantrasyonlarının ortalama inhibisyon zon çapları

T-Test							
		Average	Number of strains	Standard deviation		Standard error of the mean	
Inhibition zone (for %100% honey concentration)		3.5423	29	0.26188		0.04863	
Inhibition zone (for %50% honey concentration)		3.3032	29	0.25195		0.04679	
Reliability of the average values of the obtained inhibition zone diameters							
Average		Standard deviation	Standard error of the Mean (SEM)	Difference at 95% confidence interval		Degrees of Freedom	Two-tailed significance level (p)
				Min	Max		
100% and 50% Honey Concentration Inhibition Zone	0.23909	0.06091	0.01131	0.21593	0.26226	28	.000

Table 4. Statistical evaluation of the antimicrobial activity of 100% honey samples against *L. monocytogenes* Strains

Çizelge 4. %100 bal örneklerinin *L. monocytogenes* suşlarına karşı antimikrobiyal aktivitesinin istatistiksel değerlendirmesi

Honey type	Concentration	Average	Standard deviation	
FFH	100%	3.5675	.31388	
CFH		3.4929	.26155	
AH		3.5919	.27678	
PH		3.3989	.69953	
The reliability of the average inhibition zone diameters formed by 100% honey samples against <i>L. monocytogenes</i> strains				
Within-Groups Effect Test				
Sources	Sum of Squares	Degrees of Freedom	Mean Square	<i>p</i>
Greenhouse-Geisser	0.656	1.725	0.38	0.143

Table 6. Comparison of the antimicrobial effect spectra of 100% honey samples with each other

Çizelge 6. %100 bal örneklerinin antimikrobiyal etki spektrumlarının birbiriyle karşılaştırılması

Pairwise Comparison						
Comparison of Samples with Each Other		Mean Diameter Differences	Standard Error	p	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	0.075	0.065	1	-0.111	0.261
	3	-0.024	0.063	1	-0.203	0.154
	4	0.169	0.096	0.544	-0.105	0.442
2	1	-0.075	0.065	1	-0.261	0.111
	3	-0.099	0.043	0.175	-0.221	0.023
	4	0.094	0.117	1	-0.239	0.427
3	1	0.024	0.063	1	-0.154	0.203
	2	0.099	0.043	0.175	-0.023	0.221
	4	0.193	0.103	0.423	-0.098	0.484
4	1	-0.169	0.096	0.544	-0.442	0.105
	2	-0.094	0.117	1	-0.427	0.239
	3	-0.193	0.103	0.423	-0.484	0.098

(1: Filtered Floral honey, 2: Comb Floral Honey, 3: Pine Honey, 4: Acacia Honey)

The average values of the inhibition zone diameters showing the antimicrobial activity of the 50% concentration honey samples against *L. monocytogenes* strains are presented in Table 7.

Table 5. Antimicrobial activity of honey kinds at 100% concentration against *L. monocytogenes* strains (zone diameter: cm)

Çizelge 5. %100 konsantrasyondaki farklı bal türlerinin *L. monocytogenes* suşlarına karşı antimikrobiyel aktivitesi (zon çapı: cm)

	1	2	3	4	5	6	7	8	9	10
3Ç	3.5 a <i>ABC</i>	3.6 abc <i>ABC</i>	1.5 ab <i>D</i>	3.9 ab <i>ABC</i>	3.55 ab <i>ABC</i>	3.65 b-e <i>ABC</i>	3.65 e-ı <i>ABC</i>	3.7 ab <i>ABC</i>	3.45 a <i>ABC</i>	3.55 ab <i>ABC</i>
77-1	3.75 a <i>NS</i>	4 a <i>NS</i>	3.45 a <i>NS</i>	4 ab <i>NS</i>	3.75 a <i>NS</i>	3.9 a-d <i>NS</i>	3.95 a-f <i>NS</i>	2.1 abc <i>NS</i>	2.3 ab <i>NS</i>	2.1 abc <i>NS</i>
88-3	1.75 ab <i>B</i>	3.75 ab <i>A</i>	3.65 a <i>A</i>	3.5 abc <i>A</i>	3.35 abc <i>A</i>	3.75 a-d <i>A</i>	3.95 a-f <i>A</i>	3.8 ab <i>A</i>	3.3 a <i>A</i>	3.65 ab <i>A</i>
100-1P	4 a <i>AB</i>	3.6 abc <i>AB</i>	3.4 a <i>AB</i>	4 ab <i>AB</i>	3.2 a-d <i>B</i>	3.55 b-e <i>AB</i>	3.7 d-ı <i>AB</i>	3.6 ab <i>AB</i>	4.1 a <i>AB</i>	3.65 ab <i>AB</i>
104-1P	3.45 a <i>İ-K</i>	3.45 abc <i>İ-K</i>	3.7 a <i>E-İ</i>	0 d <i>L</i>	3.25 a-d <i>K</i>	3.5 b-e <i>I-K</i>	4 a-f <i>BCD</i>	3.6 ab <i>G-J</i>	3.95 a <i>B-E</i>	3.9 ab <i>B-F</i>
107-2	3.85 a <i>ABC</i>	3.95 a <i>ABC</i>	3.8 a <i>ABC</i>	2.2 c <i>BCD</i>	3.7 a <i>ABC</i>	0 f <i>D</i>	0 j <i>D</i>	2.15 abc <i>BCD</i>	0 b <i>D</i>	1.95 bc <i>CD</i>
114-1P	1.7 ab <i>B</i>	1.8 cd <i>B</i>	3.95 a <i>A</i>	4.15 ab <i>A</i>	3.45 ab <i>A</i>	4 ab <i>A</i>	4.1 a-d <i>A</i>	3.75 ab <i>A</i>	3.65 a <i>A</i>	3.75 ab <i>A</i>
115-1P	3.65 a <i>A</i>	3.7 abc <i>A</i>	3.85 a <i>A</i>	4.4 ab <i>A</i>	3.9 a <i>A</i>	4.2 a <i>A</i>	4.05 a-e <i>A</i>	4.2 a <i>A</i>	3.95 a <i>A</i>	4.2 a <i>A</i>
116-1P	3.35 a <i>G-İ</i>	3.3 abc <i>H-İ</i>	3.4 a <i>F-İ</i>	3.6 ab <i>C-İ</i>	3.35 abc <i>G-İ</i>	3.8 a-d <i>B-G</i>	4.35 a <i>A</i>	4.1 ab <i>AB</i>	3.75 a <i>B-H</i>	3.95 ab <i>A-D</i>
120-3P	3.8 a <i>B-G</i>	3.85 ab <i>A-G</i>	3.7 a <i>C-G</i>	4.3 ab <i>A</i>	3.6 ab <i>E-H</i>	3.95 abc <i>A-G</i>	3.85 c-h <i>A-G</i>	3.85 ab <i>A-G</i>	4 a <i>A-F</i>	4.15 a <i>ABC</i>
122-2P	3.65 a <i>A</i>	3.95 a <i>A</i>	3.35 a <i>A</i>	4.3 ab <i>A</i>	3.8 a <i>A</i>	3.6 bcde <i>A</i>	3.65 e-ı <i>A</i>	3.55 ab <i>A</i>	3.85 a <i>A</i>	3.55 ab <i>A</i>
123-3	3.5 a <i>A</i>	3.5 abc <i>A</i>	3.55 a <i>A</i>	3.75 ab <i>A</i>	3.45 ab <i>A</i>	3.65 b-e <i>A</i>	3.85 c-h <i>A</i>	3.8 ab <i>A</i>	1.9 ab <i>B</i>	3.85 ab <i>A</i>
124-2P	3.45 a <i>AB</i>	3.45 abc <i>AB</i>	1.75 ab <i>C</i>	3.15 bc <i>AB</i>	1.5 d <i>C</i>	3.65 b-e <i>AB</i>	3.45 hıı <i>AB</i>	3.5 ab <i>AB</i>	3.3 a <i>AB</i>	3.25 abc <i>AB</i>
127-4P	0 b <i>E</i>	0 e <i>E</i>	3.4 a <i>BCD</i>	0 d <i>E</i>	3.35 abc <i>CD</i>	3.4 cde <i>BCD</i>	3.4 ıı <i>BCD</i>	3.6 ab <i>A-D</i>	3.3 a <i>CD</i>	3.6 ab <i>A-D</i>
133-1P	3.45 a <i>G-İ</i>	3.45 abc <i>G-İ</i>	3.05 a <i>İ</i>	4.35 ab <i>A</i>	3.15 a-d <i>İİ</i>	3.6 b-e <i>E-İ</i>	3.95 a-f <i>A-F</i>	3.85 ab <i>B-G</i>	3.8 a <i>C-G</i>	3.6 ab <i>E-İ</i>
134-1P	3.55 a <i>D-İ</i>	3.45 abc <i>F-İ</i>	3.35 a <i>HIİ</i>	4.15 ab <i>ABC</i>	3.4 ab <i>G-İ</i>	4.05 ab <i>A-E</i>	3.9 b-g <i>A-H</i>	4.25 a <i>A</i>	3.6 a <i>C-İ</i>	3.8 ab <i>A-İ</i>
135-1	3.4 a <i>A</i>	3.3 abc <i>A</i>	3.4 a <i>A</i>	4.1 ab <i>A</i>	3.45 ab <i>A</i>	4 ab <i>A</i>	3.7 d-ı <i>A</i>	2.05 abc <i>A</i>	3.95 a <i>A</i>	3.35 abc <i>A</i>
136-1P	0 b <i>G</i>	0 e <i>G</i>	3.35 a <i>DEF</i>	4.1 ab <i>ABC</i>	3.25 a-d <i>F</i>	3.35 de <i>DEF</i>	3.5 g-ı <i>A-F</i>	3.3 ab <i>EF</i>	3.9 a <i>A-F</i>	3.4 abc <i>C-F</i>
137-3P	3.5 a <i>AB</i>	3.5 abc <i>AB</i>	1.6 ab <i>C</i>	4 ab <i>AB</i>	1.55 cd <i>C</i>	3.55 b-e <i>AB</i>	3.5 g-ı <i>AB</i>	3.55 ab <i>AB</i>	3.65 a <i>AB</i>	3.4 abc <i>AB</i>
142-1	1.85 ab <i>BCD</i>	1.95 bcd <i>A-D</i>	1.75 ab <i>CD</i>	4.75 a <i>AB</i>	1.8 bcd <i>CD</i>	0 f <i>D</i>	0 j <i>D</i>	0 c <i>D</i>	2.15 ab <i>A-D</i>	0 d <i>D</i>
150-3P	3.8 a <i>AB</i>	3.8 ab <i>AB</i>	3.6 a <i>AB</i>	0 d <i>D</i>	3.7 a <i>AB</i>	4 ab <i>A</i>	4.1 a-d <i>A</i>	3.7 ab <i>AB</i>	3.85 a <i>AB</i>	3.9 ab <i>AB</i>
151-1P	3.65 a <i>E-G</i>	3.7 abc <i>E-G</i>	3.55 a <i>E-G</i>	4 ab <i>B-F</i>	3.55 ab <i>E-G</i>	3.6 b-e <i>E-G</i>	3.6 f-ı <i>E-G</i>	3.65 ab <i>E-G</i>	3.9 a <i>B-G</i>	3.5 ab <i>FGH</i>
152-3P	3.5 a <i>FG</i>	3.65 abc <i>C-G</i>	3.55 a <i>EFG</i>	4.05 ab <i>ABC</i>	3.5 ab <i>FG</i>	3.65 b-e <i>C-G</i>	3.85 c-h <i>A-F</i>	3.6 ab <i>D-G</i>	3.95 a <i>A-E</i>	3.8 ab <i>A-G</i>
197-1P	3.15 a <i>A-E</i>	1.35 de <i>EF</i>	0 b <i>F</i>	3.5 abc <i>AB</i>	3.75 a <i>A</i>	3.15 e <i>A-E</i>	3.15 ı <i>A-E</i>	1.55 bc <i>C-F</i>	3.65 a <i>A</i>	1.45 cd <i>DEF</i>
217-2P	3.65 a <i>E-İ</i>	3.8 ab <i>C-H</i>	3.25 a <i>İİ</i>	4.05 ab <i>A-F</i>	3.55 ab <i>F-İ</i>	3.85 a-d <i>C-G</i>	4.15 abc <i>A-E</i>	3.85 ab <i>C-G</i>	4.2 a <i>A-D</i>	3.9 ab <i>C-G</i>
219-3P	3.55 a <i>FGH</i>	3.65 abc <i>E-H</i>	3.55 a <i>FGH</i>	3.8 ab <i>C-G</i>	3.45 ab <i>GH</i>	3.85 a-d <i>C-F</i>	4.3 ab <i>A</i>	4.05 ab <i>A-D</i>	0 b <i>I</i>	4.05 ab <i>A-D</i>
241-1P	1.85 ab <i>B</i>	3.55 abc <i>A</i>	3.1 a <i>A</i>	3.45 abc <i>A</i>	3.3 abc <i>A</i>	3.4 cde <i>A</i>	3.4 ıı <i>A</i>	3.4 ab <i>A</i>	3.55 a <i>A</i>	3.45 abc <i>A</i>
287-1P	3.8 a <i>B-G</i>	3.9 a <i>B-F</i>	3.25 a <i>İJ</i>	4.05 ab <i>A-D</i>	3.05 a-d <i>İ</i>	3.8 a-d <i>B-G</i>	4.05 a-e <i>A-D</i>	4.1 ab <i>ABC</i>	3.75 a <i>C-G</i>	4 ab <i>A-E</i>
ATCC	3.75 a <i>ABC</i>	3.6 abc <i>A-D</i>	1.75 ab <i>DE</i>	4.4 ab <i>A</i>	3.2 a-d <i>A-D</i>	0 f <i>E</i>	0 j <i>E</i>	0 c <i>E</i>	2 ab <i>CD</i>	0 d <i>E</i>

Two-way ANOVA; ns: not significant. Means with different lowercase letters in the columns indicate significant differences among strains for each honey type; Means with different uppercase letters in the rows indicate substantial differences among kinds of honey in each strain. The numbers are averages of all replicates.

Table 5. Antimicrobial activity of honey kinds at 100% concentration against *L. monocytogenes* strains (zone diameter: cm) (Continue)

Çizelge 5. %100 konsantrasyondaki farklı bal türlerinin *L. monocytogenes* suşlarına karşı antimikrobiyel aktivitesi (zon çapı: cm)

	11	12	13	14	15	16	17	18	19	20
3Ç	3.6 a <i>ABC</i>	3.65 a <i>ABC</i>	3.55 def <i>ABC</i>	3.1 cde <i>C</i>	4.05 a <i>AB</i>	3.7 b-g <i>ABC</i>	3.8 d <i>ABC</i>	3.6 a <i>ABC</i>	3.6 ab <i>ABC</i>	3.9 abc <i>ABC</i>
77-1	4.45 a <i>NS</i>	4.05 a <i>NS</i>	3.95 bc <i>NS</i>	3.7 bc <i>NS</i>	4.3 a <i>NS</i>	3.9 b-e <i>NS</i>	4.1 bcd <i>NS</i>	2.25 ab <i>NS</i>	4.05 a <i>NS</i>	3.85 abc <i>NS</i>
88-3	1.85 b <i>B</i>	3.8 a <i>A</i>	3.35 efg <i>A</i>	3.65 bcd <i>A</i>	3.55 ab <i>A</i>	3.45 efg <i>A</i>	3.25 e <i>A</i>	3.25 a <i>A</i>	3.45 ab <i>A</i>	3.55 abc <i>A</i>
100-1P	3.2 a <i>B</i>	4 a <i>AB</i>	3.8 bcd <i>AB</i>	3.45 b-e <i>AB</i>	3.8 ab <i>AB</i>	3.8 b-g <i>AB</i>	4.15 bcd <i>AB</i>	3.85 a <i>AB</i>	3.9 a <i>AB</i>	3.75 abc <i>AB</i>
104-1P	3.9 a <i>B-F</i>	3.9 a <i>B-F</i>	3.8 bcd <i>C-H</i>	3.8 ab <i>C-H</i>	0 c <i>L</i>	0 h <i>L</i>	0 f <i>L</i>	4 a <i>BCD</i>	3.95 a <i>B-E</i>	4.1 abc <i>B</i>
107-2	4.25 a <i>ABC</i>	4.5 a <i>AB</i>	4.35 a <i>ABC</i>	4.4 a <i>ABC</i>	2.4 b <i>ABC</i>	4.2 ab <i>ABC</i>	4.6 a <i>AB</i>	0 b <i>D</i>	4.7 a <i>A</i>	4.5 a <i>AB</i>
114-1P	4 a <i>A</i>	4 a <i>A</i>	3.85 bcd <i>A</i>	3.95 ab <i>A</i>	3.95 ab <i>A</i>	3.8 b-g <i>A</i>	3.95 cd <i>A</i>	3.75 a <i>A</i>	3.75 a <i>A</i>	3.8 abc <i>A</i>
115-1P	4 a <i>A</i>	3.9 a <i>A</i>	4.1 ab <i>A</i>	3.75 b <i>A</i>	4.1 a <i>A</i>	3.95 a-e <i>A</i>	3.95 cd <i>A</i>	4.25 a <i>A</i>	3.9 a <i>A</i>	3.9 abc <i>A</i>
116-1P	4 a <i>ABC</i>	3.95 a <i>A-D</i>	3.85 bcd <i>B-F</i>	3.85 ab <i>B-F</i>	3.65 ab <i>B-I</i>	3.5 d-g <i>D-I</i>	3.95 cd <i>A-D</i>	3.9 a <i>A-E</i>	3.9 a <i>A-E</i>	3.9 abc <i>A-E</i>
120-3P	4.2 a <i>AB</i>	3.9 a <i>A-G</i>	3.85 bcd <i>A-G</i>	3.55 bcd <i>F-I</i>	4.1 a <i>A-D</i>	3.85 b-f <i>A-G</i>	3.8 d <i>B-G</i>	4 a <i>A-F</i>	3.95 a <i>A-G</i>	3.8 abc <i>B-G</i>
122-2P	4.1 a <i>A</i>	3.95 a <i>A</i>	3.75 bcd <i>A</i>	3.5 bcd <i>A</i>	3.95 ab <i>A</i>	4.15 abc <i>A</i>	4.35 abc <i>A</i>	4.2 a <i>A</i>	3.75 a <i>A</i>	3.8 abc <i>A</i>
123-3	4.1 a <i>A</i>	4 a <i>A</i>	3.9 bcd <i>A</i>	3.95 ab <i>A</i>	3.9 ab <i>A</i>	3.75 b-g <i>A</i>	4.15 bcd <i>A</i>	3.7 a <i>A</i>	1.9 bc <i>B</i>	4.35 ab <i>A</i>
124-2P	3.35 a <i>AB</i>	3.35 a <i>AB</i>	3.2 g <i>AB</i>	2.9 e <i>B</i>	3.35 ab <i>AB</i>	3.25 fg <i>AB</i>	3.25 e <i>AB</i>	3.6 a <i>AB</i>	3.2 abc <i>AB</i>	3.25 bc <i>AB</i>
127-4P	3.4 a <i>BCD</i>	3.45 a <i>BCD</i>	3.3 efg <i>CD</i>	3.45 b-e <i>BCD</i>	0 c <i>E</i>	0 h <i>E</i>	0 f <i>E</i>	3.4 a <i>BCD</i>	3.3 abc <i>CD</i>	3.35 abc <i>CD</i>
133-1P	4.3 a <i>AB</i>	4 a <i>A-E</i>	3.9 bcd <i>A-G</i>	3.65 bcd <i>E-H</i>	4 ab <i>A-E</i>	4.15 abc <i>A-D</i>	3.9 d <i>A-G</i>	3.7 a <i>D-H</i>	3.75 a <i>C-G</i>	4 abc <i>A-E</i>
134-1P	4.25 a <i>A</i>	3.75 a <i>A-I</i>	3.75 bcd <i>A-I</i>	3.7 bc <i>A-I</i>	4.1 a <i>A-D</i>	4.15 abc <i>ABC</i>	3.9 d <i>A-H</i>	3.8 a <i>A-I</i>	3.95 a <i>A-G</i>	3.75 abc <i>A-I</i>
135-1	4.2 a <i>A</i>	4.15 a <i>A</i>	3.9 bcd <i>A</i>	3.5 bcd <i>A</i>	4.2 a <i>A</i>	3.95 a-e <i>A</i>	3.95 cd <i>A</i>	4.2 a <i>A</i>	4.1 a <i>A</i>	3.9 abc <i>A</i>
136-1P	3.7 a <i>A-F</i>	3.5 a <i>A-F</i>	3.6 cde <i>A-F</i>	3.5 bcd <i>A-F</i>	3.75 ab <i>A-F</i>	3.55 c-g <i>A-F</i>	3.3 e <i>EF</i>	3.9 a <i>A-F</i>	4.2 a <i>A</i>	3.8 abc <i>A-F</i>
137-3P	3.8 a <i>AB</i>	3.6 a <i>AB</i>	3.55 def <i>AB</i>	3.7 bc <i>AB</i>	3.95 ab <i>AB</i>	3.95 a-e <i>AB</i>	4.2 a-d <i>A</i>	3.8 a <i>AB</i>	3.8 a <i>AB</i>	4.25 ab <i>A</i>
142-1	0 c <i>D</i>	2 b <i>A-D</i>	0 h <i>D</i>	0 f <i>D</i>	4.8 a <i>A</i>	4.55 a <i>ABC</i>	4.6 a <i>ABC</i>	2.2 ab <i>A-D</i>	4.5 a <i>ABC</i>	4.4 ab <i>ABC</i>
150-3P	4.1 a <i>A</i>	3.7 a <i>AB</i>	3.9 bcd <i>AB</i>	3.9 ab <i>AB</i>	0 c <i>D</i>	0 h <i>D</i>	0 f <i>D</i>	3.9 a <i>AB</i>	3.8 a <i>AB</i>	3.85 abc <i>AB</i>
151-1P	3.8 a <i>C-H</i>	3.5 a <i>FGH</i>	3.6 cde <i>E-G</i>	3.7 bc <i>E-G</i>	3.65 ab <i>E-G</i>	4.1 a-d <i>A-F</i>	4.45 ab <i>AB</i>	4 a <i>B-F</i>	3.95 a <i>B-G</i>	4.35 ab <i>A-D</i>
152-3P	3.95 a <i>A-E</i>	4.05 a <i>ABC</i>	3.8 bcd <i>A-G</i>	3.7 bc <i>B-G</i>	4.05 a <i>ABC</i>	3.6 b-g <i>D-G</i>	4.2 a-d <i>A</i>	3.65 a <i>C-G</i>	3.6 ab <i>D-G</i>	4.05 abc <i>ABC</i>
197-1P	0 c <i>F</i>	0 c <i>F</i>	0 h <i>F</i>	0 f <i>F</i>	3.4 ab <i>ABC</i>	3.2 g <i>A-E</i>	3.35 e <i>A-D</i>	3.55 a <i>AB</i>	1.7 c <i>B-F</i>	1.7 d <i>B-F</i>
217-2P	4.05 a <i>A-F</i>	4 a <i>A-F</i>	3.8 bcd <i>C-H</i>	3.95 ab <i>B-G</i>	4.45 a <i>AB</i>	3.8 b-g <i>C-H</i>	4.05 bcd <i>A-F</i>	3.95 a <i>B-G</i>	3.95 a <i>B-G</i>	3.9 abc <i>C-G</i>
219-3P	3.9 a <i>B-F</i>	3.85 a <i>C-F</i>	3.9 bcd <i>B-F</i>	4 ab <i>A-E</i>	3.9 ab <i>B-F</i>	4.05 a-e <i>A-D</i>	3.9 d <i>B-F</i>	0 b <i>I</i>	0 d <i>I</i>	0 e <i>I</i>
241-1P	3.45 a <i>A</i>	3.35 a <i>A</i>	3.25 fg <i>A</i>	3.05 de <i>A</i>	3.25 ab <i>A</i>	3.25 fg <i>A</i>	3.25 e <i>A</i>	3.55 a <i>A</i>	3.5 ab <i>A</i>	3.9 abc <i>A</i>
287-1P	3.95 a <i>A-F</i>	3.95 a <i>A-F</i>	3.9 bcd <i>B-F</i>	3.8 ab <i>B-G</i>	4.3 a <i>A</i>	3.9 b-e <i>B-F</i>	4.05 bcd <i>A-D</i>	3.95 a <i>A-F</i>	4.1 a <i>ABC</i>	3.9 abc <i>B-F</i>
ATCC	4.25 a <i>AB</i>	4.3 a <i>A</i>	4.35 c <i>A</i>	3.9 ab <i>ABC</i>	4.2 a <i>AB</i>	4.15 abc <i>AB</i>	4.1 bcd <i>AB</i>	2.3 ab <i>BCD</i>	3.45 ab <i>A-D</i>	2.95 c <i>A-D</i>

Two-way ANOVA; ns: not significant. Means with different lowercase letters in the columns indicate significant differences among strains for each honey type; Means with different uppercase letters in the rows indicate substantial differences among kinds of honey in each strain. The numbers are averages of all replicates.

Table 5. Antimicrobial activity of honey kinds at 100% concentration against *L. monocytogenes* strains (zone diameter: cm) (Continue)

Çizelge 5. %100 konsantrasyondaki farklı bal türlerinin *L. monocytogenes* suşlarına karşı antimikrobiyel aktivitesi (zon çapı: cm)

	21	22	23	24	25	26	27	28	29	30
3Ç	3.95 a <i>ABC</i>	4.2 a <i>A</i>	3.8 c-f <i>ABC</i>	3.85 bc <i>ABC</i>	3.5 a <i>ABC</i>	3.65 a <i>ABC</i>	3.7 e-1 <i>ABC</i>	3.95 a-d <i>ABC</i>	3.35 k <i>ABC</i>	4 bc <i>ABC</i>
77-1	4.15 <i>NS</i>	4 a <i>NS</i>	4.05 a-e <i>NS</i>	3.9 bc <i>NS</i>	3.65 a <i>NS</i>	3.65 a <i>NS</i>	3.75 d-h <i>NS</i>	3.75 d-g <i>NS</i>	3.95 b-g <i>NS</i>	4 bc <i>NS</i>
88-3	3.7 ab <i>A</i>	4.4 a <i>A</i>	4.4 ab <i>A</i>	4.1 ab <i>A</i>	3.7 a <i>A</i>	4.05 a <i>A</i>	3.7 e-1 <i>A</i>	3.9 b-e <i>A</i>	3.8 e-1 <i>A</i>	3.85 abc <i>A</i>
100-1P	4.3 a <i>A</i>	4.05 a <i>AB</i>	4.05 a-e <i>AB</i>	4.35 ab <i>A</i>	3.9 a <i>AB</i>	3.35 a <i>AB</i>	3.75 d-h <i>AB</i>	3.8 c-f <i>AB</i>	3.7 g-j <i>AB</i>	3.6 de <i>AB</i>
104-1P	3.9 a <i>B-F</i>	3.9 a <i>B-F</i>	4 a-e <i>BCD</i>	3.85 bc <i>B-G</i>	3.55 a <i>H-J</i>	3.95 a <i>B-E</i>	4.05 abc <i>BC</i>	3.75 d-g <i>D-I</i>	3.95 b-g <i>B-E</i>	3.55 de <i>H-J</i>
107-2	4.6 a <i>AB</i>	4.2 a <i>ABC</i>	4.3 abc <i>ABC</i>	4.3 ab <i>ABC</i>	4 a <i>ABC</i>	3.85 a <i>ABC</i>	4.25 ab <i>ABC</i>	3.95 a-d <i>ABC</i>	4.1 a-d <i>ABC</i>	3.6 de <i>ABC</i>
114-1P	4.3 a <i>A</i>	3.95 a <i>A</i>	4 a-e <i>A</i>	4.05 bc <i>A</i>	3.4 a <i>A</i>	3.8 a <i>A</i>	3.3 i <i>A</i>	3.3 i <i>A</i>	4.2 ab <i>A</i>	3.75 cd <i>A</i>
115-1P	4.15 a <i>A</i>	3.6 ab <i>A</i>	0 h <i>D</i>	0 f <i>D</i>	1.9 b <i>BC</i>	1.7 b <i>C</i>	3.85 c-f <i>A</i>	3.75 d-g <i>A</i>	3.95 b-g <i>A</i>	3.85 abc <i>A</i>
116-1P	3.75 ab <i>B-H</i>	3.85 a <i>B-F</i>	3.95 a-f <i>A-D</i>	3.9 bc <i>A-E</i>	3.8 a <i>B-G</i>	3.7 a <i>B-I</i>	3.65 f-1 <i>B-İ</i>	3.45 hii <i>E-İ</i>	3.85 d-1 <i>B-F</i>	3.85 abc <i>B-F</i>
120-3P	3.95 a <i>A-G</i>	4.3 a <i>A</i>	3.95 a-f <i>A-G</i>	3.9 bc <i>A-G</i>	3 ab <i>İ</i>	3.85 a <i>A-G</i>	3.5 hii <i>GI</i>	4.05 ab <i>A-E</i>	3.7 g-j <i>C-G</i>	3.7 cde <i>C-G</i>
122-2P	3.8 ab <i>A</i>	3.95 a <i>A</i>	3.65 def <i>A</i>	4.25 ab <i>A</i>	1.6 b <i>B</i>	1.7 b <i>B</i>	4.05 abc <i>A</i>	4 abc <i>A</i>	3.65 h-j <i>A</i>	3.4 e <i>A</i>
123-3	4.45 a <i>A</i>	4 a <i>A</i>	4.25 abc <i>A</i>	4.3 ab <i>A</i>	3.55 a <i>A</i>	3.55 a <i>A</i>	4 bcd <i>A</i>	3.75 d-g <i>A</i>	3.6 i-k <i>A</i>	3.85 abc <i>A</i>
124-2P	3.45 ab <i>AB</i>	3.25 ab <i>AB</i>	3.65 def <i>AB</i>	3.5 cde <i>AB</i>	3.35 a <i>AB</i>	3.4 a <i>AB</i>	3.5 hii <i>AB</i>	3.45 hii <i>AB</i>	3.85 d-1 <i>AB</i>	3.9 abc <i>AB</i>
127-4P	3.4 ab <i>BCD</i>	3.3 ab <i>CD</i>	3.4 fg <i>BCD</i>	3.3 de <i>CD</i>	3.55 a <i>A-D</i>	3.6 a <i>A-D</i>	3.55 g-i <i>A-D</i>	3.35 ii <i>CD</i>	3.5 ijk <i>A-D</i>	3.55 de <i>A-D</i>
133-1P	3.9 a <i>A-G</i>	3.85 a <i>A-G</i>	3.95 a-f <i>A-F</i>	4 bc <i>A-E</i>	3.5 a <i>F-İ</i>	3.6 a <i>E-I</i>	3.85 c-f <i>A-G</i>	3.45 hii <i>G-İ</i>	4.15 abc <i>A-D</i>	3.8 cd <i>C-G</i>
134-1P	3.8 ab <i>A-I</i>	4 a <i>A-F</i>	3.95 a-f <i>A-G</i>	3.9 bc <i>A-H</i>	3.45 a <i>F-İ</i>	3.45 a <i>F-İ</i>	3.8 c-g <i>A-I</i>	3.65 fgh <i>B-İ</i>	3.5 ijk <i>E-İ</i>	3.75 cd <i>A-İ</i>
135-1	2.3 b <i>A</i>	2.2 b <i>A</i>	0 h <i>B</i>	0 f <i>B</i>	3.5 a <i>A</i>	3.75 a <i>A</i>	3.95 cde <i>A</i>	4 abc <i>A</i>	3.5 ijk <i>A</i>	3.6 de <i>A</i>
136-1P	4 a <i>A-E</i>	4.05 a <i>A-D</i>	4.2 a-d <i>A</i>	4.15 ab <i>AB</i>	3.85 a	3.35 a <i>DEF</i>	3.55 g-i <i>A-F</i>	3.25 i <i>F</i>	3.9 c-h <i>A-F</i>	4.15 ab <i>AB</i>
137-3P	4.05 a <i>AB</i>	4.1 a <i>AB</i>	3.8 c-f <i>AB</i>	4.05 bc <i>AB</i>	3.6 a <i>AB</i>	3.6 a <i>AB</i>	3.95 cde <i>AB</i>	4.15 a <i>A</i>	4.15 abc <i>A</i>	3.75 cd <i>AB</i>
142-1	4.45 a <i>ABC</i>	4.25 a <i>ABC</i>	4.4 ab <i>ABC</i>	4.1 ab <i>ABC</i>	3.55 a <i>ABC</i>	3.8 a <i>ABC</i>	3.45 ii <i>ABC</i>	3.7 efg <i>ABC</i>	3.9 c-h <i>ABC</i>	4.15 ab <i>ABC</i>
150-3P	3.75 ab <i>AB</i>	3.75 a <i>AB</i>	3.95 a-f <i>AB</i>	4.05 bc <i>A</i>	3.75 a <i>AB</i>	3.75 a <i>AB</i>	3.95 cde <i>AB</i>	3.8 c-f <i>AB</i>	3.85 d-1 <i>AB</i>	4.45 a <i>A</i>
151-1P	4.1 a <i>A-F</i>	4.15 a <i>A-E</i>	4.4 ab <i>ABC</i>	4.65 a <i>A</i>	3.5 a <i>FGH</i>	3.65 a <i>E-G</i>	3.9 c-f <i>B-G</i>	3.55 gh1 <i>E-G</i>	3.75 f-i <i>D-H</i>	3.9 abc <i>B-G</i>
152-3P	4.1 a <i>AB</i>	3.75 a <i>B-G</i>	4 a-e <i>A-D</i>	4.2 ab <i>A</i>	3.75 a <i>B-G</i>	3.55 a <i>EFG</i>	3.75 d-h <i>B-G</i>	3.95 a-d <i>A-E</i>	4.05 a-e <i>ABC</i>	3.7 cde <i>B-G</i>
197-1P	3.55 ab <i>AB</i>	3.3 ab <i>A-D</i>	3.1 g <i>A-E</i>	3.25 e <i>A-D</i>	3.95 a <i>A</i>	3.8 a <i>A</i>	3.7 e-1 <i>A</i>	4.05 ab <i>A</i>	3.95 b-g <i>A</i>	3.6 de <i>AB</i>
217-2P	4 a <i>A-F</i>	3.9 a <i>C-G</i>	4.5 a <i>A</i>	4.25 ab <i>ABC</i>	3.8 a <i>C-H</i>	3.6 a <i>F-I</i>	3.05 j <i>İ</i>	3.45 hii <i>G-İ</i>	3.45 jk <i>G-İ</i>	3.95 bc <i>B-G</i>
219-3P	4.05 a <i>A-D</i>	4.25 a <i>AB</i>	4.05 a-e <i>A-D</i>	4 bc <i>A-E</i>	3.8 a <i>C-G</i>	3.55 a <i>FGH</i>	3.85 c-f <i>C-F</i>	3.95 a-d <i>A-E</i>	3.8 e-1 <i>C-G</i>	3.9 abc <i>B-F</i>
241-1P	3.5 ab <i>A</i>	3.4 ab <i>A</i>	3.55 efg <i>A</i>	3.8 bcd <i>A</i>	3.6 a <i>A</i>	3.65 a <i>A</i>	3.5 hii <i>A</i>	3.3 i <i>A</i>	3.6 i-k <i>A</i>	3.7 cde <i>A</i>
287-1P	3.85 a <i>B-G</i>	3.5 ab <i>G-İ</i>	3.85 b-f <i>B-G</i>	3.8 bcd <i>B-G</i>	3.7 a <i>D-H</i>	3.75 a <i>C-G</i>	4.05 abc <i>A-D</i>	3.85 b-f <i>B-G</i>	4 a-f <i>A-E</i>	4.15 ab <i>AB</i>
ATCC	4.15 a <i>AB</i>	4.3 a <i>A</i>	3.65 def <i>A-D</i>	4.1 ab <i>AB</i>	4.15 a <i>AB</i>	3.75 a <i>ABC</i>	4.3 a <i>A</i>	3.75 d-g <i>ABC</i>	4.25 a <i>AB</i>	4 bc <i>AB</i>

Two-way ANOVA; ns: not significant. Means with different lowercase letters in the columns indicate significant differences among strains for each honey type; Means with different uppercase letters in the rows indicate substantial differences among kinds of honey in each strain. The numbers are averages of all replicates.

Table 5. Antimicrobial activity of honey kinds at 100% concentration against *L. monocytogenes* strains (zone diameter: cm) (Continue)

Çizelge 5. %100 konsantrasyondaki farklı bal türlerinin *L. monocytogenes* suşlarına karşı antimikrobiyel aktivitesi (zon çapı: cm)

	31	32	33	34	35	36	37	38	39	40
3Ç	3.6 b-e ABC	3.55 gh ₁ ABC	3.65 a-f ABC	4.15 a AB	3.25 ab BC	3.35 efg ABC	4.2 a A	3.9 bcd ABC	3.7 a ABC	3.3 de ABC
77-1	4.05 bc NS	3.95 a-e NS	3.85 abc NS	3.55 b-e NS	3.1 ab NS	3.6 a-f NS	4.1 ab NS	3.35 f- ₁ NS	3.35 abc NS	3.4 de NS
88-3	4.15 b A	3.45 h ₁ A	3.6 b-g A	3.5 c-e A	3.5 ab A	3.25 fgh A	3.85 a-f A	3.8 b-e A	3.55 ab A	3.5 cde A
100-1P	3.6 b-e AB	3.3 ₁ AB	3.9 ab AB	3.85 a-e AB	3.5 ab AB	3.1 gh B	3.55 d- ₁ AB	3.8 b-e AB	1.75 c C	3.6 b-e AB
104-1P	3.5 cde I-K	3.65 e-h F-J	3.85 abc B-G	3.5 c-e I-K	3.55 ab H-J	3.45 c-g İ-K	3.8 a-g C-H	3.6 d-g G-J	3.4 abc J-K	4.35 a A
107-2	3.85 bcd ABC	4.05 abc ABC	3.4 e- ₁ ABC	3.9 a-d ABC	3 ab ABC	3.5 b-g ABC	3.5 e-i ABC	3.75 b-e ABC	3.05 abc ABC	3.85 a-e ABC
114-1P	3.55 cde A	4 a-d A	3.15 ₁ A	3.85 a-e A	3.45 ab A	3.85 abc A	3.6 c-h A	3.7 c-f A	3.35 abc A	3.5 cde A
115-1P	3.9 bcd A	4.2 ab A	3.65 a-f A	3.85 a-e A	3.5 ab A	3.8 a-d A	3.1 ii AB	3.2 h ₁ A	3.35 abc A	3.75 a-e A
116-1P	3.95 bc A-D	3.5 gh ₁ D-İ	3.95 a A-D	3.8 a-e B-G	0 c J	3.95 a A-D	3.25 hii I-İ	3.8 b-e B-G	3.2 abc İ	3.45 cde E-İ
120-3P	3.75 b-e B-G	3.95 a-e A-G	3.8 a-d B-G	3.9 a-d A-G	3.65 ab D-H	3.55 a-f F-I	3.6 c-h E-H	3.6 d-g E-H	3.25 abc Hİ	3.15 e II
122-2P	3.75 b-e A	3.6 f- ₁ A	3.7 a-e A	3.35 d-e A	3.9 a A	3.95 a A	3.45 f-i A	3.05 ₁ A	3.7 a A	3.2 de A
123-3	3.8 bcd A	3.55 gh ₁ A	3.5 d-h A	3.8 a-e A	3.6 ab A	3.75 a-e A	3.4 f-i A	3.75 b-e A	3.45 abc A	3.45 cde A
124-2P	3.6 b-e AB	3.9 b-f AB	3.8 a-d AB	3.9 a-d AB	3.75 ab AB	3.35 efg AB	3.05 i AB	4.4 a A	3.9 a AB	3.35 de AB
127-4P	3.7 b-e ABC	3.55 gh ₁ A-D	3.2 h ₁ D	3.75 a-e ABC	3.35 ab CD	3.2 fgh D	3.8 a-g AB	3.35 f- ₁ CD	3.35 abc CD	3.9 a-d A
133-1P	3.7 b-e D-H	3.75 c-h C-G	3.45 e- ₁ G-İ	3.8 a-e C-G	3.85 ab A-G	3.25 fgh HII	4.2 a ABC	4 bc A-E	3.6 ab E-I	3.7 a-e D-H
134-1P	3.2 e İ	3.3 ₁ II	3.85 abc A-I	4.05 abc A-E	4.2 a AB	3.7 a-e A-İ	3.8 a-g A-I	4 bc A-F	3.8 a A-I	3.6 b-e C-İ
135-1	3.2 e A	3.45 h ₁ A	3.5 d-h A	3.9 a-d A	3.8 ab A	3.4 d-g A	3.95 a-e A	3.85 b-e A	3.6 ab A	3.7 a-e A
136-1P	3.7 b-e A-F	3.8 c-g A-F	3.55 c-g A-F	3.85 a-e A-F	3.45 ab B-F	3.9 ab A-F	3.6 c-h A-F	3.6 d-g A-F	3.6 ab A-F	3.85 a-e A-F
137-3P	3.65 b-e AB	4.05 abc AB	3.2 h ₁ AB	4.1 ab AB	2.3 b BC	3.35 efg AB	3.65 b-h AB	3.55 d-h AB	3.85 a AB	3.85 a-e AB
142-1	3.55 cde ABC	3.7 d-h ABC	3.5 d-h ABC	3.6 a-e ABC	3.3 ab ABC	3.85 abc ABC	3.65 b-h ABC	3.6 d-g ABC	3.75 a ABC	4.3 ab ABC
150-3P	3.75 b-e AB	4.25 a A	3.85 abc AB	3.7 a-e AB	4 a A	2.85 h B	3.4 f-i AB	3.75 b-e AB	1.85 bc C	4.15 abc A
151-1P	3.55 cde E-G	3.75 c-h D-H	3.65 a-f E-G	3.65 a-e E-G	3.95 a B-G	3.35 efg G-H	3.6 c-h E-G	3.85 b-e B-H	3.25 abc H	3.85 a-e B-H
152-3P	3.75 b-e B-G	3.9 b-f A-F	3.7 a-e B-G	3.8 a-e A-G	3.4 ab G	3.75 a-e B-G	4.05 abc ABC	3.5 eh FG	4.2 a A	3.55 cde EFG
197-1P	3.6 b-e AB	3.6 f- ₁ AB	3.3 gh ₁ A-D	4.15 a A	3.3 ab A-D	3.4 d-g ABC	3.35 g-i A-D	3.2 h ₁ A-E	3.65 a A	4.15 abc A
217-2P	3.75 b-e C-H	3.9 b-f C-G	3.8 a-d C-H	3.7 a-e D-I	3.3 ab HII	3.25 fgh II	3.45 f-i G-İ	3.7 c-f D-I	3.7 a D-I	3.7 a-e D-I
219-3P	3.7 b-e D-H	3.95 a-e A-E	3.65 a-f E-H	3.4 d-e H	3.45 ab GH	3.55 a-f FGH	3.65 b-h E-H	4.1 ab ABC	3.75 a C-H	3.7 a-e D-H
241-1P	3.5 cde A	3.3 ₁ A	3.8 a-d A	3.55 b-e A	3.05 ab A	3.1 gh A	4 a-d A	3 ₁ A	3.8 a A	3.85 a-e A
287-1P	3.35 de H-J	3.65 e-h E-I	3.35 f- ₁ H-J	3.3 e IIJ	3.25 ab İJ	3.25 fgh İJ	3.25 hii İJ	3.6 d-g F-İ	3.65 a E-I	3.85 a-e B-G
ATCC	4.65 a A	3.95 a-e ABC	3.65 a-f A-D	3.3 e A-D	3.85 ab ABC	3.55 a-f A-D	4.15 a AB	3.3 gh ₁ A-D	3.65 a A-D	3.7 a-e ABC

Two-way ANOVA; ns: not significant. Means with different lowercase letters in the columns indicate significant differences among strains for each honey type; Means with different uppercase letters in the rows indicate substantial differences among kinds of honey in each strain. The numbers are averages of all replicates.

Table 7. Statistical Evaluation of the Antimicrobial Activity of 50% Honey Samples Against *L. monocytogenes* strains

Çizelge 7. %50 bal örneklerinin *L. monocytogenes* suşlarına karşı antimikrobiyal aktivitesinin istatistiksel değerlendirmesi

Honey Type	Concentration	Average	Standard deviation
FFH	50%	3.3141	0.30478
CFH		3.3157	0.24943
AH		3.3382	0.25343
PH		3.108	0.71056

The reliability of the average inhibition zone diameters formed by 50% honey samples against *L. monocytogenes* strains

Within-Groups Effect Test				
Sources	Sum of Squares	Degrees of Freedom	Mean Square	<i>p</i>
Greenhouse-Geisser	1.012	1.674	0.605	0.063

When examining the differences in the average inhibition zone diameters formed by the 50% concentrations of the honey types, it was found that the highest antimicrobial effect was exhibited by pine honey, followed by comb floral honey, filtered floral honey, and acacia honey in descending order. Table 6 also presents the reliability of the average inhibition zone diameters formed by the 50% concentration honey samples against *L. monocytogenes* strains. The statistical analysis at the 95% confidence interval revealed that the differences in the inhibition zone diameters between the comb floral honey, filtered floral honey, acacia honey, and pine honeys were not statistically significant ($p>0.05$). Therefore, it was statistically observed that there were no significant differences in the antimicrobial effects of the honey types on the *L. monocytogenes* strains. In Table 8, the 50% concentrations of the honey types were compared with each other individually, and it was concluded that the differences in the average inhibition zone diameters were not statistically significant ($p>0.05$). As a result, it was statistically observed that the antimicrobial effects of the honey types on the test strains did not differ from each other.

Table 8. Comparison of the antimicrobial effect spectra of 50% honey samples with each other

Çizelge 8. %50 bal örneklerinin antimikrobiyal etki spektrumlarının birbiriyle karşılaştırılması

Comparison of Samples with Each Other		Pairwise Comparison			95% Confidence Interval	
		Mean Diameter Differences	Standard Error	<i>p</i>	Lower Bound	Upper Bound
1	2	-0.002	0.062	1	-0.178	0.175
	3	-0.024	0.062	1	-0.199	0.151
	4	0.206	0.103	0.33	-0.086	0.498
2	1	0.002	0.062	1	-0.175	0.178
	3	-0.022	0.039	1	-0.134	0.089
	4	0.208	0.117	0.514	-0.123	0.538
3	1	0.024	0.062	1	-0.151	0.199
	2	0.022	0.039	1	-0.089	0.134
	4	0.23	0.109	0.261	-0.079	0.539
4	1	-0.206	0.103	0.33	-0.498	0.086
	2	-0.208	0.117	0.514	-0.538	0.123
	3	-0.23	0.109	0.261	-0.539	0.079

(1: Filtered Floral honey, 2: Comb Floral Honey, 3: Pine Honey, 4: Acacia Honey)

The findings of our study align with several previous studies examining the antimicrobial activity of honey samples against *L. monocytogenes* strains. Silici et al. (2010) investigated the antimicrobial effects of honey samples at concentrations of 10%, 25%, 50%, and 75%, reporting no inhibition at the 10% and 25% concentrations, but varying degrees of inhibition at the 50% and 75% concentrations. Similarly, Polat (2011) observed the antimicrobial activity of honey at concentrations of 100%, 80%, 60%, and 40%, but no antimicrobial effect at the 20% concentration. Consequently, antimicrobial activity increased progressively with higher concentrations. Moussa et al. (2012) measured the antimicrobial effects of honey samples diluted to 100%, 70%, 50%, and 30%, and emphasized that undiluted samples exhibited the highest antimicrobial activity. Borum (2016) similarly found that undiluted honey samples showed the strongest antibacterial and antifungal effects at concentrations of 100%, 50%, and 25%. Nayaka et al. (2020), in line with our study, reported the highest antimicrobial activity in undiluted honey samples when tested at concentrations of 100%, 80%, 60%, 40%, and 20%, further confirming the trend observed in our study.

Table 9 presents the variance analysis results of honey samples at 100% and 50% concentrations. Statistically, the antimicrobial activity of honey samples at 100% concentration is found to be more significant.

Table 10 presents the effect size of honey samples at 100% concentration.

Table 9. Variance analysis of the antimicrobial activity of honey samples at 100% and 50% concentrations.

Çizelge 9. %100 ve %50 konsantrasyondaki bal örneklerinin antimikrobiyel aktivitesinin varyans analizi

<i>Variance analysis of 100% concentrations</i>					
Source	Sum of Squares	df	Mean Square	F	Sig.
Strains	153.916	28	5.497	17.251	.000
100 %	101.592	39	2.605	8.175	.000
Strains*100%	1429.006	1092	1.309	4.107	.000
Error	369.625	1160	.319		
Total	2054.139	2319			
<i>Variance analysis table of 50% concentrations</i>					
Source	Sum of Squares	df	Mean Square	F	Sig.
Strains	721.510	28	25.768	1.385	.088
50%	630.350	39	16.163	.869	.700
Strains*50%	21179.161	1092	19.395	1.043	.241
Error	21576.770	1160	18.601		
Total	44107.791	2319			

Tablo 10. Effect size of 100% concentrations

Çizelge 10. %100 konsantrasyonun etki büyüklüğü

	Eta	Eta Squared
Zon diameter * 100%	.222	.049
Zon diameter* Strains	.274	.075

The results from these studies, as well as from our own, demonstrate that higher concentrations of honey generally exhibit stronger antimicrobial effects against *L. monocytogenes*, supporting the hypothesis that dilution reduces the antimicrobial potency of honey. Additionally, Fratianni et al. (2021) conducted an evaluation of the effects of various monofloral honeys of Italian origin on *L. monocytogenes*. The findings revealed that *L. monocytogenes* was generally susceptible to the inhibitory effects of all tested honeys. Notably, the sensitivity of the bacteria reached 90% in the presence of ivy honey, while blackberry honey and snowberry honey demonstrated inhibitory effects exceeding 90%. Oğur & Dayan (2022) investigated the antimicrobial activity of natural honey against *L. monocytogenes* in the Bitlis region. The agar well diffusion method was employed to evaluate the antimicrobial effects of honey at concentrations of 10%, 25%, 50%, and 100%. The results demonstrated significant antimicrobial activity, with a 50% honey concentration producing an inhibition zone of 34.00 ± 1.10 mm against *L. monocytogenes*. The largest inhibition zones were observed at the 100% concentration. In contrast to our study, Çakır & Dervişoğlu (2022) explored the antimicrobial effects of honey collected from various regions of Bingöl province using the disk diffusion method. Honey samples were tested at three concentrations (500, 250, and 125 mg/mL) for their antibacterial activity against *L. monocytogenes*. The results indicated that none of the honey samples, regardless of concentration, exhibited antibacterial activity against *L. monocytogenes*.

The antimicrobial properties of honey have been extensively studied against various pathogenic bacteria. The results obtained in our study also support similar studies conducted on other pathogens. Mahendran and Kumarasamy (2015) evaluated the antibacterial activity of twelve honey samples from different origins against Gram-positive bacteria, including *S. aureus* and *Streptococcus pyogenes*, as well as Gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Their results showed that among the twelve honey samples, summer honey (S1) and winter honey (W1) exhibited the highest antibacterial activity, particularly against *S. aureus*. Süerdem & Akyalçın (2017) examined the antimicrobial activity of six different honey samples against various Gram-positive (*Enterobacter faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25923, *Bacillus cereus*) and Gram-negative (*E. coli* ATCC 25922, *S. Typhimurium* ATCC 51812) bacteria. Their results demonstrated that all tested honey samples exhibited antimicrobial activity against the studied pathogens, except for *E. faecalis* and *E. coli* ATCC 25922, which were found to be resistant. The inhibition zones ranged between 10 mm and 40 mm in diameter, indicating variable antibacterial activity among the honey samples. Similarly, Guruvu et al. (2021) investigated the antimicrobial effects of commercial honey and Bharat honey against three different pathogens, namely *P. aeruginosa* ATCC 27853, *E. coli* ATCC 35218, and *S. aureus* ATCC 25923, using seven different dilutions. Their findings indicated that undiluted honey samples exhibited higher antimicrobial effects against these pathogens compared to the diluted samples. Additionally, Özkırım et al. (2021) investigated the antimicrobial activity of oak honey against *E. coli* ATCC 35218, *S. aureus* ATCC 29213,

and *P. aeruginosa* ATCC 27853. Their findings revealed that *S. aureus* was the most susceptible strain, followed by *P. aeruginosa* and *E. coli*, respectively.

CONCLUSION

The growing interest in natural products as potential treatments for antibiotic-resistant bacteria and the diseases they cause has increasingly captured the attention of researchers. The results of the study showed that it was statistically significant that the 100% concentrations exhibited higher antimicrobial activity compared to the 50% concentrations ($p<0.05$). The highest average inhibition zone diameter in undiluted samples was observed in pine honey, followed by flower honey, comb honey, and acacia honey in decreasing order. The study emphasizes the need for comprehensive, broad-spectrum research and clinical trials to validate the findings.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors express no conflict of interest associated with this work.

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Ordu İli Yöresel Bitkilerinin (*Brassica oleracea* var. *viridis*, *Smilax excelsa*, *Conium maculatum*) İnsan Serviks Kanseri (HeLa) Hücre Hattına Karşı Antikanser Etkilerinin ve DNA Koruyucu Aktivitelerinin Araştırılması

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ÖZET

Bu çalışmada, Ordu iline ait yöresel bitkilerinden *Brassica oleracea* var. *viridis*, *Smilax excelsa* ve *Conium maculatum*'un etanolik özütlerinin DNA hasarına karşı koruyucu potansiyelleri ve insan serviks adenokarsinomu (HeLa) hücre hattına karşı antikanser aktiviteleri araştırılmıştır. Özütler, farklı konsantrasyonlarda hazırlanarak antikanser aktiviteleri, HeLa hücre hattında 3-(4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromid (MTT) testi ile değerlendirilmiştir. Özütlerin, hidroksil radikali (-OH) ile indüklenen pBR322 plazmid DNA hasarını engelleme potansiyelleri de araştırılmıştır. Özütlerin HeLa hücreleri ile yapılan MTT analizleri neticesinde, farklı konsantrasyonlarla yapılan uygulamalarda, hücre canlılığının doza bağlı olarak azaldığı gözlenmiştir. *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin 24 saatteki yaklaşık IC₅₀ değerleri sırasıyla 3.88, 2.20 ve 1.48 mg mL⁻¹, 48 saatteki yaklaşık IC₅₀ değerleri ise sırasıyla 1.82, 1.13 ve 0.39 mg mL⁻¹ olarak tespit edilmiştir. Ayrıca, -OH ile indüklenen DNA hasarını önleme açısından özütlerin farklı (20, 10 ve 5 mg mL⁻¹) konsantrasyonlarının güçlü koruyucu aktivite gösterdiği bulunmuştur. Bu bulgular, *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* etanolik özütlerinin DNA koruyucu ve antikanser etkilerine dair öncü veriler sunmakta olup, ileri çalışmalarda bu etkilerden sorumlu aktif bileşiklerin belirlenmesi ve terapötik potansiyellerinin detaylı olarak incelenmesi gerektiğini ortaya koymaktadır.

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Brassica oleracea var. *viridis*
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HeLa
pBR322 Plazmid DNA
Smilax excelsa

Investigation of the Anticancer Effects on Human Cervical Cancer (HeLa) Cell Line and the DNA Protective Activities of Traditional Plants from Ordu Province (*Brassica oleracea* var. *viridis*, *Smilax excelsa*, *Conium maculatum*)

ABSTRACT

This study investigates the protective potential of ethanolic extracts of local plants from Ordu province, *Brassica oleracea* var. *viridis*, *Smilax excelsa*, and *Conium maculatum*, against DNA damage and their anticancer activities in HeLa (cervix adenocarcinoma) cells. The extracts were prepared at varying concentrations, and their anticancer effects were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in HeLa cells. The ability of these extracts to prevent hydroxyl radical (-OH)-induced damage to pBR322 plasmid DNA was also examined. MTT assay results showed that cell viability decreased in a dose-dependent manner when treated with different concentrations of the extracts. The approximate IC₅₀ values for *S. excelsa*, *B. oleracea* var. *viridis*, and *C. maculatum* extracts at 24 hours were determined to be 3.88, 2.20, and 1.48 mg mL⁻¹, respectively, while at 48 hours, these values were 1.82, 1.13, and 0.39 mg mL⁻¹. Furthermore, the extracts demonstrated strong protective activity at various concentrations (20, 10, and 5 mg mL⁻¹) in preventing OH-induced DNA damage. These findings provide preliminary data on the DNA protective and anticancer effects of ethanol extracts of *S. excelsa*, *B.*

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oleracea var. *viridis*, and *C. maculatum*, indicating that future studies should focus on identifying the active compounds responsible for these effects and investigating their therapeutic potential in more detail.

- Atıf Şekli:** Kaynak, B., Aydoğdu, G., Kolören, Z., & Kolören., O (2025). Ordu İli Yöresel Bitkilerinin (*Brassica oleracea* var. *viridis*, *Smilax excelsa*, *Conium maculatum*) İnsan Serviks Kanseri (HeLa) Hücre Hattına Karşı Antikanser Etkilerinin ve DNA Koruyucu Aktivitelerinin Araştırılması. *KSÜ Tarım ve Doğa Derg* 28 (4), 945-954. <https://doi.org/10.18016/ksutarimdog.vi.1651205>.
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GİRİŞ

Organizmadaki anormal hücrelerin kontrolsüzce büyümesi kanser olarak tanımlanmaktadır. Kanser, dünyada her yıl milyonlarca insanın ölümünden sorumludur. Organizmada kanser geliştiğinde eski hücreler, ölmeyerek kontrolsüzce büyür ve yeni anormal hücreler meydana getirir. Akciğer kanseri, meme kanseri, rahim ağzı kanseri ve prostat kanseri dünya çapında insanlar arasında en çok görülen kanser türleridir. Günümüzde kanser tedavilerinde kullanılan cerrahi müdahale, kemoterapi ve radyoterapi gibi tedavi seçenekleri mevcuttur fakat bunlar normal hücrelere de zarar verebilmektedir (Bai ve ark., 2016; Rawat & Reddy, 2022).

Serviksten kaynaklanan bir kanser türü olan servikal kanser, gelişmekte olan ülkelerde, kadınlarda kansere bağlı ölümlerin en yaygın nedenlerinden biridir ve dünya çapında kadınları etkileyen dördüncü en yaygın ve ölümcül kanserdir (Arslan ve ark., 2025). Başlangıç aşamasında genellikle hiçbir belirti göstermeyen hastalık sonraki aşamada, anormal vajinal kanama, pelvik ağrı, cinsel ilişki esnasında ağrı gibi semptomları içermektedir. Radyoterapi etkin bir tedavi seçeneği olmasına rağmen, hastaların 1/3 kadarında hastalık ilerler ya da tekrarlar. Ayrıca kemoterapi, cerrahi tedavi ve immünoterapi gibi mevcut tedavi yöntemleri, yüksek maliyetli oluşu, özgül olmayışı ve birçok yan etkiler sergilemesi sebebiyle sınırlı kalmaktadır. Bu noktada etkinliği artırılmış, doğal, yeni ve güvenilir tedavi yaklaşımlarının geliştirilmesine ihtiyaç duyulmaktadır (Yi ve ark., 2015; Potikanond ve ark., 2017; Aliyazicioglu ve ark., 2019; Golalipour ve ark., 2024).

Kanserin esas sebeplerinden biri de sağlıklı hücrelerde reaktif oksijen türlerinin (ROS) birikmesidir. Bu süreçte, oksidatif stresin etkilerini önemli oranda azaltan antioksidan moleküller, sağlıklı hücrelerin kanser hücrelerine dönüşmesini engelleyebilir. Bitkilerdeki fitokimyasal bileşiklerin antioksidan özellikleri buna katkıda bulunmaktadır. Kanser tedavilerinde kullanılan ilaçların ciddi yan etkiler sergilemesi ve tedavi seçeneklerinin yetersiz oluşu nedeniyle kanser teşhisi konmuş bireylerin yaşamları üzerinde önemli bir etkiye sahip yeni ve etkili alternatif tedavi arayışı halen devam etmektedir. Bu bağlamda halen medikal alanda kullanılan birçok ilaç, geleneksel şifalı bitkilerden elde edilmektedir. Bu bitkiler kanser de dâhil olmak üzere çeşitli hastalıkların tedavisinde kullanılmaktadır. Bitkilerin doğal ortamlarda yetişmesi, kolay erişilebilir olmaları, ekonomik olmaları, daha az yan etkiye sahip olmaları, daha etkin olmaları ve direnci yenme yeteneklerine sahip olmaları nedeniyle kanser tedavisinde bitkisel ürünlerin araştırılması hayati önem taşımaktadır (Zhai ve ark., 2021; Al-Yassine ve ark., 2023).

Smilax excelsa L., Smilacaceae familyasına ait, çok yıllık ve çalı formunda dikenli bir bitki olup, 800 m yüksekliğe kadar olan makilik, çalılık ve ormanlık alanlarda yetişmektedir. Türkiye’de Karadeniz, Batı Marmara, Güney Ege ve Akdeniz Bölgeleri’nde yayılış göstermektedir. Halk arasında öz diken, zimbilaçi, boylu gıcır, zimilacı, zimilas, dikenucu, silcan, kırçan, melocan, saparna, merülçen ve melevcan olarak bilinen bitki, çiğ olarak, turşu şeklinde veya kavurması yapılarak tüketilmektedir (Topdaş ve ark., 2021; Topkara ve ark., 2024). Antioksidan, antikanser, antimutajenik ve antimikrobiyal etkilere (Ozsoy ve ark., 2008; Topkara ve ark., 2024) sahip olan bitki, mide ağrısı ve hazımsızlığın geleneksel medikal tedavisinde de kullanılmaktadır (Raul ve ark., 2017; Topkara ve ark., 2024). Bununla beraber romatizma, diyabet, ülser ve bazı deri hastalıklarının tedavisinde de kullanılmaktadır (Wu ve ark., 2010; Khaligh ve ark., 2016; Topkara ve ark., 2024). Yapılan bazı araştırmalarda Sarialtin ve ark. (2023), *S. excelsa* özütünün insan prostat (PC3), kolorektal (HCT116) ve akciğer (A549) karsinom hücreleri üzerinde düşük ve yüksek konsantrasyonlarda (50-1000 µg mL⁻¹) özellikle PC3 hücrelerinin büyümesini inhibe ettiği ve *S. excelsa*’nın prostat kanserinin tedavisine potansiyel ajan olabileceğini belirtmiştir. Ayrıca Al-Yassine ve ark. (2023), *S. excelsa* özütünün C6 glioblastoma (beyin tümör hücreleri) hücre hattı üzerinde antikanser etkisini araştırmışlar ve *S. excelsa*’nın kanser tedavisinde etkili terapötik ajan olarak kullanılabileceğini belirtmişlerdir.

Brassica oleracea var. *viridis*, Brassicaceae familyası içerisinde yer alan yaklaşık 50-60 cm boyolanabilen, kazık köklü, açık sarı çiçekleri olan sert saplı, geniş ve kat kat yaprakları bulunan çok yıllık bir bitkidir. Türkiye’de özellikle Karadeniz Bölgesi’ndeki halkın temel besin kaynağıdır. Nemli, yüksek deniz ve göl kenarlarında daha iyi yetişmektedir (Yeşiltaş, 2018). Bitkinin yaprakları, kardiyotonik etkili olup mide rahatsızlıklarında, gut ve

romatizmal hastalıkların tedavisinde kullanılmaktadır. Tohumlar antihelmintik, diüretik ve müshil etkilidir (Chopra ve ark., 1986; Anonim, 2025a). Yapraklar enfekte yaraların dezenfeksiyonlarında lapa olarak kullanılabilir (Chevalier, 1996; Anonim, 2025a). *B. oleracea*'nın bazı alttürlerinin incelendiği çalışmalarda; Hafidh ve ark. (2013) tarafından *Brassica oleracea* L. var. *capitata* f. *rubra* özütünün HepG2 (insan karaciğer kanseri hücresi) ve HeLa hücrelerinde antikanser potansiyeli araştırılmış ve özütün, her iki hücrede de antiproliferatif aktivite gösterdiğini, antikanser tedavisine yeni bir ajan olabileceğini vurgulamışlardır. Ayrıca Lučić ve ark. (2023), *Brassica oleracea* var. *acephala* özütünün HeLa hücrelerinde antikanser potansiyelini incelemişler ve özütün HeLa hücreleri üzerinde antiproliferatif bir etki gösterdiğini belirtmişlerdir.

Conium maculatum, Apiaceae familyasına ait otsu formda bulunan beyaz çiçekleri olan uzun, dallı bir bitkidir. Halk arasında Baldıran, Ağı otu, Ağu otu, Hırhındilik, Körek, Şemsiye otu, Yılan otu gibi isimlendirilmektedir. Türkiye'nin hemen hemen tüm bölgelerinde, özellikle koruluklarda ve dere kenarlarında yetişmektedir (Anonim, 2025b). Bitki insanlar ve hayvanlar için ciddi toksik etkileri ile bilinmektedir. Koniin, N-Metil-koniin, konhidrin, psödokonhidrin ve γ -Konikisin olmak üzere çeşitli alkaloidler, toksik karakterden sorumludur. Nörotoksikoz, titreme, kusma ve kas felci gibi bulgular bitkinin toksik belirtilerinden bazılarıdır. Son derece zehirli olmasına rağmen, bitkinin özütü uzun süredir çeşitli hastalıklar için doğal tedavi olarak kullanılmaktadır. Prostat ve testis ödemi, uçuk, erizipel, meme tümörleri gibi hastalıkların tedavisinde kullanılmakla beraber antispazmodik, sedasyon ve analjezik etkileri de mevcuttur. Ayrıca rahim ağzı kanserinin tedavisinde yaprak özütü kullanılmaktadır (Vetter, 2004; Al-Snafi, 2016; Gyaneshwari ve ark., 2023). Mondal ve ark. (2014), Fransa'dan (Lyon) hazır olarak temin ettikleri *C. maculatum* özütünü, farklı kanser hücre hatlarında (HeLa, melanom (A375), HepG2 ve A549) antikanser aktivitesini araştırmışlar ve özütün HeLa hücrelerinde çarpıcı biçimde daha fazla antiproliferatif bir etki gösterdiğini belirtmişlerdir. Venkateswaran ve ark. (2023), *C. maculatum* özütünde bulunan 25 fitokimyasal bileşiğin ilaç benzeri aktivite göstererek metastatik kolorektal kanser (mCRC) hücreleri üzerinde antiproliferatif etki gösterdiğini vurgulamışlardır.

Bu çalışmanın amacı, Ordu iline ait yöresel bitkilerinden olan *Brassica oleracea* var. *viridis*, *Smilax excelsa* ve *Conium maculatum* bitkilerinden elde edilen etanolik özütlerinin hidroksil radikali ($\cdot$ OH) ile indüklenen DNA hasarını engelleme potansiyellerini ve doza bağlı olarak HeLa hücre hattında antikanser aktivitelerini araştırmaktır.

MATERYAL ve METOD

Bitki Özütlerinin Hazırlanması

Bitkiler Ordu ilindeki yöresel pazarlardan temin edilmiştir. Bitkilerin her biri 50'şer g hassas terazide tartılıp, 150'şer mL etanolle bir öğütücüde birleştirilerek ayrı ayrı öğütülmüştür. Karışımlar 150 rpm çalkalayıcıda %40 nem ve 20°C sıcaklıktaki ortamda 72 saat bekletilmiş, süre sonunda filtre kâğıdından süzildükten sonra evaporatörde 35°C'de etanol uzaklaştırılmıştır. Son konsantrasyonlar 20'şer mg mL⁻¹ olarak hesaplanmıştır (Kaynak ve ark., 2019).

Özütlerin DNA Koruyucu Aktiviteleri

B. oleracea var. *viridis*, *S. excelsa* ve *C. maculatum* bitkilerinin etanolik özütleri $\cdot$ OH ile indüklenen DNA hasarına karşı koruyucu potansiyellerini araştırmak için 15 μ L son hacimde 0.25 μ g μ L⁻¹ pBR322 plazmid DNA'sı (Thermo Scientific SD0041, Waltham, United States) 2.5 mM Na₂-EDTA, 2.5 mM Fe₂SO₄, %2 H₂O₂ ve 20, 10, 5 mg mL⁻¹ konsantrasyonlardaki özütler, 50 mM fosfat tamponunun (pH 7) bulunduğu bir reaksiyon karışımı hazırlanarak 37°C'de 30 dakika inkübe edildikten sonra, örnekler elektroforez için %1 agaroz (0.5 mg mL⁻¹ etidyum bromür içeren) yüklenmiştir. Elektroforez yürütme tamponu (1X Tris asetik EDTA; TAE) içerisinde yürütülmüştür. UV ışığında görüntüler kaydedilmiştir (Kaynak ve ark., 2025).

Özütlerin MTT Antikanser Aktiviteleri

B. oleracea var. *viridis*, *S. excelsa* ve *C. maculatum* bitkilerinin etanolik özütleri farklı konsantrasyonlarda hazırlanarak memeli hücrelerinde antikanser aktiviteleri HeLa hücre hattı (CCL2 ATCC) ile test edilmiştir. HeLa hücreleri %10 Fetal Sığır Serum (FBS) ile 2 mM l-glutamin, 100 μ g mL⁻¹ streptomisin, 100 U mL⁻¹ penisilin içeren Dulbecco's Modified Eagle Medium (DMEM) kültür ortamında 37°C'de %5 CO₂ içeren inkübatörde T75'lik hücre kaplarında büyütülmüştür. HeLa hücrelerinin 96 kuyucuklu kültür kaplarına kuyucuk başına 10⁴ hücre olarak %10 FBS içeren DMEM besiyerine ekimi yapılmıştır. Sonraki gün hücrelere özüt, farklı konsantrasyonlarda uygulanarak 24 saat bekletilmiştir. Elabscience MTT Cell Proliferation and Cytotoxicity Assay Kiti kullanılmıştır. 24 ve 48 saatlik inkübasyon sonrası tüm kuyucuklara 50 μ L serumsuz besiyeri ile 50 μ L MTT reaktifi eklenmiştir. Kontrol için, hücre içermeyen bir kuyucuğa 50 μ L MTT reaktifi eklenmiştir. %5 CO₂ ve 37°C inkübatörde 3 saat inkübe edildikten sonra kuyucuklara 150 μ L MTT solvent eklenmiştir. 15 dakika orbital çalkalayıcıda

çalkalanmıştır. Süre sonunda Tecan i-control™ Microplate Reader'da 570 nm'de absorbansı okutulmuştur (Kaynak ve ark., 2025).

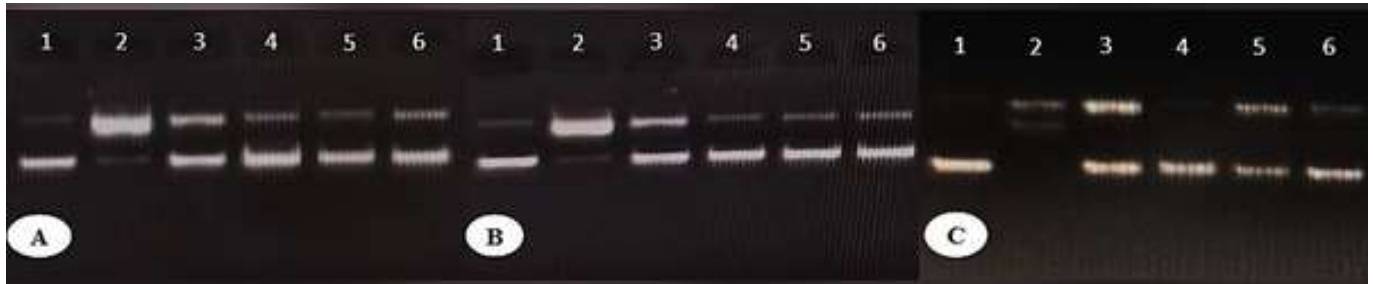
İstatistik Analizler

Kontrol (K) ile farklı konsantrasyonlardaki özütlerin HeLa hücrelerinde antikanser aktivitelerine ait veri girişi, grafikler ve tanımlayıcı veri analizi Microsoft Excel ile IBM SPSS Statistics 27.0.1 programları ile yapılmıştır. Veriler ortalama (Mean) $\pm$ standart sapma (SD) olarak ifade edilmiştir. $p < 0.01$ değeri istatistiksel olarak anlamlı kabul edilmiştir. HeLa hücrelerinde letal aktivite gösteren özütlerin farklı konsantrasyonlarının, IC₅₀ değerlerinin hesaplanmasında logaritmik regresyon analizi kullanılmıştır.

BULGULAR

Özütlerin DNA Koruyucu Aktiviteleri

B. oleracea var. *viridis*, *S. excelsa* ve *C. maculatum* bitkilerinin etanolik özütleri -OH ile indüklenmiş DNA hasarını engelleme potansiyellerini araştırmak amacıyla -OH ile indüklenmiş pBR322 plazmid DNA'sı agaroz jel elektroforeziyle incelenmiştir. pBR322 plazmid DNA'sı stressiz koşullar altında süper sarmal durumdadır. -OH ile oluşturulan DNA hasarı neticesinde DNA sirküler ve lineer duruma geçmektedir. Trolox gibi antioksidan ajan kullanılan pozitif kontrollerinde olduğu gibi DNA hasarı engellendiğinde DNA tekrar süper sarmal hale dönmektedir (Acharya & Ghaskadbi, 2013). *B. oleracea* var. *viridis*, *S. excelsa* ve *C. maculatum* özütleri 20, 10, 5 mg mL⁻¹ konsantrasyonlarının -OH ile oluşturulan DNA hasarına karşı koruyucu potansiyellerinin oldukça güçlü oldukları bulunmuştur (Şekil 1).



Şekil 1. *S. excelsa* (A), *B. oleracea* var. *viridis* (B) ve *C. maculatum* (C) özütlerinin pBR322 plazmid DNA'sı üzerinde -OH'nin neden olduğu DNA hasarına karşı DNA koruyucu aktivitelerini gösteren agaroz jel elektroforezi görüntüleri

Figure 1. Agarose gel electrophoresis images showing the DNA protective activities of extracts of *S. excelsa* (A), *B. oleracea* var. *viridis* (B), and *C. maculatum* (C) against DNA damage induced by -OH on pBR322 plasmid DNA.

1: pBR322 plazmid DNA

2: pBR322 plazmid DNA+Na₂EDTA+H₂O₂+Fe₂SO₄

3: pBR322 plazmid DNA+Na₂EDTA+H₂O₂+Fe₂SO₄ +Trolox

4-6: pBR322 plazmid DNA+Na₂EDTA+H₂O₂+Fe₂SO₄ + farklı konsantrasyonlardaki *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütleri (sırasıyla, 20, 10, 5 mg mL⁻¹).

Özütlerin Antikanser Aktivitesi

S. excelsa, *B. oleracea* var. *viridis* ve *C. maculatum* bitkilerinin etanolik özütleri farklı konsantrasyonlarda HeLa hücre hattında 24 ve 48 saat inkübasyona bırakılmıştır. Özütlerin doza bağlı olarak hücre canlılığını azalttığı gözlenmiştir. 24 saat sonra *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin yaklaşık IC₅₀ değerleri sırasıyla 3.88, 2.20 ve 1.48 mg mL⁻¹ olarak tespit edilmiştir (Şekil 2).

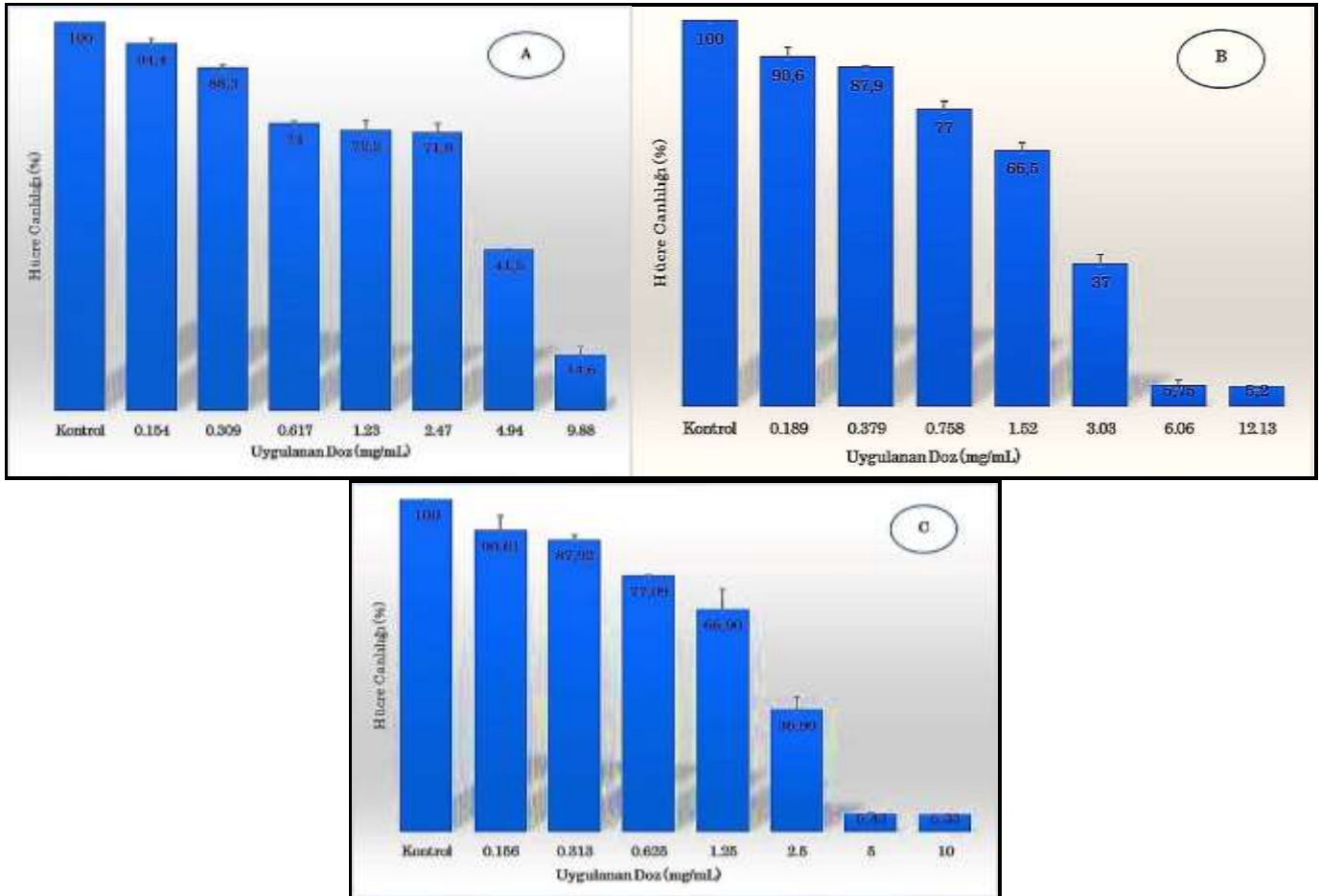
S. excelsa, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin 48 saat sonra yaklaşık IC₅₀ değerleri sırasıyla 1.82, 1.13 ve 0.39 mg mL⁻¹ olarak tespit edilmiştir (Şekil 3). Tüm özütlerin HeLa hücrelerinde antiproliferatif etkiye sahip olduğu bulunmuştur.

C. maculatum özütünün HeLa hücreleri üzerinde uygulanan özüt dozlarının canlılık üzerine etkileri incelendiğinde, özüt dozu arttıkça canlılık oranlarının azaldığı tespit edilmiştir. En yüksek canlılık 0.156 mg mL⁻¹'de, en düşük canlılık 10 mg mL⁻¹'de ölçülmüştür. Bekleme süresi arttıkça da canlılık oranlarının azaldığı belirlenmiştir. En yüksek canlılık 24. saatte, en düşük canlılık 48. saatte ölçülmüştür (Çizelge 1).

B. oleracea var. *viridis* özütünün HeLa hücreleri üzerinde uygulanan özüt dozlarının canlılık üzerine etkileri

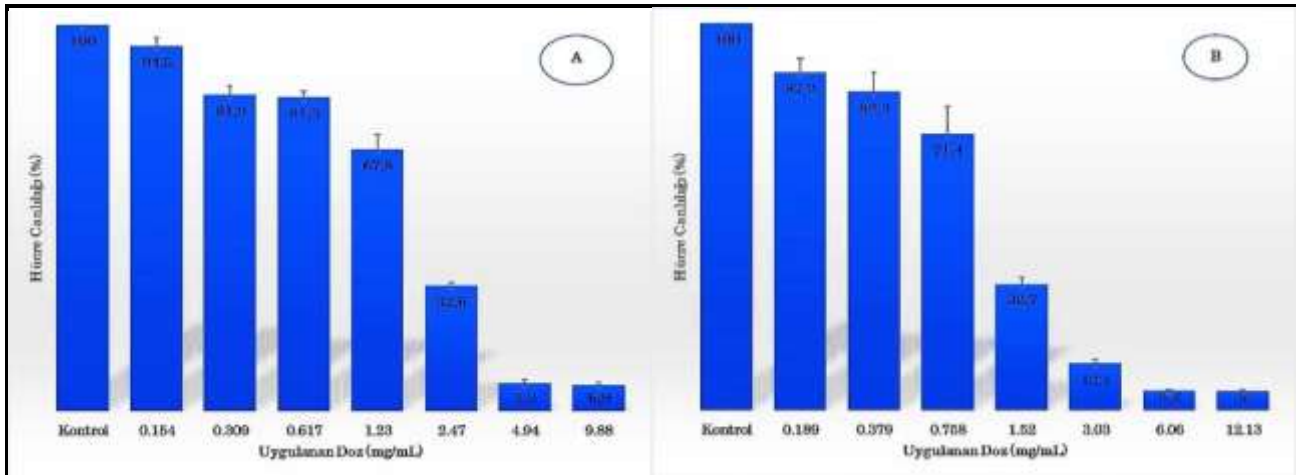
incelendiğinde, özüt dozu arttıkça canlılık oranlarının azaldığı tespit edilmiştir. En yüksek canlılık 0.189 mg mL^{-1} 'de, en düşük canlılık 12.13 mg mL^{-1} 'de ölçülmüştür. Bekleme süresi arttıkça da canlılık oranlarının azaldığı belirlenmiştir. En yüksek canlılık 24. saatte, en düşük canlılık 48. saatte ölçülmüştür (Çizelge 2).

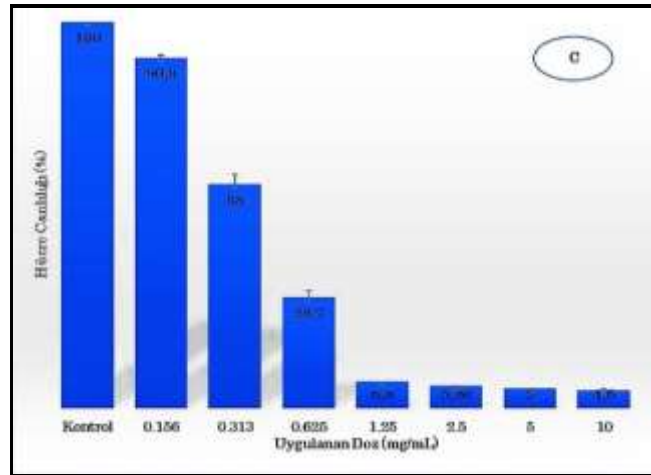
S. excelsa özütünün HeLa hücreleri üzerinde uygulanan özüt dozlarının canlılık üzerine etkileri incelendiğinde, özüt dozu arttıkça canlılık oranlarının azaldığı tespit edilmiştir. En yüksek canlılık 0.154 mg mL^{-1} 'de, en düşük canlılık 9.88 mg mL^{-1} 'de ölçülmüştür. Bekleme süresi arttıkça da canlılık oranlarının azaldığı belirlenmiştir. En yüksek canlılık 24. saatte, en düşük canlılık 48. saatte ölçülmüştür (Çizelge 3).



Şekil 2. *S. excelsa* (A), *B. oleracea* var. *viridis* (B) ve *C. maculatum* (C) özütlerinin farklı konsantrasyonlarının HeLa hücreleri üzerinde 24 saatteki antikanser aktiviteleri

Figure 2. Anticancer activities of different concentrations of *S. excelsa* (A), *B. oleracea* var. *viridis* (B), and *C. maculatum* (C) extracts on HeLa cells at 24 h.





Şekil 3. *S. excelsa* (A), *B. oleracea* var. *viridis* (B) ve *C. maculatum* (C) özütlerinin farklı konsantrasyonlarının HeLa hücreleri üzerinde 48 saatteki antikanser aktiviteleri
Figure 3. Anticancer activities of different concentrations of *S. excelsa* (A), *B. oleracea* var. *viridis* (B) and *C. maculatum* (C) extracts on HeLa cells at 48 h.

Çizelge 1. *C. maculatum*'ün farklı doz ve sürelerde HeLa hücre canlılığına etkisi

Table 1. The effect of *C. maculatum* on HeLa cell viability at different doses and durations.

Doz (mg mL ⁻¹)	24 Saat	48 Saat
Kontrol	100.00±0.00 ^a	100.00±0.00 ^a
0.156	90.61±4.37 ^b	90.81±0.65 ^b
0.313	87.92±1.41 ^b	58.01±2.60 ^e
0.625	77.09±0.12 ^c	28.70±1.61 ^g
1.25	66.90±6.05 ^d	6.80±0.10 ^h
2.5	36.90±3.70 ^f	5.80±0.97 ^h
5	5.43±0.40 ^h	5.01±0.10 ^h
10	5.33±0.02 ^h	4.60±0.46 ^h

a,b,...,h gibi farklı harfle gösterilen değerler arasında istatistiksel farklılık bulunmaktadır ($p<.01$).

Çizelge 2. *B. oleracea* var. *viridis*'in farklı doz ve sürelerde HeLa hücre canlılığına etkisi

Table 2. The effect of *B. oleracea* var. *viridis* on HeLa cell viability at different doses and durations.

Doz (mg mL ⁻¹)	24 Saat	48 Saat
Kontrol	100.00±0.00 ^a	100.00±0.00 ^a
0.189	90.60±4.38 ^{ab}	87.20±3.70 ^{bc}
0.379	87.90±1.49 ^{bc}	82.30±5.13 ^{bed}
0.758	77.01±0.19 ^{cde}	71.40±7.24 ^{de}
1.52	66.50±5.75 ^e	32.70±1.80 ^f
3.03	37.00±3.01 ^f	12.10±1.00 ^g
6.06	5.75±0.29 ^g	5.20±0.69 ^g
12.13	5.20±0.18 ^g	5.00±0.45 ^g

a,b,...,h gibi farklı harfle gösterilen değerler arasında istatistiksel farklılık bulunmaktadır ($p<.01$).

Çizelge 3. *S. excelsa*'nın farklı doz ve sürelerde HeLa hücre canlılığına etkisi

Table 3. The effect of *S. excelsa* on HeLa cell viability at different doses and times.

Doz (mg mL ⁻¹)	24 Saat	48 Saat
Kontrol	100.00±0.00 ^a	100.00±0.00 ^a
0.154	94.40±1.31 ^{ab}	94.54±2.21 ^{ab}
0.309	88.30±0.80 ^{bc}	81.90±1.89 ^{cd}
0.617	74.00±0.52 ^e	81.34±1.54 ^d
1.23	72.28±2.56 ^e	67.86±4.84 ^e
2.47	71.88±2.02 ^e	32.62±0.92 ^g
4.94	41.50±0.30 ^f	7.30±0.43 ⁱ
9.88	14.60±1.90 ^h	6.90±0.74 ⁱ

a,b,...,i gibi farklı harfle gösterilen değerler arasında istatistiksel farklılık bulunmaktadır ($p<.01$).

TARTIŞMA

Günümüzde kanser tedavilerinde kullanılan ilaçların olumsuz etkileri ve zamanla kanser hücreleri karşısında yetersiz kalması, tedavi seçeneklerinin sınırlı olması gibi bazı sorunlar araştırmacıları ekosistemde doğal olarak yetişen bitkilere yönlendirmektedir. Bu nedenle bitki özütlerinin biyolojik aktivitelerinin araştırıldığı çalışmalar son yıllarda giderek artış göstermektedir.

Brassica cinsine ait bazı türlerin [*Brassica rapa* var. *rapa* (Arora & Vig, 2015), *Brassica oleracea* var. *italica* (Chaudhary ve ark., 2018), *Brassica oleracea* var. *capitata* f. *rubra* (Alani ve ark., 2021)], *Smilax* cinsine ait bazı türlerin [*Smilax china* (Hussain ve ark., 2022), *Smilax zeylanica* (Shailasree ve ark., 2014)] ve Apiaceae familyasına ait bazı türlerin [*Trachyspermum ammi* (Qureshi & Barabde, 2018), *Ligusticum chuanxiong* (Tang ve ark., 2022), *Prangos aricakensis* (Gokcimen ve ark., 2024)] özütlerinin pBR322 plazmid DNA'sı üzerinde DNA koruyucu aktivitelerinin araştırıldığı çalışmalara rastlanılmıştır. Ancak yapılan literatür taramasında *Conium* cinsine ait türlerin pBR322 plazmid DNA'sı üzerinde DNA koruyucu aktivitelerinin araştırıldığı çalışmalara rastlanılmamıştır. Çalışmada da *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* türlerine ait etanolik özütlerin -OH ile oluşturulan DNA hasarına karşı pBR322 plazmid DNA'sı üzerinde DNA koruyucu aktiviteleri araştırılmıştır. Özütlerin 20, 10, 5 mg mL⁻¹ konsantrasyonlarının DNA hasarına karşı güçlü koruyucu aktiviteleri ilk kez bu yayınlara gösterilmiştir (Şekil 1). Bu bitkilerin antioksidan kapasitelerinin yüksek olması, -OH'lere karşı plazmid DNA'yı stabilize etmiş olabileceğini düşündürmektedir.

Bazı araştırmacılar *Brassica* cinsine ait bazı türlerin [*B. oleracea* var. *capitata* f. *rubra*, HeLa ve HepG2 hücrelerinde (Hafidh ve ark., 2013), *B. oleracea* var. *alboglabra*, BGC-823 (insan mide kanseri) hücrelerinde (Ngo ve ark., 2022), *B. oleracea* var. *acephala* ve *b. incana*, HeLa hücrelerinde (Lučić ve ark., 2023)] antikanser aktivitelerini incelemiştir.

Çalışmada, Ordu ilinden temin edilen *B. oleracea* var. *viridis*'in kullanılması, bu türün yerel varyetelerinin farmakolojik potansiyelinin araştırılması açısından önemlidir. Biyolojik aktiviteleri yeterince çalışılmamış yerel bitkiler, yeni doğal antikanser ajanlarının keşfi için önemli bir kaynak olabilir. Bu yönüyle, çalışmamız *B. oleracea* var. *viridis* özütünün HeLa hücrelerinde antikanser etkisinin bu hücre hattında test edildiği ilk araştırma olması bakımından literatüre yeni ve özgün bir katkı sunmaktadır. Literatür incelendiğinde, *Smilax* cinsine ait bazı türlerin [*S. china* (A-549, MCF-7, MDA-MB-231 (metastatik meme adenokarsinomu), U87-MG (epitel kökenli bir glioblastoma), AGS (gastrik adenokarsinomu), MKN-74 ve SNU-601 (insan gastrik karsinomu) hücrelerinde (Kim ve ark., 2022), *S. fluminensis* B16-F10 (Fare melanom) hücrelerinde (Fetter ve ark., 2022), *S. excelsa* C6 glioblastoma hücrelerinde (Al-Yassine ve ark., 2023)] ve Apiaceae familyasına ait bazı türlerin ise [*Ferulago mughlea* PC-3 ve kolorektal (SW-480) karsinom hücrelerinde (Filiz ve ark., 2018), *Seseli petraeum* A549 hücrelerinde (Cinar ve ark., 2020), *Oliveria decumbens* MCF-7 hücrelerinde (Shariatzadeh ve ark., 2022)] antikanser aktivitelerinin incelendiği çalışmalar yapmıştır. Ancak mevcut literatürde *S. excelsa*'nın HeLa hücreleri üzerindeki antikanser etkisine dair bir çalışmaya rastlanmamıştır. Bu yönüyle, çalışmamız *S. excelsa*'nın servikal kanser hücrelerine karşı sitotoksik etkisinin ilk kez raporlandığı bir araştırma olma özelliği taşımaktadır. Bu çalışmada, *S. excelsa* etanolik özütünün HeLa hücrelerinde anlamlı düzeyde sitotoksikite göstermesi, *Smilax* cinsinin farklı kanser hücre hatlarındaki antikanser potansiyelini desteklemekte ve bu türün yeni bir terapötik aday olabileceğine işaret etmektedir. Yapılan literatür araştırmasında *C. maculatum* ile ilgili farklı hücre hatları (HeLa, melanom (A375), HepG2 ve A549) antikanser aktivitelerinin araştırıldığı çalışmalara [Mondal ve ark. (2014), Venkateswaran ve ark. (2023)] rastlanılmıştır. Çalışmada da *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* türlerine ait etanolik özütlerin memeli hücrelerinde antikanser aktivitesi MTT analizi ile HeLa hücre hattı kullanılarak iki farklı zaman diliminde (24 ve 48 saat) test edilmiştir. Özütlerin hücre canlılığı üzerinde test edilen dozlara bağlı bir azalma gözlenmiş ve 24 saat sonra *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin yaklaşık IC₅₀ değerleri sırasıyla 3.88, 2.20 ve 1.48 mg mL⁻¹ olarak tespit edilmiştir (Şekil 2). 48 saat sonra yaklaşık IC₅₀ değerleri sırasıyla 1.82, 1.13 ve 0.39 mg mL⁻¹ olarak tespit edilmiştir (Şekil 3). Elde edilen bu sonuçlar, özütlerin antikanser etkinliğinin hem doza hem de uygulama süresine bağlı olarak arttığını göstermektedir. Ayrıca, literatürde sınırlı sayıda çalışması bulunan *B. oleracea* var. *viridis* ve *S. excelsa* türlerinin HeLa hücreleri üzerindeki sitotoksik etkilerinin belirlenmesi, bu türlerin potansiyel doğal antikanser ajanlar olarak değerlendirilmesine olanak sağlamaktadır.

SONUÇ ve ÖNERİLER

Çalışmada *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* türlerine ait etanolik özütlerin -OH ile oluşturulan DNA hasarına karşı pBR322 plazmid DNA'sı üzerinde DNA koruyucu aktiviteleri araştırılmıştır. Özütlerin 20, 10, 5 mg mL⁻¹ konsantrasyonlarının DNA hasarına karşı güçlü koruyucu aktiviteleri ilk kez bu yayınlara gösterilmiştir.

Ayrıca *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* türlerine ait etanolik özütlerin memeli hücrelerinde

antikanser aktiviteleri MTT analizi ile HeLa hücre hattı kullanılarak iki farklı zaman diliminde (24 ve 48 saat) test edilmiştir. Özütlerin hücre canlılığı üzerinde test edilen dozlara bağlı bir azalma gözlenmiş ve 24 saat sonra *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin yaklaşık IC₅₀ değerleri sırasıyla 3.88, 2.20 ve 1.48 mg mL⁻¹ olarak tespit edilmiştir. 48 saat sonra ise yaklaşık IC₅₀ değerleri sırasıyla 1.82, 1.13 ve 0.39 mg mL⁻¹ olarak tespit edilmiştir. Tüm özütlerin HeLa hücrelerinde antiproliferatif etkiye sahip olduğu bulunmuştur.

Elde edilen veriler ışığında *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin HeLa hücrelerine karşı antikanser aktivitesi ve oksidatif stres ile oluşan DNA hasarına karşı koruyucu aktivitesi tespit edilmiştir. Bu noktadan hareketle kontrollü şartlar altında *S. excelsa*, *B. oleracea* var. *viridis* ve *C. maculatum* özütlerinin DNA hasarına bağlı hastalıkların önlenmesinde ve servikal kanser tedavisinde potansiyel ajanlar olabileceği kanısına varılmıştır. Ancak çalışmanın sonraki aşamasında, söz konusu olan bitkilerin biyolojik aktiviteden sorumlu fitobileşiklerinin tespit edilmesi, farklı kanser hücrelerinde de test edilmesi ve deney hayvanlarında non-toksik olup olmadığının in-vivo çalışmalar ile ortaya konulması gerektiği düşünülmektedir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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The Synopsis of the Genus *Biarum* Schott (Araceae) in Türkiye, With Complementary Notes and a Key to the Species

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ABSTRACT

The aim of this study is to conduct systematic research on *Biarum*, which is one of the taxonomically problematic genera in Türkiye. It was determined that the number of *Biarum* taxa in Türkiye is 12. It is decided that *Biarum x cinarense*, *B. ditschianum*, *B. davisii* subsp. *marmarisense*, *B. rifatii*, and *B. eximium* are the endemic taxa to Türkiye, and the endemism rate is 41.6%. In this study, taxonomic notes, diagnostic keys, general distribution as well as their conservation status of each species are given. Some of the new collections and herbaria records of Türkiye and abroad are included. The checklist of the genus is given with all the changes, synonyms, and related revisions. Taxonomical changes that have occurred since the publication of Flora of Turkey are included. New findings and a key to the 12 species of *Biarum* genus in Türkiye are provided.

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Türkiye'deki *Biarum* Schott (Araceae) Cinsinin Sinopsisi, Tamamlayıcı Notlar ve Tür Teşhis Anahtarı

ÖZET

Bu çalışmanın amacı, Türkiye'de taksonomik olarak sorunlu cinslerden biri olan *Biarum* cinsi üzerinde sistematik bir araştırma yapmaktır. Türkiye'deki *Biarum* takson sayısının 12 olduğu belirlenmiştir. *Biarum x cinarense*, *B. ditschianum*, *B. davisii* subsp. *marmarisense*, *B. rifatii* ve *B. eximium*'un Türkiye'ye endemik taksonlar olduğu ve endemizm oranının %41,6 olduğu belirlenmiştir. Bu çalışmada, her türün taksonomik notları, teşhis anahtarları, genel dağılımları ve koruma durumları verilmiştir. Türkiye ve yurtdışından bazı yeni koleksiyonlar ve herbaryum kayıtları eklenmiştir. Cinsin kontrol listesi, tüm değişiklikler, sinonimler ve ilgili revizyonlar birlikte verilmiştir. Türkiye Florası'nın yayınlanmasından bu yana meydana gelen taksonomik değişiklikler eklenmiştir. Türkiye'deki *Biarum* cinsine ait 12 türü kapsayan yeni bulgular ve bir teşhis anahtarı anahtarı verilmiştir.

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INTRODUCTION

In Türkiye, the Araceae family has 10 genera, and the number of taxa has increased to 44 (Yıldırım, 2018). Nine taxa belonging to the family are endemic to Türkiye (Yıldırım & Erdem, 2019).

The genus *Biarum* consists of 22 species of tuberous plants found in semiarid regions of Southern Europe, North Africa, the Near and Middle East (POWO, 2025). The center of diversity is the Middle East, where 75% of the species are endemic (Boyce, 2008).

In the treatment of genus *Biarum* in the Flora of Turkey, Mill (1984) accepted 6 taxa. Later, Boyce (1987) described a new endemic taxon from Türkiye, known as *Biarum davisii* Turrill subsp. *marmarisensis* P.Boyce. Moreover, Bogner and Boyce (1989) described a new endemic species from Türkiye, namely *B. ditschianum* Bogner & P.C.Boyce. Since then, Boyce has studied the revision of genus *Biarum* worldwide (Boyce, 2006). According to this

revision (Boyce, 2006), *Biarum davisii* subsp. *marmarisensis* is reevaluated as a distinct species with the name of *Biarum marmarisense* (P.C. Boyce) P.C. Boyce. *Biarum pyrami* (Schott) Engler is reevaluated as *B. pyrami* (Schott) Engler var. *pyrami*. On the other hand, it is reported that *Biarum crispulum* (Schott) Engl. and *B. kotschyi* (Schott) B.Mathew ex H.Riedl have a natural distribution in Türkiye (Boyce, 2006). Akan & Balos (2008) published a new record for Türkiye, namely *Biarum syriacum* (Spreng.) H.Riedl. Later, Yıldırım et al. (2016) published a new record for Türkiye, namely *Biarum aleppicum* J. Thiébaud. In addition, *Biarum rifatii* Yıldırım & Altıoğlu was published as new species for Türkiye (2016). *Biarum marmarisense* reevaluated as *Biarum davisii* subsp. *marmarisensis* (Yıldırım, 2018). Çeçen et al. (2022), published *Biarum* × *cinarensense* as a new natural hybrid from Türkiye. Thus, the total number of *Biarum* taxa recorded in Türkiye has now increased to 12. Endemism rate is 41.6% in Türkiye (Çeçen et al., 2022).

In Türkiye, the genus members known as; sucukotu, yılanpancarı, ağukınası, kârdi, kâri, ziliké ereba, asalan, kurt kulağı, fise pire (Alpınar, 1985, 1987; Balos & Akan, 2007; Akan et al., 2008, 2013; Altay & Çelik, 2011; Altay et al., 2015; Kocabaş & Gedik, 2016).

The problems of genus were stated in the Flora of Turkey, and it is stated that the taxa in *Biarum* have different flower and leaf periods, making it difficult to diagnose the species. Therefore, it is emphasized that work on these genera is necessary (Davis & Hedge, 1975; Mill, 1984; Davis et al., 1988).

Studies conducted on the *Biarum* genus in recent years are chronologically as follows: Boyce (2006), Akan et al. (2008), Akan & Balos (2008), Boyce (2008), Yıldırım et al. (2016), Yıldırım & Altıoğlu (2016), Akyol et al. (2018), Yıldırım (2018), Çeçen et al. (2019), Altuntaş (2020), Balos et al. (2021), Tel & Akan (2021), Çeçen et al. (2022), Bağatur et al. (2023). The aim of this study is to conduct systematic research on *Biarum*, which is one of the taxonomically problematic genera in Türkiye.

MATERIAL and METHOD

The research material consists of taxa of the *Biarum* genus collected from different localities of Türkiye between 2020 and 2022. The specimens have been identified by the Flora of Turkey (Mill, 1984; Davis et al., 1988; Alpınar, 2000; Yıldırım, 2018).

In addition, the specimens kept in the herbaria of AEF, ANK, DUOF, EGE, GAZI, HUB, HARRAN, ISTE, and NGBB were examined. Type specimens kept in abroad herbaria, such as E, K, B, G, BM, P, and W herbaria, were also examined (Thiers, 2019). Herbarium specimens in Türkiye and from abroad were examined, but only some of them could be given here. The specimens determined as lectotypes in the study of Boyce (2008), which was the most fundamental revision made before this study, were taken as a basis.

Red list categories of endemic taxa were revised according to IUCN Red List Categories (Ekim et al., 2000; Yıldırım, 2018; IUCN, 2022). These categories are stated with their abbreviations: CR: critically endangered, EN: endangered, VU: vulnerable, NT: near threatened, LC: least concern.

Turkish names of plants belonging to the taxa were given according to Güner et al. (2012), Yıldırım (2018), Çeçen et al. (2022).

The location, altitude, date, habitat, collector number, and endemism status of the plant were given.

The grid system proposed by Davis (1965) was used for the distribution of each species.

Specimens collected during this study were kept in the HARRAN herbarium, Arts and Science Faculty of Harran University, Department of Biology, Şanlıurfa.

Important taxonomic characters of the genus *Biarum* were determined (Figure 1). These were determined as shape of tuber, plant height, leaf shape, leaf limb length, leaf width, petiole length and surface features, spathe shape, spathe color, spathe dimensions, spathe length, spathe condition, spadix length, spadix appendix shape, spadix appendix length and color, male flower region length, sterile flower region length and female flower region length (Boyce, 2008).

RESULTS

Biarum Schott in Schott & Endlicher, Melet. Bot. 17 (1832) nom. cons.

Type: *Biarum tenuifolium* (L.) Schott.

In the treatment of Boyce (2008) and the International Plant Names Index and World Checklist of Vascular Plants (2024), 6 synonyms are given for *Biarum*:

Homaia Adans., Fam. Pl. (Adanson) 2: 470 (1763).

Homaia Raf., Fl. Tellur. 3: 63 (1837).

Ischarum (Blume) Rchb., Deut. Bot. Herb. -Buch 32 (1841).

Cyllenium Schott., Gen. Aroid. t. 9 (1858).

Leptopetion Schott., Gen. Aroid. t. 8 (1858).

Stenurus Salisb., Gen. Pl. [Salisbury] 5 (1866).

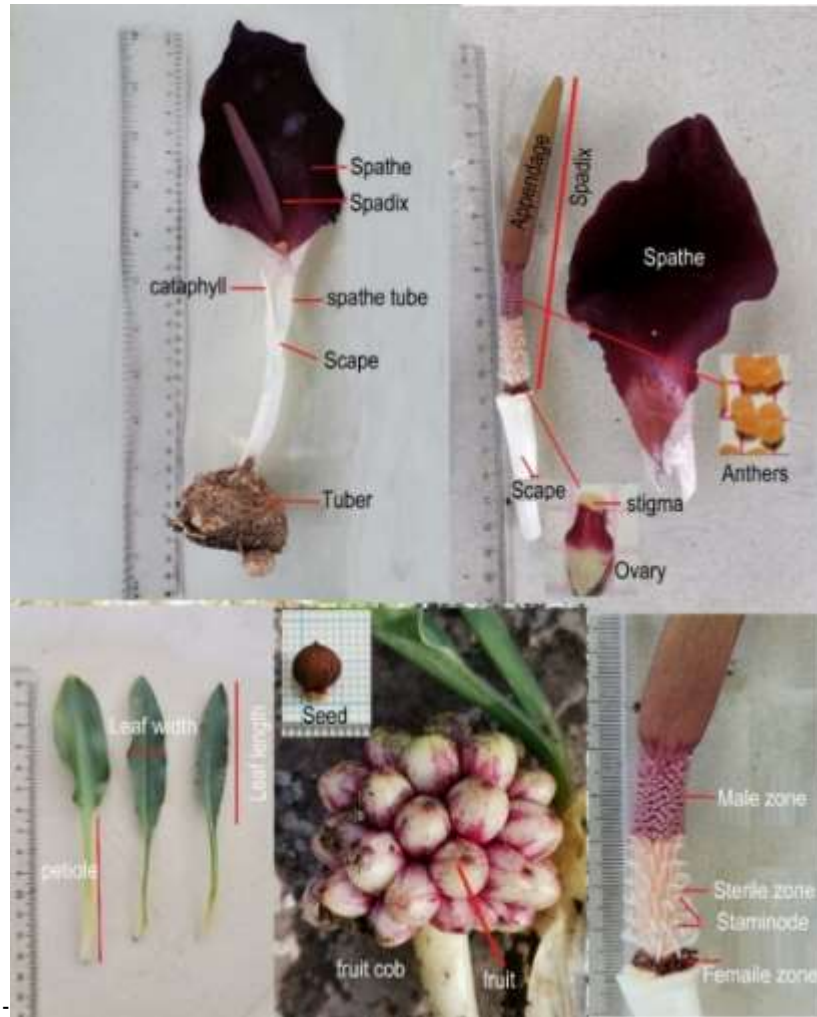


Figure 1. General morphological characteristics of the genus *Biarum*
Şekil 1. *Biarum* cinsinin genel morfolojik özellikleri

Identification key of the Turkish *Biarum* species:

1. The flowering period is the spring or summer months
 2. Staminodes are present in both the upper and lower regions of the male flower region.....*B. tenuifolium* subsp. *zelebori*
 2. Staminodes are located in the region between male and female flowers, or staminodes are absent
 3. Appendix is quite thick, yellow, with a downward-pointing hair-covered area at its base; spathe palm is quite blunt and much shorter than spathe tube.....*B. ditschianum*
 3. Appendix not as above, glabrous at base; has spathe, longer or shorter than the tube
 4. Sterile flowers present; leaves 11–27, leaf limb linear to linear-elliptic; spathe tube inner surface upper half light purple, lower half white*B. syriacum*
 4. Sterile flowers absent (rarely 1–2); leaves 3–7; lamina elliptic to oblanceolate-elliptic, inner surface of spathe tube dark shiny reddish-purple*B. rifatii*
1. The flowering period is the autumn or winter months
 5. Spathe tube swollen, globose to oblong
 6. No sterile flower.....*B. davisii* subsp. *marmariense*
 6. Sterile flower present
 7. Spathe limb narrow (0.7-1.2 cm).....*B. crispulum*
 7. Spathe limb wide (1.9-8 cm)
 8. Spathe tube swollen rectangular-cylindrical; spathe limb linear-lanceolate; 7–12 cm x 1.9–6 cm; spadix appendix narrowly fusiform to fusiform-cylindrical, 6.5–13 cm x 2–9 mm.....*B. bovei*
 8. Spathe tube globose to globose-rectangular; spathe limb considerably expanded at the base, lanceolate, 9–24 x 2.2–8 cm; spadix appendix cylindrical-nebulate, 10–28 cm x 3–10 mm.....*B. pyrami*
 5. The tube of the spathe is not swollen or slightly swollen, the scapula tube is not swollen or slightly swollen
 9. Scapulae present throughout the sterile floral region.....*B. eximium*
 9. Scapulae extend halfway into the sterile region or slightly
 10. Outer cataphylls not fibrous, ivory colored.....*B. carduchorum*
 10. Outer cataphylls fibrous, brown

11. Leaves 7–28; leaf limb width 0.6–1.9 cm *B. aleppicum*
11. Leaves 4–11; leaf limb width 1.2–3 cm *B. x cinarense*

1. ***Biarum aleppicum*** J.Thiébaud, Bull. Soc. Bot. France 95:21 (1948). (Figures 2-3, 26) /**Urfa yılanpancarı**

Lectotype: Syria, Alep, *Fr. Louis s.n.* (lectotype P00733219!, isolectotype K, photo!), lectotype designated by Boyce (2006).

Synonym:

Biarum bovei var. *aleppicum* (J.Thiébaud) Gombault [*in sched. nom. nud.*].

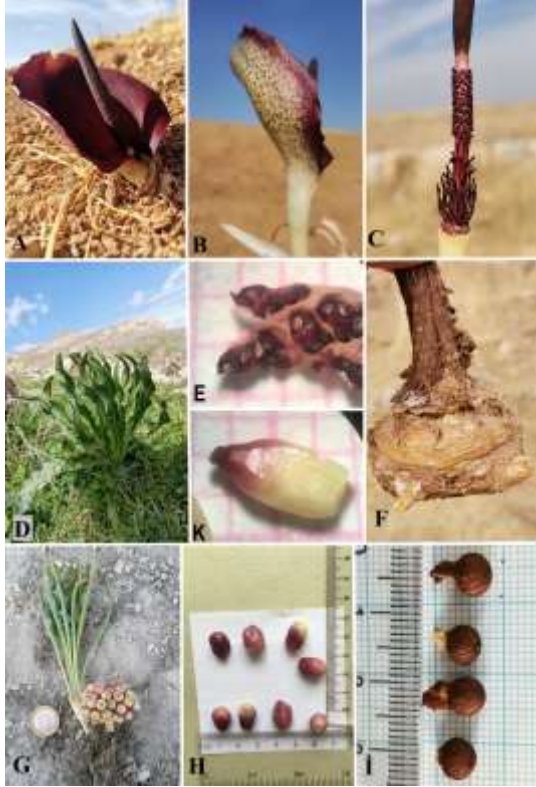


Figure 2. *Biarum aleppicum* A- flower front part, B- flower back part, C- flower reproductive organs, D- leaves, E- common flower, K- female flower, F- tuber, G- fruit cob and leaves, H- fruit, I- seeds

Şekil 2. *Biarum aleppicum* A- çiçeğin ön kısmı, B- çiçeğin arka kısmı, C- çiçek üreme organları, D- yapraklar, E- ortak çiçek, K- dişi çiçek, F- yumru, G- meyve koçanı ve yapraklar, H- meyve, I- tohumlar



Figure 3. Lectotype specimen of *Biarum aleppicum* (P00733219)
Şekil 3. *Biarum aleppicum*'un lektotip örneği (P00733219)

<http://coldb.mnhn.fr/catalognumber/mnhn/p/p00733219>

Examined specimens: **C7 Şanlıurfa:** Birecik, Zeytinbahçe village, limestone slopes, 350 m, 08.10.2020, *C. Çeçen 1114* (HARRAN!); Birecik-Mezra village, 1 km S of Akarçay, steppe, 480 m, 23.04.2015, *H. Yıldırım 3186* (EGE!); **Diyarbakır:** Mardin-Çınar 55 km, roadside, 660 m., 13.10.2020, *C. Çeçen 1123* (HARRAN!); **C8 Mardin,** Nusaybin to Midyat, Yemişli village, steppe, 950 m, 24.10.2020, *Balos 4900* (HARRAN!); Mazıdağı, Bağyaka village, slopes of dried stream, 825 m, 27.03.2021, *Balos 5049 & Geçit* (HARRAN!); Artuklu, Sultançayırı district, 1140 m, 28.03.2021, *Balos 5079* (HARRAN!).

Note: *Biarum aleppicum* is close to *B. carduchorum*, which is distributed in the Eastern and Southeastern Anatolia regions of Türkiye, and *B. angustatum* (Hook.f.) N.E.Br., which is distributed from SW Syria to Israel. *B. aleppicum* was known to be distributed only in Northwestern Syria until 2016. However, it was published as a new record from Türkiye by Yıldırım et al. (2016). *B. aleppicum* is also similar to *B. eximium*. However, it is distinguished from this species by the spathe tube being separated to the base, the stigmas not having any style, the staminodes being dense at the base, and the leaf limb being thinner. In *B. aleppicum*, it is easily distinguished from other species by the fact that the stigma is sessile. In addition, the fruit cob of *B. carduchorum* is white, while in *B. aleppicum* it is purplish.

2. ***Biarum bovei*** Blume, Rumphia 1: 114 (1836) (Figures 4-5, 26) /**Yılanpancarı**

Holotype: Lebanon, mountains of Lebanon, 1832, *Bové N. 379* (Holotype L0041870!, isotype L0041869!, L0041871!, P00733220!, P00733221!, K00400398!).

Synonyms:

Biarum bovei var. *blumei* Engl., Monogr. Phan. [A.DC. & C.DC.] 2: 577 (1879).

Biarum homaid Blume, Rumphia 1: 115 (1836)



Figure 4. A- *Biarum bovei* in field, B- flower back, C. leaves, D- flower reproductive organs, E- tuber, F- fruit cob, H- male flower part, J- female flower part, K- N- female flower, L- male flower, M- seed, O- spadix and scape
Şekil 4. A- *Doğada Biarum bovei*, B- çiçek sırtı, C. yapraklar, D- çiçek üreme organları, E- yumru, F- meyve koçanı, H- erkek çiçek kısmı, J- dişi çiçek kısmı, K- N- dişi çiçek, L- erkek çiçek, M- tohum, O- spadiks ve sap



Figure 5. *Biarum bovei* holotype (L0041870)
Şekil 5. *Biarum bovei* holotip (L0041870)

<https://data.biodiversitydata.nl/naturalis/specimen/L%20%200041870>

Caladium bovei Steud., Nomencl. Bot., ed. 2, 1: 249 (1840)

Biarum kotschyi (Schott) B.Mathew ex Riedl, Aroideana 3(1): 28 (1980).

Homaïda bovei Kuntze, Revis. Gen. Pl. 2: 742 (1891).

Ischarum bovei Schott, Syn. Aroid. 7 (1856).

Examined specimens: **B7 Tunceli:** Ovacık, above Paşadüzü village, Korti stream, 28.10.1980, *Ş. Yıldırım* 4107 (HUB 34097!); **Malatya:** Erkenek, Ağözü district, 13.10.1989, *E. Aktoklu* 1653 (ISTE 94601!); **B8 Elazığ:** Karakoçan-Sarıcan, Horşi district, steppe, 1550 m., 02.10.2021, *C. Çeçen & V. Sonay* 1185 (HARRAN!); **B9 Van:** Gevaş, Göründü to Altınış village, 09.10.2003, *M. Koyuncu* 14070 (AEF 25691!); **C6 Kahramanmaraş:** Elbistan, 22.10.1986, *S. Bayrı* (ISTE 57651!); Göksun, Höbek mountain, above Döngel cave, 23.10.1980, *B. Yıldız* 2344 (HUB!); **C7 Şanlıurfa:** Eyyübiye to Akçakale, hills, 550 m., 15.11.2020 *C. Çeçen & M. Balos* 1145 (HARRAN!); Halfeti, Savaşan village, steppe, 560 m., 05.03.2022 (at leaf) *C. Çeçen* 1192 & *M. Balos* (HARRAN!); **C8 Mardin:** W of Midyat, Acırlı village, 920 m, 20.11.2020 *C. Çeçen M. Balos & H.Akan* 1151 (HARRAN!).

Note: *B. bovei* is a species with a fairly wide distribution. In field studies, it was observed that spathe length, spadix appendix size and thickness, and width of the leaf limb of *B. bovei* varied. It is similar to *B. crispulum*, *B. dispar*, *B. kotschyi*, and *B. pyrami* in that the spathe tube is swollen and the flower and leaf are seen at the same time. *B. kotschyi* was discussed within the variation limits of *Biarum bovei* by Yıldırım (2008). Boyce (2008) stated that *Biarum bovei* is confused with *B. kotschyi* and *B. pyramid*, both in the natural area and in herbarium specimens. He stated that the main reason for this is the scarcity of real *B. bovei* in herbaria and the fact that most of the type specimens are pressed in a way that makes comparison difficult. Examination was carried out on fresh specimens collected from Mardin and Şanlıurfa, and the description of the species was expanded. The observations showed that the spathe size of *B. bovei* varies. In Midyat, town of Mardin, and Karaköprü, town of Şanlıurfa specimens, it was seen that the spathe was thin and long, the outer surface of the spathe was green, and the leaves were thin. It was seen that the specimens near Yemişli village between Mardin-Nusaybin and the specimens in Eyyübiye district of Şanlıurfa had larger spathes and narrower leaf limbs. In some specimens, it was seen that the outer surface of the leaf limb was spotted, and the leaf limb was wider. Since *B. bovei* and *B. carduchorum* share the same habitats, it is predicted that the specimens with spotted spathe may be hybrids of *B. bovei* and *B. carduchorum*. A large-scale study at the phylogenetic population level is needed to clarify this situation.

3. *Biarum carduchorum* (Schott) Engl., Monogr. Phan. 2: 575 (1879) (Figures 6-7, 26) / **Kardi**

Lectotype: Türkiye, Schirwan (Şirvan), Kotschy *s.n.* (holotype W destroyed; lectotype W, designed by Boyce (2006), Schott's leones no. 1825).



Figure 6. *Biarum carduchorum* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower

Şekil 6. *Biarum carduchorum* A- habitus, B- çiçeğin ön görünümü, C- çiçek üreme organları, D- yapraklar, E- koçan, F- herbaryum örneği H- yumru, J- meyve, K- tohumlar, L- dişi çiçek, M- erkek çiçek



Figure 7. *Biarum carduchorum* Epitype (K000400388)

Şekil 7. *Biarum carduchorum* Epitip (K000400388)

<http://specimens.kew.org/herbarium/K000400388> (© RBG Kew)

Epitype: Türkiye, B7 Tunceli, Sakaltutan mountain, E side; 10 km NE of Pertek; hills, soft limestone, fallow fields and *Quercus* shrub. Common, local, alt. 1550 m, Flowers mahogany brown, 23 September 1966, A. C & Watson 2404 (Epitype K000400388!) (Epitype designed here).

Synonyms:

Biarum platyspathum Bornm., Repert. Spec. Nov. Regni Veg. 5: 57 (1908).

Biarum carduchorum var. *platyspathum* (Bornm.) Engl., Pflanzenr. (Engler) IV, 23F: 137 (1920).

Biarum platyspathum var. *bakhtyarum* Parsa, Kew Bull. 4: 36 (1949).

Homaida carduchorum Kuntze, Revis. Gen. Pl. 2: 742 (1891).

Cyllenium carduchorum Schott, Prodr. Syst. Aroid. 65 (1860).

Examined specimens: **B8 Elazığ:** Karakoçan, Sarıcan district, steppe, 1200 m, 02.10.2021, *C. Çeçen 1186* (HARRAN!); **C7 Adıyaman:** Kahta, Çiçek village, dry riverbank, 650 m, *C. Çeçen 1181* (HARRAN!); **Diyarbakır:**

Çınar to Mardin 5 km, nearby dam lake, 660 m, 13.10.2020, *C. Çeçen & M. Balos 1125* (HARRAN!); **Şanlıurfa:**

Şanlıurfa-Viranşehir, district Çoban stream, steppe, 690 m, 13.10.2020, *C. Çeçen & M. Balos 1118* (HARRAN!);

C8 Mardin: Mardin to Diyarbakır 15 km, stony slopes, 1040 m, 13.10.2020, *C. Çeçen 1121 & M. Balos* (HARRAN!).

Note: *B. carduchorum* is distributed from the Eastern and Southeastern Anatolian Regions of Türkiye to Southern Iran. It is similar to *B. aleppicum*, *B. angustatum* and *B. eximium* in terms of the mottled outer surface of the spathe. It can be easily distinguished from *B. aleppicum* and *B. eximium* by the union of the spathe tube in the upper quarter and the condition of the sterile flowers. It is easily distinguished from *B. angustatum* by the upward position of the sterile flowers and the wider leaves. It was revealed for the first time that the tuber was offspringless for *B. carduchorum*.

4. *Biarum* × *cinarense* Çeçen, H. Akan, Yıldırım & Balos, Ann. Bot. Fenn. 59(1): 67 (2022) (Figures 8-9, 26) / Çınar yılanpancarı

Holotype: Türkiye. Diyarbakır Province, Çınar district, Mardin-Diyarbakır road 5 km from Çınar, around Göksu dam, Akçomak village road, stony steppe areas, 660 m, 13 October 2020 *C. Çeçen 1135 & Balos* (Holotype HARRAN!; isotype HARRAN!, EGE!).

Examined specimens: Diyarbakır: Çınar-Diyarbakır 25 km, steppe, 660 m, 13.10.2020, *C. Çeçen 1135 & Balos* (HARRAN!; EGE!); ibid., 28.03.2021 (at leaf), *C. Çeçen 1161* (HARRAN!).



Figure 8. *Biarum x cinarense* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower

Şekil 8. *Biarum x cinarense* A-habitus, B- çiçeğin ön görünümü, C- çiçeğin üreme organları, D- yapraklar, E- koçan, F- herbarium örneği H- yumru, J- meyve, K- tohum, L- dişi çiçek, M- erkek çiçek



Figure 9. *Biarum x cinarense* holotype (C. Çeçen & M. Balos 1135, HARRAN!)

Şekil 9. *Biarum x cinarense* holotipi (C. Çeçen & M. Balos 1135, HARRAN!)

Note: *Biarum x cinarense* was identified and published as a new natural hybrid species recently (Çeçen et al., 2022). It is morphologically a hybrid species between *B. aleppicum* and *B. carduchorum*. It resembles to *B. aleppicum* in terms of its tuber structure, hard outer cataphylls, spathe color, and frilled leaves. It is easily distinguished from *B. aleppicum* by the long lanceolate spathe, wider leaf limb, lighter color, and fewer leaves. It is easily distinguished from its parent, *B. carduchorum*, by having hard cataphylls and different male, female, and sterile regions. The species epithet is derived from the Çınar district of Diyarbakır province in Türkiye, where the species was first gathered. It is a local endemic to Diyarbakır, SE Türkiye.

5. *Biarum crispulum* (Schott) Engl., Bot. Jahrb. 5: 334 (1884) (Figures 10-11, 26) /Eğri yılanpancarı

Holotype: 'Iraq, ad Arcem Semiramidis' (but see note below), Kotschy, cult. Schoenbrunn (holotype W destroyed)

Lectotype: Türkiye: Adana, Kassan Oghlu (Hasanoğlu) Gorumse (Gürümze) valley, 21 May 1859, lectotype Kotschy 442, G-BOIS) chosen by Boyce (2006).

Synonyms:

Calla orientalis L., Sp. Pl., ed. 2. 2: 1373 (1763), nom. rej.

Arum carsaami Kunth, Enum. Pl. [Kunth] 3 25 (1841).

Eminium carsaamii (Kunth) Schott, Syn. Aroid. 17 (1856), nom. illeg.

Ischarum carsaamii (Kunth) Schott, Prodr. Syst. Aroid. 67 (1860), nom. illeg.

Biarum bovei var. *carsaamii* (Kunth) Boiss., Fl. Orient. 5: 34 (1882).

Biarum orientale (L.) Druce, Rep. Bot. Exch. Club Soc. Brit. Isles 3: 415 (1913 publ. 1914).

Ischarum crispulum Schott, Prodr. Syst. Aroid. 68 (1860).

Examined Specimens: Türkiye, B6 Adana: Saimbeyli, Himmetli, 12.04.1957, P.H. Davis 26644 (K 000400399 photo!), Adana, Kassan Oghlu (Hasanoğlu), Gorumse (Gürümze) valley, 21 May 1859, Kotschy 442 (G-BOIS, photo!), C6 Hatay, Hatay, Belen pass, stony slopes, 740 m, 14.11.2020, C. Çeçen & M. Balos 1143 (HARRAN!),

Note: *B. crispulum* is morphologically similar to *B. bovei*. It is easily distinguished from *B. bovei* by its narrow, toothed margins and smaller size. It is clearly distinguished from the other species by its synanthetic leaves and narrow, curved, and curled spathe edges. *B. crispulum* was collected by us for the first time after 55 years since the specimen was collected by Cheese in 1967 from Belen Pass in the Amanos Mountains, within the borders of Hatay, Türkiye. The type specimen of *B. crispulum* was investigated and epityped here. Its description was expanded by making measurements on fresh specimens of the species. The female flower dimensions were revealed for the first time it is decided that the tuber was offspringless in this study.



Figure 10. *B. crispulum* türünün A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower

Şekil 10. *B. crispulum* ve A- Habitus, B- Çiçeğin ön görünümü, C- Çiçek üreme organları, D- Yapraklar, E- Koçan, F- Herbarium örneği H- Yumru, J- Meyve, K- Tohumlar, L- Dişi çiçek, M- Erkek çiçek



Şekil 11. Lectotype of *B. crispulum* (G-BOIS, photo!)
Şekil 11. *B. crispulum*'un lektotipi (G-BOIS, foto!)

6. *Biarum ditschianum* Bogner and P.C.Boyce, Willdenowia 18: 409 (1989) (Figures 12-13, 26) / **Güdük yılanpancarı**
Holotype: Türkiye, Antalya, Xanthos hill; in holes and crevices in limestone, 30 m, 24 IV 1988, Koenen Bonn 22592 (holotype K; isotypes B100217094!, K000400410!, M0198907!, BONN 22592!).

Examined specimens: **C3 Antalya:** Aksu, district Karaçalı, fallow fields, 30 m, 23.04.2021, *C. Çeçen & Balos 1171* (HARRAN!), **Antalya:** Kaş-Kalkan, district Yeşilköy, 30.04.2016, *Yıldırım 3843* (EGE!); Ksantos, 24.04.1988, *Koenen 22592* (B 100217094, photo!), **C4 Konya:** Seydişehir to Tınaztepe, 04.05.2014, *T. Körüklü 20973* (ANK!)

Note: *B. ditschianum* can be easily distinguished from other *Biarum* species by its unusually shaped flower. The spathe is very small and almost imperceptibly reduced above the tube. Its most striking feature is the dark, thick, hollow yellow spadix. None of the other species have this feature.

7. *Biarum eximium* (Schott and Kotschy) Engl., Monogr. Phan. 2: 576 (1879) (Figures 14-15, 26) / **Adanapancarı**
Lectotype: Türkiye: İter Cilicicum in Tauri alpes "Bulgar Dagħ." prope Adana, in via romana versus Minaret Chan, 60 m, 28 ix 1853, *Kotschy 343* (holotype W destroyed, lectotype G-BOIS, isolectotype K000400392!, P02075091!, P01736146!, S06-17893!, MW0734476! MPU014375). (Lectotype designated by Yıldırım et al. (2018).

Synonyms:

Homaida eximia (Schott & Kotschy) Kuntze, Revis. Gen. Pl. 2: 742 (1891)

Ischarum eximium Schott & Kotschy, Oesterr. Bot. Wochenbl. 4: 81 (1854)

Examined specimens: **C5 Adana,** around Kozan, stony slopes, 85 m, 16.10.2020, *C. Çeçen & M. Balos 1136* (HARRAN!); Kozan, Ağlıboğaz village, steppe, 200 m, 16.10.2020, *C. Çeçen 1137* (HARRAN!); **Adana:** Pozantı to Fındıklı 8 km, 16.04.2010, *Z. Tıraş* (ISTE 103390!) (at leaf); **Mersin:** Silifke, 37 km NW of Silifke, 10.10.1985, *F. Sorger 85-49-1* (W 1991-03021, photo!); Mersin, 04.1896, *Siehe 22* (E 00330073 photo!)

Note: *Biarum eximium* differs from all other species of the subgenus *Ischarum* in that the staminodes are evenly distributed between the male and female flower regions. It is morphologically similar to *Biarum aleppicum*. However, it is distinguished from *B. aleppicum* by the staminodes completely covering the area between the female and male flowers, the purplish and shiny velvety texture of the spathe inner surface, and the stalkless and thicker spadix appendix. The holotype of this species in Vienna has disappeared; a lectotype was determined from the isotypes by Yıldırım (2018).

8. *Biarum davisii* Turill subsp. *marmarisense* P.C.Boyce, Aroideana 10(4): 14 (1987) (Figures 16-17, 26) / **Adasucukotu**

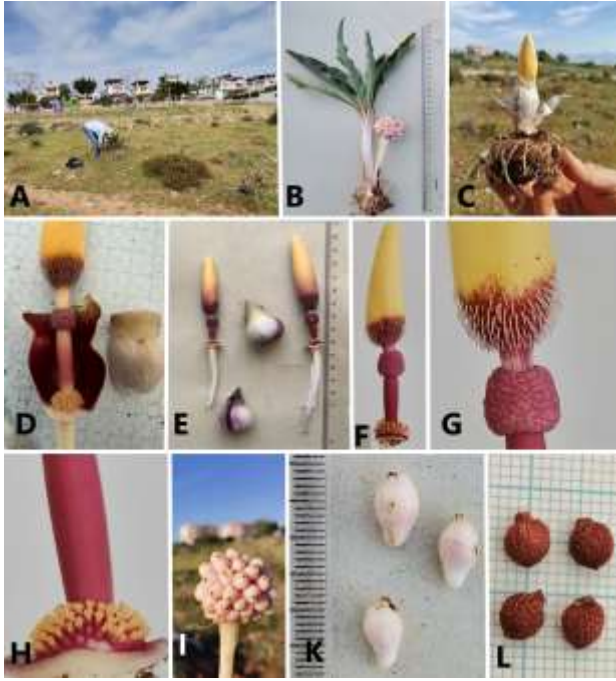


Figure 12. *B. ditschianum* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower

Şekil 12. *B. ditschianum* A- Habitus, B- Çiçeğin ön görünümü, C- Çiçek üreme organları, D- Yapraklar, E- Koçan, F- Herbarium örneği H- Yumru, J- Meyve, K- Tohumlar, L- Dişi çiçek, M- Erkek çiçek



Figure 14. *B. eximium* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower, N- anthers

Şekil 14. *B. eximium* A- habitus, B- çiçeğin ön görünümü, C- çiçeğin üreme organları, D- yapraklar, E- koçan, F- herbarium örneği H- yumru, J- meyve, K- tohumlar, L- dişi çiçek, M- erkek çiçek, N- anterler



Figure 13. Isotype of *B. ditschianum* (K000400410)
Şekil 13. *B. ditschianum*'un izotipi (K000400410)
<http://specimens.kew.org/herbarium/K000400410>
(© RBG Kew)



Figure 15. Lectotype of *B. eximium* (Kotschy 343, G-BOIS)
Şekil 15. *B. eximium*'un lektotipi (Kotschy 343, G-BOIS)

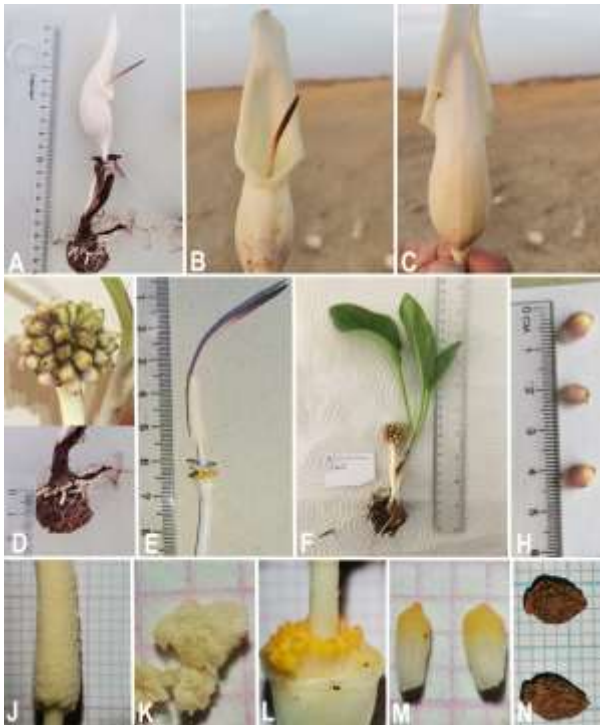


Figure 16. *B. davisii* subsp. *marmarisense* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower, N- seeds

Şekil 16. *B. davisii* subsp. *marmarisense* A- habitus, B- çiçeğin ön görünümü, C- çiçeğin üreme organları, D- yapraklar, E- koçan, F- herbarium örneği H- yumru, J- meyve, K- tohumlar, L- dişi çiçek, M- erkek çiçek, N- tohumlar



Figure 17. Isotype of *B. davisii* subsp. *marmarisense* (K000400411)
Şekil 17. *B. davisii* subsp. *marmarisense*'nin izotipi (K000400411)
<http://specimens.kew.org/herbarium/K000400411>
(© RBG Kew)

Holotype: Türkiye: Muğla, Marmaris, Bozburun, Taşlıca köyü, T.Baytop, Zeybek, G.Görk (holotype.: EGE 8796!; **isotype:** ISTE 47726, K000400411!, E00196510!)

Synonym: *Biarum marmarisense* (P.C.Boyce) P.C. Boyce, Aroideana 29: 34 (2006).

Examined specimens: C2 Muğla, Ortaca, Dere köy village, slopes, steppe, 20 m, 25.10.2020, *C. Çeçen 1141* (HARRAN!); ibid., 20.10.2021, *C. Çeçen 1191* (HARRAN!), **Muğla:** Fethiye, Ölüdeniz, Kelebekler valley, Kozagaç village, 18.10.2015, *Yıldırım 3760, 3764* (EGE!); Marmaris, Bayır to Söğüt, 18.10.2015, *Yıldırım 3752* and *3754* (EGE!); Bozburun, Taşlıca village 17.10.2013, *S. Aslan 4581* (DUOF 5485); Bozburun, Taşlıca Köyü, 01.11.1981, *T. Baytop* (ISTE-47726).

Note: Boyce, who described the subspecies *Biarum davisii* subsp. *marmariense*, changed its status in his later study (Boyce, 2006) and transferred it to species level as *Biarum marmarisense* (Boyce, 2006 and 2008). Later, this species was reduced to subspecies level as subsp. *marmarisense* by Yıldırım (2018). The specimens we examined confirm this situation. Although Boyce (2008) mentioned the existence of *Biarum davisii* subsp. *marmariense* in Greece, Yıldırım (2018) reports that this information needs confirmation.

9. *Biarum pyrami* (Schott) Engl., Monog. Phanerog. 2: 576 (1879) (Figures 18-19, 26) / **Ağukınası**

Type: Juxta arcem Semeramidis [plantae ad Pyramum (Ceyhan River) in monte Nur lectae: inter Messis (Misis) et castellum Scheih Meran (Yilankale), 60 m, J, Kotschy s.n. (holotype W destroyed, isotypes G (sterile fragments).

Epitype: W. Schott, Icones Aroideae no. 2161 (W!). (designated by Boyce, 2008): Bot. Mag. (Kew Mag.) 209:93 (2008).

Synonyms:

Ischarum pyrami Schott, Prodr. Syst. Aroid. 66 (1860).

Ischarum nobile Schott, Prodr. Syst. Aroid. 66 (1860).

Homaida pyrami (Schott) Kuntze, Revis. Gen. Pl. 2: 742 (1891)

Examined specimens: C2 Muğla: Fethiye, 27.03.1956, *P.H. Davis 25434* (E 00330080, photo!); Fethiye, Ölüdeniz, Karaağaç village, 10.10.2015, *Yıldırım 3746* (EGE!); C3 Burdur: Bucak, Üzümlübel village, Kandıra district, stony steppe, 800 m, 18.10.2020, *C. Çeçen & M. Balos 1138* (HARRAN!), **Burdur:** Bucak, Çobanpınar village, 21.10.2004, *A. Güner 13685* (NGBB 2126!), **Antalya:** Akseki, Dederi district, 17.11.1973, *N. Sütlüpınar* (ISTE 27131!); Antalya: Beydağı, S of Avlan Lake, 24.11.1967, *Watson 3901* (K 000400395, photo!); Antalya: Gazipaşa, Şahinlar village, 17.10.2006, *A. Dönmez 14171* (ANK!; G, photo!); Antalya: İbradı, Ürünlü village, *H. Duman 10277* (GAZI!)



Figure 18. *B. pyrami* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H-tuber, J- fruit, K- seeds, L- female flower, M- male flower, N- seeds
Şekil 18. *B. pyrami* A- habitus, B- çiçeğin ön görünümü, C- çiçeğin üreme organları, D- yapraklar, E- koçan, F- herbarium örneği H-yumru, J- meyve, K- tohumlar, L- dişi çiçek, M- erkek çiçek, N- tohumlar



Figure 19. Epitype of *B. pyrami* (Schott, *icones Aroideae* no. 2161, W)
Şekil 19. *B. pyrami*'nin epitipi (Schott, *Aroideae* resimleri no. 2161, W)

Note: *Biarum pyrami* is similar to *B. bovei*. It has a large and showy flower. It is a species that can be easily distinguished from autumn-flowering *Biarum* species by its distinctly swollen tube, broad-based lanceolate spathe and long spadix. There is a sudden narrowing between the spathe tube and the spathe. In some samples collected from the Bucak district of Burdur, it was observed that the leaves were bubbly. This feature is not found in any other species. Koach and Feinbrun (1986), in the 4th volume of *Flora of Palestine*, described a variety under the name of *B. pyrami* (var. *serotinum* Koach & Feinbrun); Boyce (2008) also accepted the existence of this variety and distinguished it from *B. pyrami* var. *pyrami* by the simultaneous emergence of leaves and inflorescences and the absence of pimple-like blisters on the leaves.

10. *Biarum rifatii* Yıldırım & Altıoğlu, *Bagbahçe Bilim Derg.* 3(2): 13 (2016) (Figures 20-21, 26) /**Rıfat sucukotu**
Holotype: Türkiye: Antalya: Kaş, Palamut village, between Ağacli Akgedik plateau and Rahat plateau, mountain slopes, 1800-2000 m, 20 vi 2016, *Yıldırım 3927* (holotype: EGE-42437!, isotype: EGE-42438!, NGBB!, ANK!)

Examined specimens: **C2 Antalya:** Kaş, Palamut village, mountain steppe, 1600 m, 24.04.2021 *C. Çeçen & Balos 1174* (at leaf) (HARRAN!); *ibid.*, 1800-1900 m, 17.06.2021, *C. Çeçen 1179* (HARRAN!), **Antalya:** Kaş, Palamut village, 20.06.016, *Yıldırım* (EGE!; ANK!; HUB!).

Note: It has no close relatives. It is a species characterized by the emergence of the inflorescence in June, the absence of sterile flowers, and the entire interior of the sphata tube being bright purple. *B. rifatii* is quite different from other species by flowering at high altitudes (2000 m) in June (Yıldırım & Altıoğlu, 2016). It shows some morphological similarities with *B. pyrami* (Yıldırım, 2018). However, it can be distinguished from *B. pyrami* by not having sterile flowers. It is a local endemic to Türkiye, around Antalya.

11. *Biarum syriacum* (Spreng.) Riedl, *Aroideana* 3: 19 (1980) (Figures 22-23, 26) /**Arap sucukotu**

Lectotype: Syria, prope Aleppo, Russell s.n. (Lectotype BM00084853!).

Isolectotype: Syria: prope Aleppo, *Russell s.n* (P02133553!). (isolectotype designed here).

Synonyms:

Biarum gramineum Banks & Sol., A. Russell, *Nat. Hist. Aleppo*, ed. 2, 2: 264 (1794), nom. superfl.

Arum syriacum Spreng., *Syst. Veg.*, ed. 16. 3: 768 (1826)

Biarum russellianum Schott, *Prodr. Syst. Aroid.*: 63 (1860), nom. superfl.

Homaida russelliana (Schott) Baill., *Hist. Pl.* 13: 456 (1895)

Arum gramineum Sol., *Nat. Hist. Aleppo*, ed. 2 [A. Russell] 2: 264 (1794).

Biarum gramineum (Banks & Sol.) Eig, *J. Bot.* 75: 189 (1937).



Figure 20. *B. rifatii* A- habitus, B- flower front view, C- flower reproductive organs, D- leaves, E- cob, F- herbarium specimen H- tuber, J- fruit, K- seeds, L- female flower, M- male flower, N- female flower

Şekil 20. *B. rifatii* A- Habitus, B- Çiçeğin ön görünümü, C- Çiçek üreme organları, D- Yapraklar, E- Koçan, F- Herbarium örneği H- Yumru, J- Meyve, K- Tohumlar, L- Dişi çiçek, M- Erkek çiçek, N- Dişi çiçek



Figure 22. *B. syriacum* A- habitus, B- flower front view, C- flower back part, D- tuber, E- fruit, F- female flower, H- male flower, J- spadix and scape, K-fruit, L-seeds, M twwhole plant, N- part that carries the reproductive organs, O- capsule

Şekil 22. *B. syriacum* A- Habitus, B- Çiçeğin ön görünümü, C- Çiçeğin arka kısmı, D- Yumru, E- Meyve, F- Dişi çiçek, H- Erkek çiçek, J- Spadix ve çiçek sapı, K- Meyve, L- Tohumlar, M- Tüm bitki, N- Üreme organlarını taşıyan kısım, O- Kapsül



Figure 21. Holotype of *Biarum rifatii* (EGE 42437!)
Şekil 21. *Biarum rifatii*'nin holotipi (EGE 42437!)



Figure 23. Holotype of *B. syriacum* (P02133553)

Şekil 23. *B. syriacum*'un holotipi (P02133553)
<http://coldb.mnhn.fr/catalognumber/mnhn/p/p02133553>

Examined specimens: **C6 Gaziantep:** Dülük Baba forest, 8 km of Gaziantep, 1100-1200 m, 19.04.2021, *C. Çeçen & Balos 1168* (HARRAN!), *ibid.*, 01.05.2005, *Akan 5565 & Balos* (HARRAN!); *ibid.*, 23.04.2015, *Yıldırım 3180 & Balos* (EGE!).

Note: *Biarum syriacum* has a very different structure compared to other species. It blooms in the leafy period in spring. The leaves are arranged to surround the flower part. It has no close relatives.

The original of the specimen whose drawing was labeled as Holotype by P. Boyce in the BM herbarium is in the Paris herbarium with the barcode number P02133553. For this reason, the specimen numbered P02133553 was selected as Epitype instead of a drawing.

12. *Biarum tenuifolium* (L.) Schott subsp. *zelebori* (Schott) P.C.Boyce in R.H.A.Govaerts & D.G.Frodin, World Checkl. & Bibliogr. Araceae: 245 (2002) (Figures 24-26) / *Sucukotu*

Lectotype: Türkiye, prope Smyrnam (Izmir). Zelebor s.n. (holotype W destroyed; lectotype W (Schott's leones Aroideae no. 1532). Lectotype designed by Boyce (2006).

Epitype: Türkiye: prope Smyrnam (Izmir). Lydia., Bornmüller, J.F.N. 10033 (B100217078!) Epitype designed here. *Synonyms:*

Biarum zeleborii Schott, Oesterr. Bot. Wochenbl. 7: 245 (1857)

Biarum tenuifolium var. *zelebori* (Schott) Engl., A.L.P.P.de Candolle & A.C.P.de Candolle, Monogr. Phan. 2: 574 (1879)

Examined specimens: **B2 İzmir:** Bornova, 1933, *O. Schwarz 698* (B 100217077 photo!); İzmir, Ilıca, 29.05.1906, *Bornmueller 10033* (K 000400419, photo!); Menderes to Gümlü, Deliömerler district, 1603.2017, *Yıldırım 4256* (EGE!); **Muğla:** Bodrum, Çömlekçi village, 24.06.2001, *Ertuğ (NGBB!)*; **C2 Aydın:** Bozdoğan-Yatağan, 15 km after Bozdoğan, 650-700 m, 25.04.2021, *C. Çeçen 1175 & Balos* (at leaf) (HARRAN!); Aydın, Inside and around the ancient city of Nisa (Nysa), in olive groves, 210-230 m, 25.04.2021, *C. Çeçen 1176 & Balos* (HARRAN!); **Aydın:** Bozdoğan-Yatağan, 10 km after Bozdoğan, 31.03.2017 (at leaf), *Yıldırım 4505* (EGE!); Sultanhisar, Aydın to Nazilli, 03.04.1956, *Davis 25589* (E 00330085, photo!).

Note: The most important feature that distinguishes this species from others is the presence of the sterile area on both the male and female flower parts. In other species, the sterile area is only on the female flowers. The presence of a thick spadix that extends well beyond the spathe is also an important distinguishing feature. It is a highly diverse species with 6 subspecies worldwide. *B. tenuifolium* subsp. *zelebori* is distinguished from other subspecies by its large and wide palm and thicker appendix.



Figure 24. *B. tenuifolium* subsp. *zelebori* A- habitat, B- flower front part, C- flower side position, D- leaves, E- fruit cob and tuber, F- part carrying reproductive organs, H- tuber, J- anthers, K- fruit, L- seed, M- female flower

Şekil 24. B. tenuifolium subsp. *zelebori* A- habitat, B- çiçeğin ön kısmı, C- çiçeğin yan pozisyonu, D- yapraklar, E- meyve koçanı ve yumru, F- üreme organlarını taşıyan kısım, H- yumru, J- anterler, K- meyve, L- tohum, M- dişi çiçek



Figure 25. Epitype of *B. tenuifolium* subsp. *zelebori* (B100217078)
Şekil 25. B. tenuifolium subsp. *zelebori* türü epitipi (B100217078)
<https://herbarium.bgbm.org/object/B100217078>

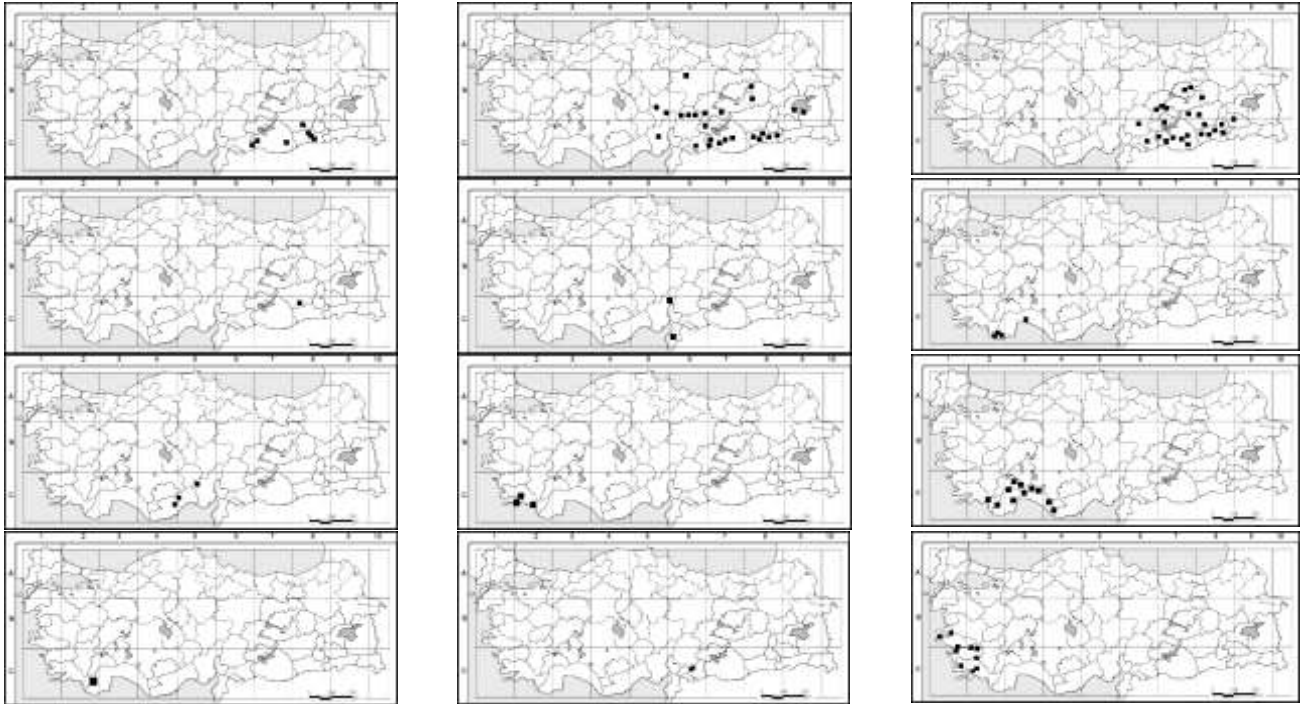


Figure 26. Distribution map of *Biarum* members in Türkiye A) *B. aleppicum* B) *B. bovei* C) *B. carduchorum* D) *B. x cinarense* E) *B. crispulum* F) *B. ditschianum* G) *B. eximium* H) *B. davisii* subsp. *marmarisense* I) *B. pyrami* J) *B. rifatii* K) *B. syriacum* L) *B. tenuifolium* subsp. *zeleborii*

Şekil 26. *Biarum* üyelerinin Türkiye'deki dağılım haritası A) *B. aleppicum* B) *B. bovei* C) *B. carduchorum* D) *B. x cinarense* E) *B. crispulum* F) *B. ditschianum* G) *B. eximium* H) *B. davisii* subsp. *marmarisense* I) *B. pyrami* J) *B. rifatii* K) *B. syriacum* L) *B. tenuifolium* subsp. *zeleborii*

DISCUSSION

In this study, a synopsis of the *Biarum* genus distributed in Türkiye was given. Both population of endemic species was determined and samples that can be known only from the type were collected and taken to herbaria. The diagnostic keys have been rearranged. Each statu of members is given below:

Biarum davisii subsp. *marmariense*, was described as subspecies by Boyce, later elevated it to species level (Boyce, 2006 and 2008). Although he used the appendix width of subsp. *marmarisense* in the distinction, studies at the population level showed that the appendix was not as thin as Boyce stated (0.5 mm), on the contrary, this situation was seen quite rarely, with spadix appendix mostly 2 mm in diameter (Yıldırım, 2018). The specimens examined by us confirmed this situation. Although Boyce (2008) mentioned the existence of *Biarum davisii* subsp. *marmariense* in Greece, Yıldırım (2018) reports that this information needs confirmation.

In this study, it's confirmed that *Biarum pyrami* populations in Türkiye have a blister.

Fruit number, seed dimensions and female flower length were revealed for the first time for *Biarum rifatii* in this study.

In this study, the description of the species *Biarum syriacum* was expanded. Its presence in the Flora of Turkey was noticed by Akan and Balos (2008) when it was collected from Gaziantep. Fruit cob, fruit number and seed sizes were determined for the first time.

For the *Biarum ditschianum*, it was revealed for the first time that the tuber had offspring.

Differences were observed in fruit size and seed dimensions of *Biarum crispulum* when compared with the studies of Yıldırım (2018) and Boyce (2008).

It was seen that there were differences in leaf limb, leaf petiole, scap length, spathe limb, fruit number and seed dimensions for *Biarum pyrami* when compared with the study of Yıldırım (2018) and Boyce (2008). The number of cataphylls was not mentioned in previous studies and was determined for the first time in this study.

It was observed that the tuber size, number of leaves, leaf stalk length, leaf limb, spathe length, spathe limb, spathe tube, appendix, male flower area, fruit cob, number of fruits, and fruit size measurements were higher for *Biarum eximium* than previous studies (Boyce, 2008; Yıldırım, 2018) It was revealed for the first time that the tuber had offspring and the inner surface of the spathe was bright velvety red and had white dots.

It was seen that the style, fruit cob, fruit size, fruit number, and seed dimensions of *B. tenuifolium* subsp. *zeleborii* were not given by Yıldırım (2018) and Boyce (2008). When compared with Boyce (2008), it was seen that there were differences in the measurements. The main reasons for the differences may be the effect of different habitats, the number of samples studied, and the result of working on dried samples.

The holotype of *Biarum eximium* in Vienna has disappeared; a lectotype was determined from the isotypes by Yıldırım (2018). Boyce (2008) states that *Biarum eximium* should be collected from Adana, the type locality. Although Boyce (2008) stated that this species was found in Jordan based on the Dinsmore 11725 (E 00330074, photo!) specimen, Yıldırım (2018) stated that this specimen is not *B. eximium* but it should be a variation of *B. bovei* because the structure of the spathe palm is narrowly lanceolate, and the intermediate region is not completely covered with sterile flowers. In our examination, it was seen that this specimen was not *B. eximium* as stated by Yıldırım (2018), and in addition, the leaf structure and style were different from those of *B. eximium*. Boyce stated that a specimen (Al-Eisawi 8861) collected by Al-Eisawi (1981) from 50 km south of Amman and located in the Jordan University Herbarium (AMM) could be *B. eximium*. He also stated that he had not seen this specimen but examined the fresh plants collected by Salmon and Lovell in 1988, who were very close to the same region. He desired that the species *B. eximium* should be collected especially from Adana, Türkiye. Based on the data we obtained, it was seen that the literature records by Boyce (2008) remained far from *B. eximium*. 170 years later, with this study, this confusion was eliminated. Measurements were made on our fresh samples collected from Adana province, and their description was expanded.

Biarum kotschyi was made a synonym of *Biarum bovei* by Yıldırım (2018).

Biarum × *cinarense* was published as a new natural hybrid from Türkiye by Çeçen et al. (2022), and it is added to the identification key in this study.

The holotype of the *Biarum carduchorum* has become extinct. The lectotype was determined by Boyce (2006). Observations and examinations of collected specimens showed that the species showed a wide variation, especially the length of the spathe limb and spadix, and the length and density of the staminodes in the sterile region were different. In some specimens, it is similar to *B. angustatum* in terms of the staminodes being directed downwards. In some specimens, both the downward and upward staminodes are in the same individual, making species identification difficult. In some collected specimens, it was observed that the outer surface of the spathe limb was canalized, and the male flower region was purplish rather than yellow. A phylogenetic population-level study is needed on *B. carduchorum*.

Some taxonomical notes obtained from this study are given for some taxa and compared with some previous studies, are given below:

The comparison of the findings of the *B. aleppicum* species with the studies of Yıldırım (2018) and Boyce (2008) is given in Table 1.

Tuber offspring, fruit cob, fruit number, fruit size, and seed measurement of *Biarum aleppicum* were not given in the study of Yıldırım (2018). It was observed that the fruit diameter and length were larger, and the style length was longer than in the study of Boyce (2008). Other differences related to *B. aleppicum* are given in Table 2.

The comparison of the *B. bovei* with previous studies is given in Table 2.

Table 1. Comparison of morphological findings for *B. aleppicum* with previous studies

Çizelge. *B. aleppicum* için morfolojik bulguların önceki çalışmalarla karşılaştırılması

Characters / Karakterler	This study / Bu çalışma	Yıldırım (2018)	Boyce (2008)
Tuber offspring / Tuber yavruları	present	not given	not given
Cataphyll number / Katafil sayısı	4-25	4-20	5-7
Spadix length (cm) / Spadiks uzunluğu (cm)	7-16	10-12	11-12
Sterile flower area length (mm) / Steril çiçek alanı uzunluğu (mm)	13-20	14-16	16
Male flower area length (mm) / Erkek çiçek alanı uzunluğu (mm)	10-24	12-15	
Fruit cob (diameter x length cm) / Meyve koçanı (çap x uzunluk cm)	2-5 x 2.5-3.5	-	c. 12
Fruit number / Meyve sayısı	20-40	-	c.2 cm dia.
Fruit size (diameter x length mm) / Meyve boyutu (çap x uzunluk mm)	9-11 x 14-16	-	4 x 5
Seed (diameter x width mm) / Tohum (çap x genişlik mm)	5 x 6	-	5 x 5-6

Table 2. Comparison of morphological findings for *B. bovei* with previous studies

Çizelge 2. *B. bovei* için morfolojik bulguların önceki çalışmalarla karşılaştırılması

Characters / Karakterler	This study Bu çalışma	Yıldırım (2018)	Boyce (2008)
Leaf stalk (length/cm) / Yaprak sapı (uzunluk/cm)	7-19	8-16	5-9
Scape length (cm) / Skapoz uzunluğu (cm)	3-17	3-14	4-7
Spadix length (cm) / Spadix uzunluğu (cm)	10-16	7-15.5	9-14
Female flower (length x width / mm) / Dişi çiçek (uzunluk x genişlik / mm)	3-3.2	-	-
Fruit cob (diameter x length / cm) / Meyve koçanı (çap x uzunluk / cm)	5-5.5	-	2.5-3
Fruit size (Diameter x length / mm) / Meyve boyutu (Çap x uzunluk / mm)	13-16 x 9-11	-	7 x 4

The fruit and seed sizes, and the style length of *B. bovei* were not given in the study of Yıldırım (2018). It was observed that the fruit cob diameter was larger, the diameter and length of the fruit were larger, and there were

differences in seed dimensions. Female flower dimensions were revealed for the first time.

Biarum members in Türkiye are generally spread on stony areas and open slopes, the altitudes vary (10-2100 m), their flowering periods generally occur in autumn, spring, and early summer months, 75% of the taxa are in the Mediterranean and 25% in the Irano-Turan regions as phytogeographic regions.

The short history of the genus in Türkiye is given in Table 3.

Table 3. Short history of *Biarum* genus in Türkiye

Çizelge 3. Türkiye'de *Biarum* cinsinin kısa tarihi

Name of study/çalışma adı	Taxa numbers Takson sayısı	Taxa names/Takson adı
Flora of Turkey 8 th vol (Mill, 1984) <i>Türkiye Florası 8.cilt (Mill, 1984)</i>	6	<i>Biarum tenuifolium</i> subsp. <i>zelebori</i> , <i>B. davisii</i> , <i>B. carduchorum</i> , <i>B. eximium</i> , <i>B. pyrami</i> and <i>B. bovei</i> .
A new subspecies of <i>Biarum</i> from Turkey (Boyce, 1987). <i>Türkiye'den yeni bir Biarum alttürü (Boyce, 1987)</i>	1 taxa added	<i>Biarum davisii</i> subsp. <i>marmarisensis</i>
A remarkable new <i>Biarum</i> from Türkiye (Bogner and Boyce, 1989) <i>Türkiye'den dikkate değer yeni bir Biarum (Bogner ve Boyce, 1989)</i>	1 species added	<i>Biarum ditschianum</i>
A new record of <i>Biarum</i> for Flora of Türkiye (Akan and Balos, 2008) <i>Türkiye Florası için Biarum'un yeni bir kaydı (Akan ve Balos, 2008)</i>	1 species added	<i>Biarum syriacum</i>
A taxonomic revision of <i>Biarum</i> (Boyce, 2008) <i>Biarum'un (Boyce, 2008) taksonomik revizyonu</i>	1 new stat. nov.	stat. nov. for <i>Biarum davisii</i> subsp. <i>marmarisense</i> (Become synonym of <i>Biarum marmarisense</i>)
A new record for Türkiye (Yıldırım et al., 2016) <i>Türkiye için yeni bir rekor (Yıldırım ve ark., 2016)</i>	1 species added	<i>Biarum aleppicum</i>
A new <i>Biarum</i> species from Türkiye (Yıldırım and Altıoğlu, 2016) <i>Türkiye'den yeni bir Biarum türü (Yıldırım ve Altıoğlu, 2016)</i>	1 species added	<i>Biarum rifatii</i>
The Illustrated Flora of Türkiye (Yıldırım, 2018) <i>Resimli Türkiye Florası (Yıldırım, 2018)</i>	1 new stat. nov.	<i>Biarum davisii</i> subsp. <i>marmarisensis</i> was accepted at subspecies level again.
The Illustrated Flora of Türkiye (Yıldırım, 2018) <i>Resimli Türkiye Florası (Yıldırım, 2018)</i>	1 new stat. nov.	stat. nov. for <i>Biarum kotschyi</i> (Become synonym of <i>B. bovei</i>)
A new natural hybrid from Türkiye (Çeçen et al., 2022) <i>Türkiye'den yeni bir doğal melez (Çeçen ve ark., 2022)</i>	1 species added	<i>Biarum</i> × <i>cinarense</i>

As can be seen from Table 1, after taxonomical changes that have occurred since the publication of Flora of Turkey, *Biarum* genus has 12 taxa in Türkiye now.

Five taxa of the genus are endemic, and the endemism rate is 41.6% in Türkiye. These endemic members are *Biarum* × *cinarense*, *B. ditschianum*, *B. davisii* subsp. *marmarisense*, *B. rifatii* and *B. eximium*. Due to the smaller number of individuals in locations and populations, they should be evaluated in the “EN” risk category. Among the non-endemic members, *B. aleppicum*, *B. bovei*, and *B. carduchorum* should be evaluated in the “LC” risk category; *B. eximium*, *B. crispulum*, *B. ditschianum*, and *B. syriacum* should be evaluated in the “EN” risk category; *B. pyrami* and *B. tenuifolium* subsp. *zelebori* should be evaluated in the “NT” category.

It is believed that this study makes contributions to the Illustrated Flora of Türkiye and provides additional notes to the *Biarum* genus in Türkiye.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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Effects of Microbial Fertilizer Applications on Yield, Fruit Quality and Leaf Characteristics of Red Chief Apple Variety

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ABSTRACT

The research was carried out on Red Chief apple variety grafted onto MM106 rootstock in the apple orchard established in 2011 at Yozgat Bozok University in 2022-2023. The study was established to determine the effects of Control (100% fertilizer), 50% reduced fertilizer, 100% reduced fertilizer (without fertilizer), and combinations of fertilizers with microbial fertilizer containing bacteria on fruit yield and quality and vegetative growth. The highest fruit weight was obtained from Control (100% fertilizer dose) + Bacteria application (170.57 g) and 50% reduced fertilizer dose + Bacteria (164.30 g) applications. The lowest fruit weight was determined from a 100% reduced fertilizer dose (without fertilizer) (103.18 g). Total soluble solids were measured with a 20.30% Brix value in the Control (%100 fertilizer) + bacteria and a 19.90% brix value in 50% reduced + bacteria applications. The highest titratable acidity was determined with 0.59% in Control (%100 fertilizer) application. The fruits from the trees of each replication were collected separately and the highest tree yield values were obtained from Control (%100 fertilizer) + bacteria (22.35 kg/tree) and Control (%100 fertilizer) applications (19.63 kg/tree). When the effects of the applications on shoot length were examined, the highest values were determined in Control (%100 fertilizer), Control (%100 fertilizer) + bacteria, and 50% reduced fertilizer + bacteria applications. The highest amount of nitrogen (N) in the leaf was determined in Control (100% fertilizer), Control (100% fertilizer) + bacteria, and 50% reduced + bacteria applications. The highest amount of P was obtained from Control (100% fertilizer) and Control (100% fertilizer) + bacteria applications. The highest amount of K was measured in 50% reduced fertilizer application. It was concluded that microbial fertilizers containing plant growth-promoting bacteria can be used in apples. Replication of the study in different climatic and geographical conditions may increase the extensibility of the results.

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Keywords

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ÖZET

Araştırma, 2022-2023 yıllarında Yozgat Bozok Üniversitesi'nde 2011 yılında kurulmuş olan elma bahçesinde yer alan MM106 anacına aşıllı Red Chief elma çeşidi üzerinde yürütülmüştür. Çalışma, kontrol (%100 gübre), %50 azaltılmış gübre, %100 azaltılmış gübre (gübresiz) ve gübrelerin bakteri içeren mikrobiyal gübre ile kombinasyonları kullanılarak meyve verim ve kalitesi ile vejetatif büyüme üzerine etkilerini belirlemek amacıyla kurulmuştur. Meyve ağırlığı en yüksek kontrol (%100 gübre dozu)+bakteri uygulaması (170.57 g) ile %50 azaltılmış gübre dozu+bakteri (164.30 g) uygulamalarından elde edilmiştir. En düşük meyve ağırlığı ise %100 azaltılmış gübre dozundan (gübresiz) (103.18 g) tespit edilmiştir. Meyve eti sertliği (kg/cm²), en yüksek kontrol (%100 gübre), %50 azaltılmış gübre ve %100 azaltılmış (gübresiz) uygulamalarında ölçülmüştür. Suda çözünabilir kuru madde içeriği %20.30 Brix değeri ile kontrol (%100 gübre) + bakteri ve %19,90

Bahçe Bitkileri

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 05.01.2025

Kabul Tarihi : 06.05.2025

Anahtar Kelimeler

Red Chief elma çeşidi

Mikrobiyal gübre

Kimyasal gübre

Yozgat

Brix değeri ile %50 azaltılmış + bakteri uygulamalarında ölçülmüştür. Titre edilebilir asit miktarı ise en yüksek %0.59 ile kontrol (%100 gübre) uygulamasında belirlenmiştir. Her tekerrüre ait ağaçlardaki meyveler ayrı ayrı toplanarak elde edilen ağaç başı verim değerleri en yüksek kontrol (%100 gübre)+bakteri (22.35 kg/ağaç) ve kontrol (%100 gübre) uygulamalarından (19.63 kg/ağaç) elde edilmiştir. Uygulamaların sürgün boyu üzerine etkisi incelendiğinde en yüksek değerler kontrol (%100 gübre), kontrol (%100 gübre)+ bakteri ve %50 azaltılmış gübre+bakteri uygulamalarında tespit edilmiştir. Yapraktaki azot (N) miktarı en yüksek kontrol (%100 gübre), kontrol (%100 gübre) + bakteri ve %50 azaltılmış + bakteri uygulamalarında tespit edilmiştir. P miktarı en yüksek kontrol (%100 gübre) ve kontrol (%100 gübre) + bakteri uygulamasından elde edilmiştir. K miktarı en yüksek %50 azaltılmış gübre uygulamasında ölçülmüştür. Bitki gelişimini teşvik eden bakterileri içeren mikrobiyal gübrelerin elmalarda kullanılabileceği sonucuna varılmıştır. Çalışmanın elma yetiştirilen farklı iklim ve bölgelerde tekrarlanması sonuçların yaygınlaştırılabilirliğini artıracaktır.

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INTRODUCTION

The apple, a plant of cold temperate climates generally between 30°-50° latitude in the world, belongs to the genus *Malus* within the subfamily Pomoideae of the family Rosaceae. The number of species included in the genus *Malus* varies from author to author. Apples are grown on all continents of the world, but the Asian continent accounts for 66.28% of world apple production, with 63,522,168 tonnes. The European continent, with 18,794,929 tonnes of production, is in second place after the Asian continent, accounting for 19.61% of world production. America is in third place with 8,857,607 tonnes, and Africa is in fourth place with 3,785,189 tonnes. The lowest production was in Oceania with 876,071 tonnes (FAO, 2024). According to the FAO, world apple production in 2022 will be 95,835,964 tonnes. China ranks first in production with 47,571,800 tonnes, followed by Turkey (4,817,500 tones), America (4,429,330 tonnes), Poland (4,264,700 tonnes), India (2,589,000 tonnes), Russia (2,379,900 tonnes), and Italy (2,256,240 tonnes).

The growth and development of a plant vary according to climate and region. Phenological observations of plants include dates such as bud swelling, bud burst, first flowering, full flowering, end of flowering, harvest date, and leaf fall date (Rana et al., 2023). As a result of these observations, decisions are made on issues such as agricultural techniques and planning, cultural practices, selection of suitable varieties, and pollinator species in the geographical region where the plant is located. Chemical fertilizers used in excessive amounts and unknowingly in cultivation cause a decrease in product quality and environmental pollution (Güneş et al., 2012; Shuqin & Fang, 2018; Rahman & Zhang, 2018). The use of fertilizers to increase fruit yield and quality in our country and in the world pollutes the environment to a great extent. For this reason, in the studies conducted, researchers are trying applications that increase yield and quality by reducing the use of chemical fertilizers and minimizing the damage to the environment (Ertürk, 2015; Tian et al., 2022).

Organisms living in the soil have a very important place in terms of soil fertility. Soil organisms consist of soil flora and soil fauna. Fungi, bacteria, actinomycetes, and algae are included in the soil flora. Protozoa, nematodes, earthworms, and other animals are included in the soil fauna. They have different functions in increasing soil fertility. Bacteria play a role in the breakdown of organic matter and the nitrogen cycle (Karaçal & Tüfenkçi, 2010; Wang et al., 2020). As a result of many studies conducted on different applications and costs that do not harm nature and reduce the use of chemical fertilizers, the use of microorganisms stands out. Bacteria found in the soil that promote plant growth are called biofertilizers. It has been determined that bacteria provide nitrogen use, phosphorus solubility, plant hormone production, and water use efficiency as a result of nitrogen fixation, increase the uptake of nutrient elements by the plant, and enzymatically reduce the level of ethylene and positively increase plant growth (Ekici et al., 2015). The use of microorganisms and their new combinations as microbial fertilizers has important consequences in agriculture (Kucharski et al., 1996; Vessey, 2003). Microbial fertilizers are used to

increase plant growth and development and productivity by increasing nutrient uptake, to improve soil structure and productivity, to help break down organic residues, and to provide resistance to diseases and pests (Çakmakçı, 2005).

With the application of reduced doses of chemical fertilizers together with microbial fertilizers, it is aimed to increase the efficiency and fertilizer use efficiency by using natural, biological, and man-made plant nutrient sources without harming the environment (Gruhn et al., 2000). Another negative effect of excessive fertilizer use is that it increases the unit cost of agricultural products. The main goal of the enterprises is to obtain a profit that will ensure the continuity of the enterprise in agricultural activities. In order to obtain high yield and quality from fruit species such as apple, they need macro elements such as N, P, and K from the soil. For this reason, producers use large amounts of fertilizer in apple cultivation. There are variable costs such as pruning, soil processing, irrigation, fertilization, spraying, thinning, harvesting, transportation, insurance, and revolving fund interest in the cultivation of fruit species such as apple. In a study conducted on apples in Isparta and Karaman, it was determined that these costs constituted approximately 70% of the total costs. Among these, it has been determined that fertilization costs constitute the third largest cost item with a rate of approximately 8-10% after spraying (20%) and harvesting (17%) (Bayav & Karli, 2020).

In today's modern fruit growing and with the existing varieties, it is not possible to grow without using chemical fertilizers. However, the use of chemical fertilizers by plants is mostly less than 50%, and this rate even decreases to 10% in P. Therefore, at least half of the applied fertilizer remains in the soil, mixes with groundwater, or is lost to the atmosphere as gas. Accordingly, continuous use of chemical fertilizers has many negative effects. Continuous use of chemical fertilizers negatively affects the physical, chemical, and biological properties of the soil and reduces soil fertility. It plays a significant role in environmental pollution. In addition, it contributes to global climate change by increasing the levels of greenhouse gases in the atmosphere. In addition, as soil conditions deteriorate, growers tend to use more fertilizer to achieve the desired yield, quality, and growth, exacerbating the negative effects. This situation leads to an increase in negative effects in a vicious circle. In addition to having such negative effects, increased fertilizer use also increases the unit cost of the product and reduces the producer's profitability. Therefore, developing applications that will increase the usability of fertilizers by plants is very important in reducing these negativities. In order to ensure sufficient vegetative growth and high yield and quality in apple cultivation, a very intensive fertilizer application is made. Accordingly, serious contributions are made to the negativities mentioned due to intensive fertilizer use during apple cultivation (Abebe et al., 2022).

It has been reported that plant growth-promoting rhizobacteria promote vegetative development in different plant species such as apple, apricot, barley, lettuce and tomato, blueberry, hazelnut, grape, cherry (Bassil et al, 1991; Rodriguez and Fraga, 1999; De Silva et al., 2000; Eşitken et al., 2003; Köse et al., 2003; Eşitken et al., 2006; Aslantaş et al., 2007; Pırlak et al., 2007; Ekici et al., 2015). Parılı (2018) observed that root application of *Bacillus subtilis* OSU-142 and *Bacillus megaterium* M-3 bacterial strains to some apple seedlings grafted on MM106 rootstock resulted in a significant increase in vegetative development of the seedlings. Kotan et al. (2021) reported that in their study using microbial fertilizer containing *Bacillus subtilis* in the cultivation of apple seedlings, the use of bacteria increased shoot length by 65.09% and shoot diameter by 22.66% compared to the control. Yaman et al. (2022) found the shoot length as 34.45 cm and 26.50% positive bacterial effect in the Red Chief / M9 combination, and as 31.88 cm and 25.53% positive bacterial effect in the Red Chief / MM106 combination.

The aim of this research is to reduce the amount of fertilizer used by applying microbial fertilizers without reducing plant growth, development, fruit yield, and quality, thus reducing the negative impact on the environment, human health, and the country's economy.

MATERIAL and METHOD

The research was carried out on the Red Chief apple cultivar grafted onto the MM106 rootstock in the apple orchard established in 2011 at Yozgat Bozok University, Gedikhasanlı Bilal Şahin Agricultural Application and Research Center (TUAM). The variety is of American origin and has weak, semi-upright trees. Its medium-sized fruits are sweet and juicy. The color of the fruit is creamy white. The outer shell color is bright red on a yellow-green base. The harvest is done in the last week of September (Anonymous, 2023a).

Yozgat is located between 34°05' - 36°10' east meridians and 38°40' - 40°18' north parallels. Its altitude above sea level is 1300 meters, and it ranks 15th among 81 provinces in terms of land width, with its 1.412.300 hectares of land. The altitude decreases from east to west of the province (Anonymous, 2023b).

The climate characteristics of the orchard located in Songun district of Yozgat province, where the research was conducted, show the typical continental climate characteristics of the Central Anatolia Region. When the meteorological data covering the years 2022-2023 of the region, where summers are hot and dry, winters are cold and rainy, are examined, it is seen that the highest monthly average temperature was 23.7°C in August 2022, and

the lowest was -2.3°C in January 2022. In 2023, the highest average temperature was measured as 23.6°C in August, and the lowest was -0.7°C in February. The average relative humidity in 2022 was 64.28%; and 64.53% in 2023. In 2022, the highest monthly average temperature was measured as 38.1°C in July, and the lowest was -21.2°C in January 2022. The lowest temperature value recorded in 2023 was recorded in February with -12.5°C , and the highest monthly average temperature value was recorded in August with 36.9°C (Figure 1 and Figure 2).

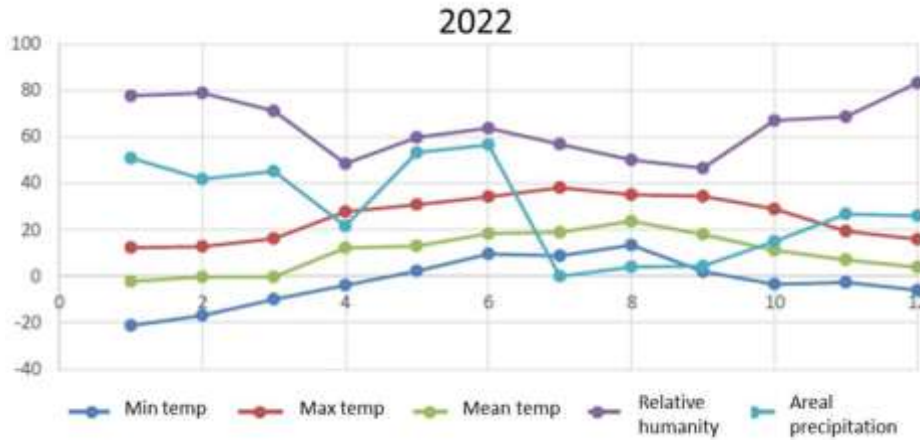


Figure 1. Meteorological Data of the Research Area for 2022

Şekil 1. Araştırma Alanının 2022 Yılı Meteoroloji Verileri

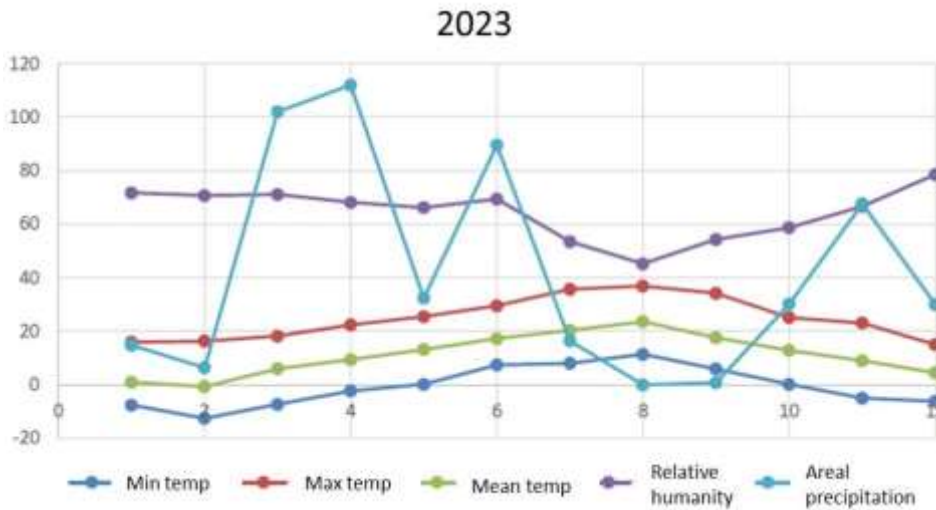


Figure 2. Meteorological Data of the Research Area for 2023

Şekil 2. Araştırma Alanının 2023 Yılı Meteoroloji Verileri

As a result of the analysis of soil samples taken from the field where the research was carried out, it was determined that the average pH was 7.8, the amount of lime was 16%, and the organic matter was below 1%. It was determined that the soils were poor or very poor in terms of organic matter, total nitrogen, available phosphorus, iron, zinc, and magnesium, and were generally clayey, slightly alkaline, and contained high levels of lime and Ca (Table 1).

In the experiment, the amounts of N, P, and K fertilizer used by the Agricultural Application and Research Centre where the study is carried out were used as a control, and in addition 50% and 100% reduced doses of these amounts were applied to the crown projection areas of the trees in April. A commercial preparation was used, Lactic acid bacteria (*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*), Phototrophic Bacteria (*Rhodopseudomonas palustris*), and *Bacillus subtilis* containing as bacterial material. This product is ready for use in agriculture and is applied to the soil.

The first application of the bacterial solution to be used in the study was made in April, when the temperature reached the point at which the roots would start to work actively. In addition, the application was repeated in June and July. In the second year of the experiment, the applications were repeated in the same period. Bacterial applications were applied as a solution to the crown projection of the trees, 1000 ml into the root rhizosphere from 8 different points opened with pipes (Figure 3).

Table 1. Soil Properties of the Experimental Area
Çizelge 1. Deneme Alanının Toprak Özellikleri

Depth (cm)	Analysis- Unit	Structure	pH	Salinity-EC %	Lime %	Organic Matter %	Total Nitrogen %	P ₂ O ₅ (kg/da)	K ₂ O (kg/da)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
0-20	Result	66.0 Clayey Loam	7.73 Slightly Alkaline	0.02 Salt-Free	10.41 Medium	0.83 Very Little	0.04 Very Poor	1.96 Very Little	41.65 Excessive	1.06 Insufficient	1.16 Sufficient	0.59 Sufficient	2.10 Sufficient	7.92 Excessive	155.1 Little	19.10
20-40	Result	69.3 Clayey Loam	7.80 Slightly Alkaline	0.02 Salt-Free	16.82 Excessive	0.57 Very Little	0.03 Very Poor	0.97 Very Little	24.3 Medium	1.02 Insufficient	0.10 Very Little	0.32 Sufficient	2.12 Sufficient	7.55 Excessive	128.4 Little	20.90
40-60	Result	66.0 Clayey Loam	7.81 Slightly Alkaline	0.02 Salt-Free	20.18 Excessive	0.66 Very Little	0.03 Very Poor	0.82 Very Little	20.15 Medium	1.13 Insufficient	0.12 Very Little	0.22 Sufficient	2.39 Sufficient	7.39 Excessive	130.3 Little	20.20

Experiment applications were presented below:

1. Control (100% fertilizer level, farm application, 8 kg/da N, 6 kg/da P₂O₅, 10 kg/da K₂O)
2. 50% reduced (4 kg/da N, 3 kg/da P₂O₅, 5 kg/da K₂O)
3. 100% reduced (without fertilizer)
4. Control (100% fertilizer level, farm application, 8 kg/da N, 6 kg/da P₂O₅, 10 kg/da K₂O) + bacteria application
5. 50% reduced (4 kg/da N, 3 kg/da P₂O₅, 5 kg/da K₂O) + bacteria application
6. 100% reduced (without fertilizer) + bacteria application

In the study, observations of phenological periods (bud swelling, bud burst, first flowering, full bloom, end of flowering, harvest date, leaf fall date) were taken. For pomological analyses, measurements and analyses were carried out using 20 randomly selected fruits from each replication. The measurements of fruit weight (g), fruit width (mm), length (mm), yield (kg.tree⁻¹), total soluble solids (brix, %), total acidity (%), fruit firmness (kg.cm⁻²), and fruit skin color obtained from the applications in the study were determined.

In order to determine the vegetative development of the trees, samples were taken from the middle part of the shoots in August, from the leaves that had reached full size, in three replications, with 20 leaves in each replication. In order to determine the shoot characteristics, shoot characteristics were measured in October, when the trees entered dormancy. Leaf chlorophyll content readings (measured using the Konica Minolta SPAD-502 Plus Brand Chlorophyll Meter, SPAD), leaf anthocyanin amount (measured by (Opti Science ACM-200 Plus Anthocyanin Meter, ACD), leaf area determination (determined by ADC Bio Scientific Area Meter AM300 device, cm²), Leaf Fresh Weight (g), Leaf Dry Weight (g) and Specific leaf weight (g.cm⁻²) were measured.



Figure 3. Application of bacterial solution
Şekil 3. Bakteri solüsyonunun uygulanması

To determine the effect of the applications on macro-micronutrient content in leaves, N (%), P (%), K (%), Mg (%), Ca (%), Fe (ppm), Cu (ppm), Zn (ppm) and Mn (ppm) were measured. Leaf samples were first prepared for analysis after cleaning, drying, and grinding processes (Kacar, 1994). Except for the nitrogen element, other analyses were carried out on plant samples obtained by the wet combustion method. Nitrogen analysis was found as the percentage total N value in plant samples by Kjeldahl method. The phosphorus amount was measured by Spectrophotometer with vanodomolybdo phosphoric yellow color method (Lott et al., 1956), magnesium, calcium, and potassium amounts were measured by Atomic Absorption Spectrometer, and the results were given as percentage (Kacar, 1972). Manganese, copper, iron, and zinc amounts were also given as mg.kg^{-1} (ppm) as a result of measurements made by Atomic Absorption Spectrometer (Lindsay & Norwell, 1978).

Statistical Analysis

In the study, phenological observations, fruit pomological characteristics, effects of applications on vegetative development, and macro-micronutrient element content in the leaf were investigated. The experiment was established using 6 applications, 3 replicates, and 3 trees in each replicate, totaling 54 trees. Irrigation was done by the drip irrigation method in all plots. Descriptive statistical methods, such as mean and standard deviation, were used to analyze each result. Results were expressed as mean $\pm$ standard deviation of three replicates, and differences between groups were distinguished at a significance level of $p \leq 0.05$. P values less than 0.05 were considered statistically significant at a 95% confidence interval. Statistical evaluation of the results obtained from each application was made according to variance analysis (ANOVA) and Duncan multiple comparison test in SPSS package program (version 20.0, SPSS Inc., Chicago).

RESULTS and DISCUSSION

Phenological observations were recorded from the bud swelling to leaf fall period.

Table 2. Phenological observations of Red Chief apple variety

Çizelge 2. Red Chief elma çeşidinin fenolojik gözlem sonuçları

Phenological Periods	2023
Bud swelling	08-10 April
Bud break	14-16 April
First bloom	20-24 April
Full bloom	25-29 April
End of bloom	29 April-1 May
Harvest date	5-10 October
Leaf fall date	11-25 November

In all applications, bud swelling started on April 8, and buds burst 6 days later. First flowering started 6 days after bud break and ended on May 1. Fruit harvest was made 164-169 days after full flowering, between October 5-10. Leaves started to turn yellow at the end of October, leaf fall started on November 11 and was completed on November 25. No effect of the applications on the phenological periods of the trees was observed.

Environmental conditions affect the phenological characteristics, fertilization biology, and pomological characteristics of apples. Therefore, in order to determine suitable cultivars for the region, the adaptation of different cultivars to the region should be investigated, and the cultivars should be selected according to the results obtained from this (Özbek, 1978). In his study in Tokat, Baytekin (2006) determined the bud break of the Red Chief variety on March 12, first flowering on April 21, full flowering on April 28, and end of flowering on May 3. In the

study on the "Red Chief" apple variety in Niğde, bud swelling was determined as March 25, bud break as March 31, beginning of flowering as April 16, full flowering as April 22, end of flowering as April 28 and harvest date as September 20, and the period from full flowering to harvest was determined as 151 days (Ceylan, 2008). In his study conducted in Elmalı, Antalya, Arıkan (2020) stated that the bud swelling of the Red Chief apple cultivar occurred on March 17, the beginning of flowering on April 2, full bloom on April 7, and the end of flowering on April 17. In previous studies (Akgül & Başayığit 2005; Ekinci 2010), it was determined that the Red Chief apple variety came to harvest in approximately 140-155 days. Kırac (2016) determined the harvest date as October 14 in his study on the 'Red Chief' cultivar. It was reported that the harvest was made 163-169 days after full bloom in Kahramanmaraş conditions. Arıkan (2020) states that the harvest was made on September 27 in Elmalı, Antalya, 173 days after full bloom. Climatic conditions and altitude are effective on fruit ripening time.

Fruit pomological characteristics

For pomological analyses, measurements and analyses were carried out using 20 randomly selected fruits from each replication. The measurements of fruit weight, fruit width, length, and fruit skin color obtained from the applications in the study are given in Table 3. It was determined that the difference between the means of all these characteristics was statistically significant ($p<0.05$).

Table 3. Fruit weight, width, length, and fruit skin color values according to the different applications

Çizelge 3. Uygulamalardan elde edilen meyve ağırlığı, meyve eni, meyve boyu ve meyve kabuğu rengi değerleri

Applications	Fruit Weight (g)*	Fruit Width (mm)*	Fruit Leng (mm)*	Fruit Skin Color		
				L*	a*	b*
1. Control	149.28±8.3 b	78.45±3.9 a	74.37±2.5 c	33.80±3.4 bc	22.13±3.9 b	7.53±2.2 b
2. 50% reduced	149.08±14.0 b	79.04±44.1a	74.94±3.9 bc	35.88±2.8 ac	27.28±4.3 a	9.37±1.6 a
3. 100% reduced	103.18±7.1 d	67.53±1.9 c	63.74±2.8 d	36.50±2.8 ab	22.65±3.1 b	9.31±1.5 a
4. Control + bacteria	170.57±16.2 a	80.06±3.0 a	79.36±3.2 a	32.92±3.4 c	23.03±3.2 b	7.41±2.1 b
5. 50% reduced+ bacteria	164.30±11.9 a	81.03±2.9 a	77.70±3.8 ab	35.47±3.2 ac	25.01±3.1 ab	9.26±1.2 a
6. 100% reduced+bacteria	135.69±9.6 c	74.49±2.0 b	74.95±3.9 bc	36.90±2.8 a	25.09±2.4 ab	9.97±1.5 a

*There is a statistical difference between the means shown with different letters in the same column ($p<0.05$).

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır ($p<0,05$).

The highest fruit weight was obtained from Control (100% fertilizer dose) + Bacteria application (170.57 g) and 50% reduced fertilizer dose + Bacteria (164.30 g) applications. The lowest fruit weight was determined from 100% reduced fertilizer dose (without fertilizer) (103.18 g).

Karakurt (2006) determined the effects of *Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7, and *Pseudomonas putida* BA-8 bacterial strains on plant development, fruit set, fruit characteristics, and plant nutrient content of some 11-year-old apple cultivars grafted onto semi-dwarf (MM-106) rootstocks. It was determined that A-18 application caused an increase in fruit weight in Granny Smith and Starking Delicious cultivars, while other bacterial applications caused a decrease.

Baytekin & Akça (2011) found the average fruit weight of a 3-year-old Red Chief variety grafted on MM106 rootstock to be 235.80 g, Arıkan et al. (2015) found the average fruit weight of a 5-year-old Red Chief tree grafted on M26 rootstock to be 176.5 g, Öztürk et al. (2011) found the average fruit weight of a Red Chief variety grafted on M26 rootstock to be 167.42-180.54 g. Kırac (2016) reported that fruit weights of the Red Chief apple variety varied between 84.8-176.5 g depending on the rootstocks, growing conditions, and age of the tree. Arıkan (2020) reported that the average fruit weights of the Red Chief apple variety were between 96.51-248.91 g (average 180.51 g) in their study. In the Yahyalı district of Kayseri province, Yaman et al. (2022) found the fruit weight of the Red Chief/M9 combination was 158.57 g, while the fruit weight of the Red Chief/MM106 was measured as 170.09 g. The effect of the bacterial application on fruit weight was positive, and this effect was found to be 6.18% in the M9 grafted and 14.51% in the MM106 grafted. The different sizes of the fruits vary according to genetics, cell number, intercellular space, cell size, climate, number of fruits on the tree, cultivation practices, and photosynthetic capacity of the trees.

In fruit growing, skin color is one of the important quality parameters. The ripening, health, decay, and harmful effects of the fruit can be understood by the color changes in the fruit skin. Color also affects the marketing of the products. Apple skin color is affected by many factors such as irrigation, nutrition, plant diseases, climate, and fruit set. In addition to these factors, day and night temperature differences are other factors affecting the fruit skin (Lakatos et al. 2012). In terms of L value, which determines fruit skin color brightness, the lowest was measured in Control (%100 fertilizer dose) +Bacteria (32.92) and Control (%100 fertilizer dose) (33.80) doses, while other applications were higher. The highest a value, which indicates a red color, was measured in 50% reduced

fertilizer (27.28); 50% reduced fertilizer+bacteria (25.01) and 100% reduced fertilizer+bacteria (25.09) applications. The lowest b value, which indicates a yellow color, was obtained from Control (%100 fertilizer dose) (7.53) and Control (%100 fertilizer dose) +Bacteria applications (7.41). Fruit skin colors of Red Chief apple were measured as L=34.63, a=25.94 and b=10.30 by Küçük (2010); L=34.70, a=27.26, b=8.73 by Ekinci (2010); L=39.99, a=26.13, b=16.79 by Baytekin & Akça (2011).

The measurements and analyses of the fruit firmness, total soluble solids (TSS), titratable acidity and yield per tree values obtained from the applications in the trial are given in Table 4. The difference between the means of these characteristics was found to be statistically significant ($p<0.05$).

Fruit firmness is one of the factors affecting the storage of fruits (Ekinci 2010; Öztürk et al. 2011; Kaynaş et al. 2011). Fruit firmness (kg/cm²) was determined in the highest bacteria-uncombined Control (100% fertilizer), 50% reduced fertilizer, and 100% reduced (without fertilizer) applications. The lowest fruit firmness was obtained from the Control (100% fertilizer) + bacteria application with 6.50 kg/cm². The average fruit firmness of Red Chief apple was determined as 11.62 lb (5.27 kg/cm²) by Arıkan et al. (2015), 8.82 lb (4.00 kg/cm²) by Satıcı (2011), and between 15.58 and 17.94 lb by Öztürk et al. (2011). Arıkan (2020) measured the average fruit firmness as 7.18 kg/cm² (15.82 lb). The values obtained were found to be above the values found by Arıkan et al. (2015) and Satıcı (2011), and similar to the values of Öztürk et al. (2011) and Arıkan (2020). Different results show that fruit firmness may be caused by many factors such as climate, altitude, rootstock, and harvest time.

Total soluble solids (TSS), an important indicator of ripening in fruits, play an important role in the aroma of fruits due to the sugars in the fruit as well as ripening (Kaynaş et al. 2011). TSS was measured at the highest in the Control (100% fertilizer) + bacteria applications Brix value of 20.30%, and in the 50% reduced + bacteria applications, with a Brix value of 19.90%. In the studies, the amount of TSS was determined as 14.16% by Satıcı (2011), 14.73% by Kırac (2016), 13.55% by Baytekin & Akça (2011), 10.3% by Öztürk et al. (2011), 11.84% by Arıkan et al. (2015), and 12.43% by Arıkan (2020). Yaman et al. (2022) measured the TSS value as 13.07% in fruits grafted onto M9 rootstock of the Red Chief variety and 13.77% in fruits grafted onto MM106 rootstock. While the effect of bacteria application on TSS was negative, this effect was found to be -7.01% in fruits grafted onto M9 and -7.50% in fruits grafted onto MM106. The ripening process of the fruit begins with the picking of the fruit, and the TSS ratio gradually decreases as time passes (Satici, 2011). Wang et al. (2022) found that soluble sugar was higher in low fertilizer and DMPP added treatments. The results revealed that 40% N reduction combined with DMPP (TN60% + D) improved fruit quality on the basis of the traditional N application rate ($P < 0.05$).

Table 4. Fruit firmness, TSS, TA, and yield values obtained from the applications

Çizelge 4. Uygulamalardan elde edilen meyve eti sertliği, suda çözünebilir kuru madde, titrasyon asitliği ve verim değerleri

Applications	Fruit firmness (kg.cm ⁻²)*	Total soluble solids (TSS) (%)*	Titratable acidity (TA) (%)*	Yield (kg.tree ⁻¹)*
1. Control	8.17±0.8 a	18.88±0.2 bc	0.59±0.02 a	19.63±1.1 ab
2. 50% reduced	7.89±0.2 a	17.47±0.9 c	0.47±0.03 b	16.78±1.4 bd
3. 100% reduced	7.61±0.2 ab	18.30±0.7 c	0.37±0.02 c	14.78±0.7 d
4. Control + bacteria	6.50±0.5 c	20.30±1.0 a	0.40±0.01 c	22.35±1.0 a
5. 50% reduced + bacteria	6.77±0.5 bc	19.90±0.2 ab	0.39±0.02 c	18.30±2.9 bc
6. 100% reduced + bacteria	6.83±0.6 bc	18.03±1.0 c	0.37±0.01 c	15.92±1.3 cd

*There is a statistical difference between the means shown with different letters in the same column ($p<0.05$).

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır ($p<0,05$).

The highest titratable acid amount was determined as 0.59% in the Control (100% fertilizer) application. In similar studies, the titratable acidity was determined as 0.23% by Satıcı (2011), 0.39% by Sabır et al. (2011) and Baytekin & Akça (2011), 0.33% by Arıkan et al. (2015), and 0.67% by Arıkan (2020). Yaman et al. (2022) found the titratable acidity value as 1.00% and -5.67% negative bacterial effect in the seedling grafted onto Red Chief/M9 rootstock, while they found 0.80% and 1.38% positive bacterial effect in the seedling grafted onto Red Chief/MM106 rootstock. In studies conducted with different fruit species, it was determined that bacterial application significantly reduced the TEA and SÇKM contents in apple (Karakurt & Aslantaş, 2010), strawberry (Eşitken et al., 2010), quince (Arıkan et al., 2013), and pomegranate (Acar, 2018) species.

The highest yield values per tree obtained by collecting the fruits from the trees of each replication separately were obtained from the Control (%100 fertilizer) +bacteria (22.35 kg/tree) and Control (%100 fertilizer) applications (19.63 kg/tree). The lowest yield value was found in the 100% reduced fertilizer (without fertilizer) application. Kırac (2016) found the yield per tree of Red Chief apple on MM106 rootstock to be 20-25 kg, Einhorn & Caspari (2004) found the yield per tree to be 20.9-27.7 kg on M26 rootstock and Lombardini et al. (2004) found the yield

per tree to be 16.17 to 20.8 kg on M9 rootstock. Arıkan (2020) obtained a yield value of 99.34 kg/tree from Red Chief apple on MM111 rootstock. In the study of Wang et al. (2022), TN40% yield was found to be 12.53% lower than TN. The results showed that reducing the N application rate to 200 kg ha⁻¹ (TN40%) negatively affected the yield and economic benefit. While the yield and yield value decreased with the reduction of N rate in the DMPP addition application, the agronomic benefit first increased and then decreased. The reason for the different yield amounts may be due to planting density, rootstock differences, and growing conditions.

Effect of applications on vegetative development

In order to determine the effect of applications on the vegetative development of trees, leaf and shoot characteristics were taken. The difference between the means of all characteristics except original leaf weight, leaf chlorophyll amount, and shoot diameter was found to be statistically significant ($p < 0.05$) (Table 5).

Table 5. Leaf area, leaf fresh and dry weight, specific leaf weight, leaf anthocyanin amount, and leaf chlorophyll amount values were obtained from the applications

Çizelge 5. Uygulamalardan elde edilen yaprak alanı, yaprak yaş ve kuru ağırlığı, özgül yaprak ağırlığı, yaprak antosiyanin miktarı ve yaprak klorofil miktarı

Applications	Leaf Area (cm ²) *	Leaf Fresh Weight (g) *	Leaf Dry Weight (g) *	Specific leaf weight (g.cm ⁻²) NS	Leaf Anthocyanin (ACI) *	Leaf Chlorophyll (SPAD) NS
1. Control	55.88±5.5 a	1.29±0.2 a	0.577±0.1 a	0.0103±0.001	11.55±3.2 ab	50.86±4.6
2. 50% reduced	53.19±7.1 ab	1.27±0.2 a	0.574±0.1 a	0.0108±0.002	11.42±2.4 ab	52.35±5.0
3. 100% reduced	46.79±7.3 c	1.10±0.2 b	0.472±0.1 c	0.0101±0.002	10.47±1.8 b	49.85±4.4
4. Control + bacteria	51.97±7.9 ac	1.19±0.2 ab	0.536±0.1 ab	0.0103±0.003	12.78±2.2 a	50.99±4.8
5. 50% reduced + bacteria	49.23±7.3 bc	1.06±0.2 b	0.495±0.1 b	0.0101±0.002	11.90±2.6 ab	50.26±3.1
6. 100% reduced + bacteria	49.59±4.7 bc	1.08±0.1 b	0.499±0.1 b	0.0101±0.002	12.08±1.3 ab	50.85±2.7

*There is a statistical difference between the means shown with different letters in the same column ($p < 0.05$). NS: Not significant

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır ($p < 0,05$).

NS: Önemli değil

The lowest leaf area was obtained in the 100% reduced (without fertilizer) application. Yaman et al. (2022) found the leaf area value as 30.79 cm² and 2.98% positive bacterial effect in the Red Chief/M9 combination, while it was 27.92 cm² and 8.67% positive bacterial effect in the Red Chief/MM106 combination.

Leaf fresh and dry weight were measured as the highest in Control (100% fertilizer), 50% reduced fertilizer and Control (100% fertilizer) + bacteria applications. Although there was no statistically significant difference between the applications in terms of specific leaf weight calculated by dividing leaf dry weight by leaf area (Zokaee-Khosroshahi et al., 2014), the highest value was obtained from 50% reduced fertilizer application. The lowest leaf anthocyanin amount was determined in 100% reduced (without fertilizer) application. All other applications were statistically in the same group. Yaman et al. (2022) found the SPAD value to be 56.35 and -0.58% negative bacterial effect in the Red Chief/M9 combination and 54.47 and -2.45% negative bacterial effect in the Red Chief/MM106 combination.

Effect of applications on macro-micronutrient content in leaves

When the effects of applications on macro and micronutrient elements in leaves were examined, the differences between the means were found to be statistically significant ($p < 0.05$) (Table 6-7).

Leaf nitrogen (N) content was highest in Control (100% fertilizer), Control (100% fertilizer) + bacteria, and 50% reduced + bacteria applications. The highest P content was obtained from Control (100% fertilizer) and Control (100% fertilizer) + bacteria applications. The highest K content was measured in 50% reduced fertilizer application. Ca content was higher in Control (100% fertilizer) + bacteria, combined with bacteria; 50% reduced fertilizer + bacteria, and 100% reduced (without fertilizer) + bacteria applications. The lowest Mg content was found in 100% reduced (without fertilizer) and 100% reduced (without fertilizer) + bacteria applications.

The lowest amount of Fe was measured in the 100% reduced (without fertilizer) application, while the other applications were statistically in the same group. The highest amount of Cu was obtained from the Control (100% fertilizer) application and the 50% reduced fertilizer application. The highest amount of Zn was determined in the 50% reduced fertilizer application. The lowest amount of Mn was found in the 50% reduced fertilizer and 100% reduced (without fertilizer) applications (Table 7).

Table 6. N, P, K, Ca and Mg values in the leaves obtained as a result of the applications

Çizelge 6. Uygulamalar sonucunda elde edilen yapraklardaki N, P, K, Ca ve Mg değerleri

Applications	N (%)*	P (%)*	K (%)*	Ca (%)*	Mg (%)*
1. Control	1.619±0.002ab	0.203±0.02 a	0.235±0.005 c	2.651±0.08 d	0.059±0.0005 a
2. 50% reduced	1.597±0.04 b	0.132±0.01 b	0.290±0.01 a	3.265±0.16 c	0.059±0.008 a
3. 100% reduced	1.532±0.02 c	0.121±0.01 b	0.220±0.01 c	3.298±0.11 bc	0.048±0.005 b
4. Control + bacteria	1.618±0.03 ab	0.191±0.02 a	0.270±0.01 b	3.467±0.03 ab	0.053±0.002 ab
5. 50% reduced + bacteria	1.655±0.02 a	0.140±0.003 b	0.235±0.02 c	3.459±0.003 ab	0.057±0.002 a
6. 100% reduced + bacteria	1.591±0.03 b	0.138±0.01 b	0.220±0.01 c	3.518±0.14 a	0.045±0.007 b

There is a statistical difference between the means shown with different letters in the same column (p<0.05).

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

Table 7. Fe, Cu, Zn and Mn values in the leaves obtained as a result of the applications

Çizelge 7. Uygulamalar sonucunda elde edilen yapraklardaki Fe, Cu, Zn ve Mn değerleri

Applications	Fe (ppm) *	Cu (ppm) *	Zn (ppm) *	Mn (ppm) *
1. Control	202.0±6.0 ab	22.40±2.2 a	16.90±1.3 b	168.0±5.2 a
2. 50% reduced	186.0±24.0ab	20.40±2.2 a	20.00±1.4 a	137.0±4.5 b
3. 100% reduced	181.0±19.0 b	9.90±0.9c	14.10±1.1 b	133.0±5.0 b
4. Control + bacteria	198.0±8.0 ab	14.50±1.3 b	17.00±2.4 b	168.0± 5.3 a
5. 50% reduced + bacteria	215.0±19.0 a	13.15±0.75 b	15.07±2.1 b	170.0±4.6 a
6. 100% reduced + bacteria	199.0±11.0 ab	12.60±0.2 b	15.00±0.8 b	154.0±4.0 ab

* There is a statistical difference between the means shown with different letters in the same column (p<0.05).

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

Karakurt (2006) determined the lowest (1.57%) value in leaf nitrogen content in the Granny Smith variety, and the highest (2.12%) value in Starkrimson Delicious variety. It was found that Starking Delicious (1724 ppm) and Granny Smith (1630 ppm) had the lowest value, while Golden Delicious (3895 ppm) had the highest value in phosphorus content. It was reported that OSU-142, OSU-7, and BA-8 applications decreased the phosphorus content in Starking Delicious variety, while A-18 caused an increase. It was determined that Starking Delicious (19359 ppm) and Granny Smith (18482 ppm) had the lowest value, while Starkspur Golden Delicious had the highest value (31790 ppm). In terms of magnesium content, Golden Delicious variety had the lowest (1186 ppm), Starkspur Golden Delicious variety had the highest (1327 ppm), and OSU-142 bacteria application (1282 ppm) had higher magnesium content than the control and other bacteria applications. He found the variety x application interaction on calcium content to be very important. It was determined that sodium content varied between 566-644 ppm, depending on variety and bacterial applications. It was determined that iron content was the lowest in Starking Delicious (17.7 ppm) and the highest in Golden Delicious (45.6 ppm) and OSU-142 (32.7 ppm) application increased the iron content compared to the control (27.9 ppm) and other bacterial applications. It was determined that Starking Delicious (25.5 ppm) and Granny Smith (26.3 ppm) had the lowest manganese content, and Starkspur Golden Delicious (54.7 ppm) had the highest manganese content. Golden Delicious (5.9 ppm) had the lowest zinc content, and Starkspur Golden Delicious (17.2 ppm) had the highest content, while A-18 (17.2 ppm) application among the bacterial applications provided a greater increase in leaf zinc content compared to the control (12.9 ppm) and other bacterial applications. Copper content was found to be lowest in Granny Smith (3.2 ppm) and highest in Golden Delicious (6.8 ppm).

Karlıdağ et al. (2007) determined that bacterial applications had a positive effect on all nutrient element contents (N, P, K, Ca, Fe, Mn, and Zn) except Mg in the leaf in their studies, where they applied three different bacterial strains and combinations to the Granny Smith apple variety. Kotan et al. (2021) examined the contents of some nutrients in the leaves with the microbial fertilizer they used in apple sapling cultivation. They reported that the use of bacteria caused an increase in all parameters in the leaf except Cu (%-19.91) compared to the control, the lowest of these increases was in the amount of Zn in the leaf with 0.33%, while the highest increase occurred in the amount of Mg with 69.69%, followed by P (%36.54), N (%27.74), K (%23.64), Mn (%20.52), Ca (%15.17) and Fe (%6.99).

The results of the statistical analysis of some fruit and leaf characteristics are given in Table 8, and the results of the statistical analysis of leaf macro and micronutrients are given in Table 9.

Table 8. Statistical results of applications on some features (Degree of freedom, F value, p value and η^2 value)

Çizelge 8. Uygulamaların bazı özellikler üzerine istatistiksel sonuçları (Serbestlik derecesi, F değeri, p değeri ve η^2 değeri)

Features	Degree of freedom	F value	Sig.(P value)	η^2
Fruit Weight (g)	59	42.714	0.000	0.798
Fruit Width (mm)	59	26.535	0.000	0.710
Fruit Length (mm)	59	25.663	0.000	0.704
Fruit Skin Color L*	59	2.608	0.035	0.195
Fruit Skin Color a*	59	3.267	0.012	0.232
Fruit Skin Color b*	59	3.803	0.005	0.260
Yield (kg.tree ⁻¹)	17	9.054	0.001	0.790
Fruit firmness (kg.cm ⁻²)	17	5.793	0.006	0.707
Total soluble solids (TSS)(%)	17	6.235	0.004	0.722
Titrateable acidity (%)	17	57.155	0.000	0.959
Leaf Fresh Weight (g)	89	4.015	0.003	0.193
Leaf Dry Weight (g)	89	3.665	0.005	0.179
Leaf Area (cm ²)	89	3.322	0.009	0.165
Leaf Anthocyanin (ACI)	89	1.624	0.162	0.088
Leaf Chlorophyll (SPAD)	89	0.621	0.684	0.036
Specific leaf weight (g.cm ⁻²)	89	0.446	0.815	0.026

Table 9. Statistical results of the applications on macro-micronutrients in leaves (Degree of freedom, F value, p value, and η^2 value)

Çizelge 9. Uygulamaların yapraklardaki makro-mikro besin elementleri üzerine istatistiksel sonuçları (Serbestlik derecesi, F değeri, p değeri ve η^2 değeri)

Macro-micro nutrients	Degree of freedom	F value	Sig.(P value)	η^2
N (%)	17	7.857	0.002	0.766
P (%)	17	22.077	0.000	0.902
K (%)	17	22.708	0.000	0.904
Ca (%)	17	28.726	0.000	0.923
Mg (%)	17	4.432	0.016	0.649
Fe (ppm)	17	1.732	0.202	0.419
Cu (ppm)	17	33.208	0.000	0.933
Zn (ppm)	17	5.223	0.009	0.685
Mn (ppm)	17	3.564	0.033	0.598

CONCLUSION

In plant production, differences in ecological conditions cause differences in phenological, pomological, and vegetative growth values of fruits. In general, fruit characteristics are affected by the total temperature of the region where the plant is grown, altitude, direction, vegetation period duration, cultivation techniques, and the time from full bloom to harvest. Fruits grown in high-altitude areas and plateaus are more durable than those grown in low-altitude areas and bottom lands, and apples grown in hot regions are larger and more flattened than those grown in cool regions. Fruits are smaller and harder as a result of the cells not being able to develop in arid regions due to a lack of water. Ecological conditions must be suitable for the genetic characteristics of the plant to emerge (Karaçalı, 1993; Westwood, 1993).

Bacteria have the ability to increase the synthesis of hormones such as auxin, gibberellin, and cytokinin in the plant, thus increasing the vegetative growth and development of the plant (Aslantaş et al., 2007; Eşitken et al., 2010; Gerçekcioğlu et al., 2018). As a result of bacterial application, the increase in the plant's leaf area increases photosynthetic efficiency, and fruit quality criteria are positively affected in parallel. Selvi et al. (2020) stated that the use of bacteria, which has productivity and low cost, is in line with the universal principles of sustainable agriculture. As a result, it was concluded that microbial fertilizers containing plant growth-promoting bacteria can be used in apples. Replication of the study in different climatic and geographical conditions may increase the extensibility of the results. It is recommended to disseminate the study to adopt microbial fertilizers as a sustainable alternative in agriculture.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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Berit ve Ahır Dağı Astragalus Türleri Üzerindeki Curculionidae Türlerinin Belirlenmesi

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ÖZET

Astragalus cinsi dünyada ve Türkiye’de en fazla yayılış gösteren, en fazla endemizm oranına sahip bir cinstir. Çok fazla tür içermesi, her türlü toprak koşullarında ve çok sert iklim şartlarında yaşayabilmesi, derine inen kökleri ile toprağı koruması, tıp, eczacılık, arıcılık vb kullanım alanları olmasına rağmen ülkemizde bu özellikleri fazla bilinmemektedir. Ekonomik ve ekolojik değeri çok fazla olan bu cinsin en önemli böcek zararlıları arasında curculionidler en başta gelmektedir. Bu araştırma 2021 yılında Kahramanmaraş ilinde yer alan Berit dağı batı bakıda ve Ahırdağı güney bakıda çeşitli *Astragalus* türleri üzerinde bulunan curculionoid türlerini belirlemek amacıyla 700-2300 m yükseltiler arasında gerçekleştirilmiştir. Bu çalışmada araştırma alanında yayılış gösteren *Astragalus* dikenli çalıları bir sopa yardımıyla plastik bir kova içerisine çarpılarak, alt kısmındaki bitki artıkları toplanıp ayıklanarak curculionoid türleri toplanmıştır. Çalışma sonunda çeşitli *Astragalus* türleri üzerinde Berit dağında 6, Ahır dağında ise 3 farklı curculionoid türü tespit edilmiştir.

Bitki Koruma

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Anahtar Kelimeler

Astragalus

Berit dağı

Ahırdağı

Curculionidae

Determination of Curculionidae Species on Berit and Ahır Mountain Astragalus Species

ABSTRACT

The genus *Astragalus* is the most widely distributed genus in the world and in Turkey and has the highest rate of endemism. Although it contains many species, can survive in all types of soil conditions and very harsh climate conditions, protects the soil with its deep roots, and has areas of use such as medicine, pharmacy, beekeeping, etc., these features are not well known in our country. Curculionids are among the most important insect pests of this genus, which has many ecosystem services. This study was carried out in 2021 between 700-2300 m elevations in order to determine the curculionoid species found on various *Astragalus* species on the western side of Berit Mountain and the southern side of Ahırdağı in Kahramanmaraş province. In this study, curculionoid species were collected by beating the *Astragalus* thorny shrubs distributed in the research area into a plastic bucket with the help of a stick, collecting and sorting the plant residues at the bottom. At the end of the study, 6 different curculionoid species were identified on various *Astragalus* species in Berit Mountain and 3 different curculionoid species in Ahır Mountain.

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GİRİŞ

Astragalus cinsi Fabaceae familyası içerisinde yer alan, dünyada damarlı bitkiler içerisinde en geniş cinstir. Çoğunlukla yarı-kurak ve kurak step alanlarda yayılış göstermektedir. (Davis ve ark., 1988; Frodin, 2004; Uzun ve ark., 2019). Dünyada yaklaşık 3000 kadar takson içermektedir (Podlech & Zarre, 2013). Dünyada endemizm oranı en yüksek cinslerden olan *Astragalus*’un Türkiye’de yaklaşık 466 kadar taksonu bulunmaktadır ve

endemizm oranı ise %47 dir (Ekici ve ark., 2015). Güneybatı Asya'daki endemik *Astragalus* türlerin çoğu, 885 tür ve 589 endemik türle İran, 319 tür ve 144 endemik türle Afganistan ve 435 tür ve 257 endemik türle Türkiye ile sınırlıdır. Toplamda bu üç ülkede 1021 endemik türe sahip yaklaşık 1459 takson bulunmaktadır; bu, en yüksek endemizm oranı anlamına gelmekte olup Eski Dünya'daki türleşmenin ana merkezidir (Maassoumi & Nasab, 2023).

Astragalus'un Tıpta, eczacılıkta vb çok fazla kullanım alanları olmasına rağmen Türkiye'de bu özellikleri çok az tanınmaktadır. Cinse ait türler, derine inen kökleri ve toprak yüzeyini kaplayan geniş dalları ile erozyonu önler. Ayrıca her çeşit toprakta ve zorlu iklim şartlarında yaşayabilmeleri sebebiyle toprağın korunması açısından oldukça önemlidirler (Kadıoğlu ve ark., 2008). Cinsin çoğu türü, sert iklim şartlarına uyumlu, çoğunlukla dikenli, çok yıllık otsu bitkiler olup salkım şeklinde yastık formlarına sahiptir (Aytaç ve ark., 2012). Bu cinsin gövdesinde bulunan “sakızı” ile de çok dikkat çekicidir. Kurutulan *Astragalus* kökleri ve kitre sakızı eczacılıkta çok farklı kullanım alanları içermektedir. Başlıca farmakolojik etkileri, anti-diyabetik, anti-inflamatuvar, anti-septik, anti-viral, anti-flegmatik, anti-oksidatif, anti-tümör, anti-aging, kardiyο koruma, nöron-koruyucu, hepato-koruyucu, gaz giderici, şişmanlatıcı, yatıştırıcı, kurutucu, yapışkan, müshil, soğutucu, çözücü ve stiptik sayılabilir. (Li ve ark., 2014; Lysiuk & Darmohray, 2016). Bal arılarının bal üretiminde *Astragalus* çiçekleri en temel ana kaynağı oluştururlar.

Kahramanmaraş ilinde *Astragalus* cinsine ait taksonları belirlemek için yapılan bir çalışmada 96 bitki taksonu belirlenmiştir. Yine aynı çalışmada *Astragalus* taksonlarının geçerli adları ile birlikte önceki eş adları da liste halinde verilmiştir. Kahramanmaraş ilinde endemik *Astragalus* takson sayısı 38 olarak tespit edilmiş ve endemizm oranı %39.5 olarak bulunmuştur (Uzun ve ark., 2019).

Türkiye’de *Astragalus*lar üzerinde yapılan çalışmada *Astragalus*lara zarar veren odun delici böceklerden 6 tür belirlenmiş bu türler ise; *Agapanthia coeruleipennis* Frivaldszky, 1878, *Xylotrechus sieversi* (Ganglbauer, 1890) (Coleoptera: Cerambycidae), *Sphenoptera anthracina* Jakovlev, 1887, *S. coracina* (Steven, 1830), *S. tragacanthae* (Klug, 1829) ve *Anthaxia truncata* Abeille de Perrin, 1900 (Coleoptera: Buprestidae) (Tonga & Sakalian, 2023).

Astragalus türleri çeşitli hayvan ve böcekler için beslenme, saklanma, kışlama yeri ve barınak bitkileri olma gibi birçok ekosistem fonksiyonlarına sahip önemli bir bitki grubudur. Çiçeklerinin bal bezelerinin fazla olması sebebiyle başta arılar ve çok farklı böcekler tarafından ziyaret edilirler. Curculionidae familyasının bir cinsi olan *Sitonini* (Coleoptera: Curculionidae: Entiminae) cinsine bağlı türler *Astragalus* cinsi üzerinde önemli zararlar sebep olurlar. *Sitonini* cinsine bağlı türler çeşitli Fabaceae bitkileri ile beslenen polifag bir gruptur, birçok türü kültür bitkileri üzerinde önemli zararlı olarak bilinmektedir (Gözüaçık ve ark., 2020).

MATERYAL ve METOD

Arazi araştırmaları Kahramanmaraş ilinde 2021 Yılı Mayıs-Ekim ayları arasında, Berit ve Ahırdağı'nın 700-2000 m rakımlarda doğal ekosistemde yürütülmüştür. Çalışmanın yürütüldüğü alanlar şekil 1 de yer alan haritada ve Google Earth görüntülerinde yer almaktadır (Şekil, 1).



Şekil1:Çalışma alanı
Figure 1. Study area

Astragalus'un altındaki bitki kalıntılarını eleyerek, dikenli yarı çahlları plastik bir kova içerisine çırparak bitki parçacıkları ile birlikte toplanan materyal ayrı ayrı plastik kaplara konmuş ve KSÜ Orman Fakültesi Orman Entomolojisi Laboratuvarına getirilmiştir. Laboratuvarda plastik kaplar içindeki bitki materyali incelenerek

curculionidler ayıklanmıştır. Elde edilen curculionidler öldürme şişelerinde öldürülerek teşhis için preparasyonu yapılarak uygun numaralı iğnelerle iğnelenmiştir. Curculionidler teşhis için Kırşehir Ahi Evran Üniversitesi Moleküler Biyoloji ve Genetik Bölümüne gönderilmiştir. Teşhisler Makalenin ikinci yazarı olan Doç. Dr. Mahmut ERBEY tarafından yapılmıştır. Teşhis için “İçanadolu Bölgesi Curculionidae (Coleoptera) familyası üzerinde taksonomik çalışmalar” (Sert, 1995) ve “Bolkar Dağlarının Curculionidae (Coleoptera) Familyası Üzerinde Taksonomik Ve Morfolojik Çalışmalar” (Erbey, 2010) adlı doktora tezlerinden yararlanılmıştır.

BULGULAR ve TARTIŞMA

Araştırma kapsamında elde edilen Curculonoid türlerinin biyolojileri, Türkiye ve Dünya yayılışları, konukçu bitkileri verilmiştir.

Berit Dağı Batı Bakısında Yayılış Gösteren *Astragalus* Türleri Üzerinde Tespit Edilen Curculonoidae Türleri

1-*Larinus minutus* Gyllenhal, 1836

Türkiye Yayılışı: Adana – Ceyhan, Karaisali, Saimbeyli and Tufanbeyli; Hatay - Central province, Altınözü, Akbezi, Arsuz, Hassa, Kırıkhan, Samandağı, Soğukoluk ve Yayladağı; Osmaniye – Düziçi ve Gavur Dağı (Lodos ve ark., 2003; Avgın & Colonnelli, 2011). Kahramanmaraş için ilk kayıttır.

Dünya Yayılışı: Doğu Akdeniz, Kafkasya ve Transkafkasya (Csiki, 1934).

İncelenen Materyal: 22.VII. 2021 7 adet *L. minutus* ergini elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E.

Konukçu Bitkiler: *Centaurea* sp. (Avgın & Colonnelli, 2011). Yabancı otlar, *Prunus domestica* L., *Cirsium* sp., *Medicago sativa* L., *Cirsium* sp., *Quercus* sp., *Sinapis* sp., *Cynara scolymus* Pers., *Lens culinaris* Medik. (Pehlivan ve ark., 2005); *Carduus nutans* L. (Güven, 2019). *Elaeagnus angustifolia* L., *Genista* sp., *Onopordum* sp., *Rosa* sp., *Rubus* sp., *Vincetoxicum* sp., *Vitis vinifera* L. (Gözübenli ve ark., 2024)

L. minutus yabancı otların biyolojik kontrolü amacıyla kullanılmaktadır. Kuzeybatı Arkansas Eyaletinde ilk kez 1991 yılında *Centaurea* sp. bitkisine karşı kullanılmış ve başarılı olmuştur (Alford, 2013). *Centaurea diffusa* Lam. bitkisine karşı Kanada, Kolombiya ve İngiltere’de *L. minutus* kullanılmıştır. Fakat bu böceğin sadece tohumlarda azalma sağladığı, buna karşın bitki yoğunluğunu azaltmada yeterli görülmediği bildirilmiştir (Myers ve ark., 2009). Kuzey Amerika ve Kanada’da *Centaurea stoebe* L. ssp. *micranthos* (Gugler)’e karşı *L. minutus* ve *L. obtusus* biyolojik mücadele ajanı olarak kullanıldığı bildirilmiştir (Carson & Landis, 2014).

2-*Polydrusus ponticus* (Faust, 1888)

Türkiye yayılışı: Ankara, Adana, Aksaray, Antalya, Adıyaman, Amasya, Bursa, Balıkesir, Bilecik, Burdur, Denizli, Çanakkale, Çorum, Diyarbakır, Gaziantep, Edirne, Elâzığ, Giresun, Hatay, Isparta, Kahramanmaraş, İzmir, Malatya, Kayseri, Kütahya, Mardin, Manisa, Osmaniye, Yalova, Nevşehir, Mersin, Samsun, Yozgat (Tezcan ve ark., 2014).

Dünya Yayılışı: Doğu Avrupa ve Batı Asya (Lodos, 1972; Avgın & Colonnelli, 2011).

İncelenen Materyal: 26.VII. 2021 13 adet ergin *P. ponticus* bireyi elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E.

Konukçu Bitkiler: *Malus sylvestris* subsp. *mitis* (Wallr.), *Ligustrum* sp., *Amygdalus communis* L., *Pyrus communis* L., *Medicago sativa* L., *Armeniaca vulgaris* Lam., *Persica vulgaris* Miller, *Pyrus elaeagnifolia* Pallas, *Crataegus* sp., *Prunus domestica* L., *Quercus* sp., *Rosa* sp., *Cupressus* sp., *Paliurus spina-christi* Mill., *Prunus avium* L., *Rubus* sp., *Cydonia vulgaris* Pers., *Prunus cerasus* L., *Persica vulgaris* Miller, *Pistacia* sp., *Humulus lupulus* L., *Morus* sp., *Triticum* sp., *Juglans regia* L., *Urtica* sp., *Alnus* sp., *Platanus* sp., *Corylus avellana* L., *Hippophae* sp., *Lens culinaris* Medik., *Populus* sp., *Salix* sp., *Pistacia vera*, *Punica granatum* L., *Celtis* sp., *Crataegus oxyacantha* L., *Ulmus* sp., *Echinops* sp., *Acacia* sp., (Tezcan ve ark., 2014). *P. ponticus* türünün Malatya yöresinde Kayısı ağaçlarının meyvelerinde önemli zararlara sebep olduğu bildirilmiştir (Öztürk & Ulusoy, 2014)

3-*Larinus turbinatus* Gyllenhal, 1835

Türkiye Yayılışı: Artvin, Afyon, Bayburt, Bitlis, Mardin, Burdur, Bingöl, Hakkâri, Van, Diyarbakır, Bingöl, Balıkesir, Iğdır (Lodos ve ark., 1978; Pehlivan ve ark., 2005; Güven, 2019). Kahramanmaraş için ilk kayıttır.

Dünya Yayılışı: Türkiye, Yunanistan, Almanya, Avusturya, Rusya, Bosna Hersek, Bulgaristan, Makedonya, Çek Cumhuriyeti, Belarus, Estonya, Fransa, Hollanda, İspanya, İsviçre, İtalya, Karadağ, Litvanya, Lüksemburg, Macaristan, Moldova, Polonya, Portekiz, Sırbistan, Slovakya, Ukrayna. Kuzey Afrika: Madeira Takımadaları. Asya: Çin, Ermenistan, Azerbaycan, Gürcistan, İsrail, İran, Kırgızistan, Kazakistan. Nearktik Bölge (Alonso-Zarazaga ve ark., 2017; Güven, 2019).

İncelenen Materyal: 26.VII. 2021 8 adet *L. turbinatus* ergin bireyi elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E.

Konukçu Bitkiler: *Carduus nutans* L. subsp. *nutans*, *Cirsium arvense* (L.) Scop. (Güven, 2019). Diyarbakır'da yapılan bir çalışmada *Cirsium arvense* (L.) Scop.'un bu türün konukçu bitkisi olduğu bildirilmiştir (Bolu, 2016).

4- *Sitona flavescens* (Marsham, 1802)

Türkiye Yayılışı: Afyon (Sultandağ), Aydın (Söke), Denizli (Center, Çivril, Çardak), Çanakkale (Gökçeada), İzmir (Menemen), Manisa (Kula, Muğla, Marmaris, Bodrum, Milas, Köyceğiz), Sakarya (Hendek), Tekirdag (Saray) (Lodos ve ark., 1978). Kahramanmaraş için ilk kayıttır.

Dünya Yayılışı: Avusturya, Almanya, Liechtenstein (Coşkuncu & Gencer, 2010).

İncelenen Materyal: 22.VII 2021 5 adet *S. flavescens* ergin bireyi elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E.

Konukçu Bitkiler: *Medicago sativa* L., *Pyrus elaeagrifolia* Pallas, *Cydonia vulgaris* Pers., Leguminosae, *Juglans regia* L., *Prunus spinosa* L. (Lodos ve ark., 1978). İngilterede yapılan bir çalışmada bu türün *Trifolium* sp. türlerine bağımlı olduğu kanıtlanmıştır. Böceğin fizyolojisinde besin türünün birincil öneme sahip olduğu ve *Trifolium* spp dışındaki tüm bitki türlerinde yumurta üretimi ve hayatta kalma oranının azaldığı tespit edilmiştir (Murray & Clements, 1994; Murray, 1996).

5- *Larinus aeruginosus* Hochhuth, 1851

Türkiye Yayılışı: Şanlıurfa (Pehlivan ve ark., 2005); Ankara (Sert & Çağatay, 1994). Kahramanmaraş için ilk kayıttır.

Dünya Yayılışı: Güneydoğu Asya, Transkafkasya, Ön Asya (Legalov, 2010).

İncelenen Materyal: 26.VII 2021 6 adet *L. aeruginosus* ergin bireyi elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E.

Konukçu Bitkiler: *Centaurea* (Pehlivan ve ark., 2005); *Salsola*, *Beta* (Broumand, 1998; Legalov ve ark., 2010). *Larinus* türlerinin farklı habitat tercihleri vardır; bunlardan bazıları kserotermik topluluklarla ilişkilidir, diğerleri ise ıslak çayırarda, meralarda veya kırsal topluluklarda yaşar. *Larinus* türleri genellikle monofag veya oligofagdır ve *Arctium* L., *Carduus* L., *Carlina* L., *Centaurea* L., *Cirsium* Mill., *Onopordum* L. cinsinin Cardueae kabilesine ait bitkilerle ilişkilidirler (Koch, 1992).

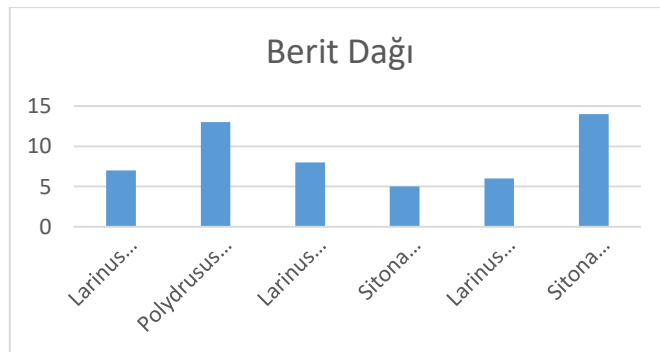
6- *Sitona puncticollis* (Hochhuth, 1851)

Türkiye Yayılışı: Adana – Kozan ve Tufanbeyli; Hatay – Akbeze ve Reyhanlı (Lodos ve ark., 2003). Kahramanmaraş (Gözüaçık ve ark., 2020)

Dünya yayılışı: Western Palaearctic (Knutelski, 2005).

İncelenen Materyal: 22.VII 2021 14 adet *S. puncticollis* ergin bireyi elde edilmiştir. Berit Dağı batı bakıdan elde edildiği koordinatlar: 38°00'35.64"N 36°49'01.18"E, 38°00'24.49"N 36°49'06.84"E, 38°00'20.06"N 36°49'25.68"E, 38°00'32.65"N 36°48'43.12"E. *S. puncticollis* Berit Dağı batı bakıda *Astragalus* türleri üzerinden Şekil 2'de de görüldüğü üzere en fazla elde edilen tür olmuştur.

Konukçu Bitkiler: Fabaceae familyası türleri özellikle *Trifolium* sp. (Dieckmann, 1980).



Şekil 2. Berit Dağı batı bakısında çeşitli *Astragalus* türleri üzerinden elde edilen Curculionidae türleri.

Figure 2. Curculionidae species obtained from *Astragalus* species in the western aspect of Berit Mountain

Ahırdağı Güney Bakı Üzerinde Yayılış Gösteren *Astragalus* Türleri Üzerinde Tespit Edilen Curculonoidae Türleri

1. *Sitona humeralis* Stephens, 1831

S. humeralis Stephens, 1831, Coleoptera takımı içerisinde yer alan Curculionidae familyasının Entiminae Schoenherr, 1823 altfamilyası içerisinde yer alır. Çalışmalar sonucunda elde edilen bulgularla birlikte, türün yayılış alanları ve konukçu bitkileri verilmiştir. *S. humeralis* Ahır Dağı güney bakıda Astragalus türleri üzerinden Şekil 3'de de görüldüğü üzere en fazla elde edilen tür olmuştur. *S. humeralis* ergin bireylerinin yonca bitkisinin (*Medicago sativa* L.) üst kısmında yer alan yaprak ve sürgün kısımlarında, larvaların ise yonca bitkisinin kılcal kök ve nodozitelerde beslenme yaparak ciddi zararlar oluşturmakta olduğu bildirilmiştir (Gözüaçık ve ark., 2021). *S. humeralis* yonca ekim sahalarında çok görülen bir tür olması bu türün çok farklı iklim şartlarına uyum sağlama kabiliyetinin olduğunu göstermektedir (Özdemir ve ark., 2020)

Türkiye yayılışı: Tekirdağ, Kırklareli, Uşak, Afyon, Balıkesir, Çanakkale, Denizli, Isparta, Yalova, İzmir, Kütahya, Manisa, Muğla, Bilecik, Burdur, Bursa, Sakarya, (Velázquez de Castro ve ark., 2010). Ankara, Adana, Kahramanmaraş, Gaziantep, Antalya, Bolu, Çorum, Düzce, Bartın, Eskişehir, Hatay, Mersin, Konya, Nevşehir, Karabük, Karaman, Kırıkkale, Kırşehir, Kayseri, Niğde (Lodos ve ark., 2003). Ankara (Sert & Çağatay, 1994) Tekirdağ (Kıvan, 1995), Ankara, Konya (Tamer ve ark., 1997), Bursa (Coşkun & Gencer, 2010; Gözüaçık ve ark., 2021).

Dünya yayılışı: Avrupa: Arnavutluk, Bosna Hersek, Bulgaristan, Estonya, Finlandiya, Beyaz Rusya, Hırvatistan, Çek Cumhuriyeti, Avusturya, Belçika, Danimarka, Fransa (Corse, Monaco dahil), Kazakistan (Ural Nehri'nin batısında), Büyük Britanya, Yunanistan, Macaristan, İtalya (Sardegna, Sicilia, San Marino dahil), Almanya, Letonya, Moldavya, Hollanda, Norveç, Rusya: Kuzey Avrupa Bölgesi, Polonya, Lüksemburg, Makedonya, Portekiz, Romanya, Slovakya, Slovenya, Sırbistan, İspanya (Cebelitarık dahil), Rusya: Güney Avrupa Bölgesi, Türkiye, Ukrayna, İsveç, İsviçre, Kuzey Afrika: Madeira Takımadaları, Asya: Ermenistan, Kıbrıs, Azerbaycan, Gürcistan, Kırgızistan, İran, Irak, Kazakistan (Ural Nehri'nin doğusu), Moğolistan, Lübnan, Mısır, Türkmenistan, Türkiye, Suriye, Tacikistan, Özbekistan, Rusya: Batı Sibirya (Alonso-Zarazaga ve ark., 2017).

İncelenen materyal: 10.10. 2021 tarihinde 8 adet ergin *S. humeralis* bireyi elde edilmiştir. Ahır Dağı güney bakıdan elde edildiği koordinatlar: 37°37'26.63"N 36°55'15.14"E, 37°37'16.14"N 36°54'48.81"E.

Konukçu bitkiler: *Vicia cracca* L., *Medicago sativa* L., *Vicia faba* L. (Lodos ve ark., 2003).

2- *Sitona crinitus* (Herbst, 1795)

İçerdikleri bitkisel protein sebebiyle baklagiller (Fabaceae), dünyada ve Türkiye'de insanların ve hayvanların beslenmelerinde büyük öneme sahip bitkilerdir. *S. crinitus* (Herbst.) baklagil bitkilerinin yetiştirilmesinde problem çıkaran zararlı böcekler içinde en önde gelenlerden biridir (Yıldırım, 2007). *Sitona crinitus* (Herbst.) baklagil bitkilerin (Fabaceae) önemli zararlılarından birisidir. Yumurtadan çıkan larvalar hemen toprağa girerler ve baklagillerin kök kısımlarındaki nodüllerin içine yerleşirler. Larvalar hem ana kökü hem yan kökleri ve nodülleri yiyerek zarar verdikleri gibi, yaptıkları faaliyetlerle bakteri ve fungusların bitkiye bulaştırarak bu zararlılar için de uygun ortam oluşturmaktadırlar (Yıldırım, 2007). *S. crinitus*'ün erginleri, kışı çeşitli bitki artıkları arasında bunun yanında topraktaki yarıklarda saklanarak yazdan itibaren sonbahar ve kışı hareketsiz şekilde geçirdikten sonra ilkbaharda havalar ısınmaya başladığında beslenmek için çeşitli baklagillerin yaprakları üzerinde beslenirler ve sonrasında, çiftleşmeye ve bunun ardından da yumurta bırakmaya başlarlar (Altay ve ark., 1972; Yıldırım, 2007).

S. crinitus (Herbst.) (Coleoptera, Curculionidae) Türkiye'de özellikle mercimeklerde zarar yaptığından dolayı bu böceğe Ziraî Mücadele Enstitüsü bu böceğe "Hortumlu Mercimek Böceği" adını vermiştir (Kılıç ve ark., 1968).

Türkiye yayılışı: Denizli, Isparta, Uşak, Afyon, Balıkesir, Çanakkale, Bilecik, Bursa, Edirne, İzmir, Yalova, Kırklareli, Manisa, Muğla, Tekirdağ, Kütahya (Lodos ve ark., 1978). Ağrı, Adıyaman, Bingöl, Elâzığ, Erzincan, Bitlis, Erzurum, Malatya, Muş, Tunceli, Hakkari, Van (Bingöl 1978). Ankara (Sert & Çağatay, 1994). Ankara, Konya (Karapınar) (Tamer ve ark., 1997). Ankara, Adana, Osmaniye, Kahramanmaraş, Antalya, Bolu, Aksaray, Çankırı, Eskişehir, Gaziantep, Çorum, Hatay, Mersin, Karabük, Karaman, Kayseri, Kilis, Kırıkkale, Kastamonu, Kırşehir, Nevşehir, Niğde, Konya, Yozgat (Lodos ve ark., 2003).

Dünyadaki yayılışı: Suriye, Türkiye, Avrupa'nın tamamı, Mısır, Kuzey Afrika, Kafkaslar ve Türkistan.

İncelenen materyal: 10.10. 2021 tarihinde 6 adet ergin *S. crinitus* bireyi elde edilmiştir. Ahır Dağı güney bakıdan elde edildiği koordinatlar: 37°37'26.63"N 36°55'15.14"E, 37°37'16.14"N 36°54'48.81"E.

Konukçu bitkiler: Acı bakla, Yonca, Bezelye, yabancı bezelye, (Balachowsky 1963; Yıldırım, 2007). *Juglans regia* L., *Medicago sativa* L., *Vicia cracca* L., *Triticum* sp., *Pyrus elaeagnifolia* Pall., *Vicia ervilia* L., *Pyrus communis* L., *Prunus persica* L., *Rubus* sp., *Prunus amygdalus* Batsch, Leguminosae, *Matricaria chamomilla* L., *Prunus domestica* L., *Citrus* sp., *Populus* sp., *Centaurea* sp., *Pinus* sp., Cruciferae, *Ulmus campestris* L., *Prunus cerasus* L., *Vicia faba* L., *Lens esculenta* Moench., *Chenopodium* sp., *Quercus* sp., *Sinapis arvensis* L., *Sesamum indicum* L., *Onobrychis sativa* L., *Pyrus malus* L., *Myrtus communis* L., *Crataegus* sp., (Lodos ve ark., 1978).

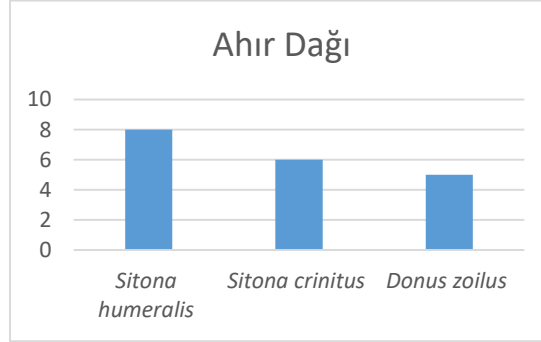
3- *Donus zoilus* (Scopoli, 1763)

Türkiye yayılışı: Dört Yol-Hatay (Pehlivan ve ark., 2005)

Dünya yayılışı: Palaearctic (Osella ve ark., 2005; Majka ve ark., 2007).

İncelenen materyal: 14.10.2021 tarihinde 5 adet ergin *D. zoilus* bireyi elde edilmiştir. Ahır Dağı güney bakıdan elde edildiği koordinatlar: 37°37'26.63"N 36°55'15.14"E, 37°37'16.14"N 36°54'48.81"E.

Konukçu bitkiler: *Medicago* sp, *Trifolium* sp. (Dieckmann, 1989). Larvalar yonca bitkilerinde beslenirler, erginler ise daha geniş bir yelpazedeki Fabaceae familyası bitkileri ile beslenirler (Majka ve ark., 2007).



Şekil 3. Ahır Dağı güney bakışında Astragalus türleri üzerinden elde edilen Curculionidae türleri.

Figure 3. Curculionidae species obtained from Astragalus species in the southern aspect of Ahır Mountain.

SONUÇ ve ÖNERİLER

Bu çalışmada çeşitli Astragalus türleri üzerinde toplam 9 farklı curculionid türü elde edilmiş ve bu türlerden 5 tanesi Kahramanmaraş için ilk kayıttır. Bu türlerden 4 tanesi *Sitona*, 3 tane *Larinus*, 1 tane *Polydrusus*, 1 tane de *Donus* cinsine aittir. *Sitona* içerisinde yer alan türler *Sitona puncticollis*, *S. flavescens*, *S. humeralis*, *S. crinitus* türleridir. Bu cinse bağlı türler çeşitli Fabaceae bitkileri ile beslenen polifag bir gruptur, birçok türü kültür bitkileri üzerinde önemli zararlı olarak bilinmektedir (Gözüaçık ve ark., 2020). Bu türlerden *S. flavescens* üzerine yapılan çalışmalarda bu türün beslenme açısından *Trifolium* spp türlerine bağımlı olduğu, farklı bitki türleri ile beslenmesi durumunda yumurta veriminin ve hayatta kalma oranının düştüğü İngiltere’de yapılan çalışmalarda ortaya konulmuştur (Murray, 1996). Çalışmada tespit edilen *Sitona* türlerinden olan *S. humeralis* ve *S. puncticollis* türleri ise yonca ekim alanlarında önemli bir zararlıdır. Çalışmada tespit edilen diğer bir tür olan *Donus zoilus* yoncalarda zarar yapan bir türdür. Yonca (*Medicago sativa* L.) hem küçükbaş hem de büyükbaş hayvan beslenmesinde son derece önemli bir yere sahip çok yıllık otsu bir bitkidir. Bu öneminden dolayı tüm dünya ve Türkiye’de yonca ekim alanları önemli bir yer tutmaktadır. Yem bitkileri arasında besin değeri en yüksek olan yem bitkisi yoncadır. Hayvan besiciliğinde %30 oranlarına kadar et ve süt verimini arttırılabilmektedir. Bunun yanında silo ve pelet yemi şeklinde kullanılabilmektedir (Radovic ve ark., 2009; Gözüaçık ve ark., 2020). Türkiye’de 2019 yılı içerisinde 641.213 ha alanda yonca ekimi gerçekleştirilmiştir (Türk, 2019). Bu rakam yoncanın ülkemizde oldukça geniş alanlarda ekiminin gerçekleştirildiğini ortaya koymaktadır. Bu ekim alanlarının en önemli zararlıları arasında başta *Sitona* türleri gelmektedir. 2018-2020 yılları arasında Gözüaçık ve arkadaşları tarafından Kahramanmaraş Ahırdağı, Ilıca ve Kümperli bölgelerinde yapılan ve sadece *Sitona* türlerini belirlemek amacıyla yaptıkları çalışmada 6 tür bulmuşlardır. Bunlar *Sitona humeralis*, *S. concavirostris*, *S. callosus*, *S. hispidulus*, *S. puncticollis*, *S. macularius* türleridir (Gözüaçık ve ark., 2020). Bu çalışmada bulunan *Sitona puncticollis* ve *S. humeralis* türleri ortak diğer iki tür *Sitona flavescens* ve *S. crinitus* ise farklıdır. Ahırdağı her iki çalışmada da ortak çalışma alanıdır.

Tohumla beslenen böceklerle olan ilginin artması, biyolojik kontroldeki rollerine dayanmaktadır. Otları doğrudan öldürebilmeleri (zayıflatarak veya strese sokarak) veya dolaylı olarak beslenme sırasında taşıdıkları patojenlerle ikincil enfeksiyon oluşturarak yabancı otların ölmelerine sebep olabilmektedirler (Wilson & Randall, 2003). *Larinus* türlerinin çoğunluğu yabancı otların potansiyel biyolojik kontrol ajanları olarak düşünülmektedir (Nikulina ve ark., 2004; Seeastedt ve ark., 2007; Skuhrovec & Gosik, 2011). Bu türler psammofil (Kumlu alanları tercih eden) otlak topluluklarında, kuru çayırda ve kırsal topluluklarda görülmektedirler. *Centaurea* bitki cinsiyle ilişkili stenotopik (Çevre koşullarındaki değişikliklere uyum sağlama yeteneği dar), termofil (Sıcaklığı seven) bir oligofaj olduğu düşünülmektedir (Koch, 1992). Çalışmanın yapıldığı Berit ve Ahır dağları hakim bitki örtüsü olan *Astragalus*, *Onopordum*, *Centaurea* gibi bitkilerdir. Erozyona ve iklim değişimine oldukça duyarlı bölgelerdir. Stenotopik böcek türleri olan *Larinus* türleri çevre koşullarındaki değişimleri izlemek amacıyla kullanılabılır. Çalışmaların yürütüldüğü Berit Dağı batı bakışı ile Ahırdağı Güney bakışı *Astragalus* türleri üzerinde bulunan curculionid çeşitliliği bakımından karşılaştırıldığında Berit Dağının daha zengin olduğu göze

çarpmaktadır. Bu zenginlikte Berit dağının içerdiği *Astragalus* tür çeşitliliğinin fazla olması gösterilebilir.

Her iki çalışma alanından elde edilen curculionidlere baktığımızda çoğunun konukçu bitki olarak Fabaceae familyası türlerini tercih ettikleri görülmektedir. Çalışmada belirlenen 9 türden *Larinus* türlerinin Fabaceae familyası dışında yabancı otlar üzerinde de beslenmektedirler.

Türkiye’de *Astragalus*lar üzerindeki böcek türlerinin belirlenmesi üzerine yapılan çalışmalar oldukça azdır. Bu çalışma doğrudan *Astragalus* türleri üzerindeki Curculionid türlerinin belirlenmesi üzerine bir çalışma olması nedeniyle diğer çalışmalardan ayrılmaktadır.

Türkiye’nin çok büyük bir kısmı kurak ve yarı kurak alanlardan oluşmaktadır. Ortalama yükselti 1132 m dir. Topraklarının çok büyük bir bölümü erozyona duyarlıdır. Böylesi bir alanda hem erozyonu önleyen, hem arıcılık ve balcılık açısından ekonomik katkısı olan bu bitkilerin tanınması ve korunması gerekmektedir. Bu maksatla bu bitkileri tehdit eden biyotik ve abiyotik faktörlerin belirlenmesi amacıyla daha fazla akademik çalışmaların yapılması gerekmektedir. Erozyona duyarlı bu alanların çevre koşullarındaki değişimleri izlemek amacıyla Stenotopik böcek türleri olan *Larinus* türlerinin kullanılması tavsiye edilebilir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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The Role of Arbuscular Mycorrhizal Fungi (AMF) in the Control of *Rhizoctonia* Root Rot in Local Bean Genotypes of the Van Lake Basin

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ABSTRACT

Beans (*Phaseolus vulgaris* L.), one of the main vegetables common in the Van Lake Basin, are frequently grown in the region with local genotypes. *Rhizoctonia solani* root rot, an important soil-borne disease that negatively affects beans, especially in this region, causes economic losses in our country and worldwide. Therefore, a viable and eco-friendly alternative to chemical control in the treatment of such significant soil-borne plant diseases is the application of Arbuscular Mycorrhizal Fungi (AMF). This study aimed to investigate the effects of commercial AMF (ERS) and *Funneliformis mosseae* (Fm) against *R. solani* (Rs) root rot disease in V29 and TR 50763 (T71) bean genotypes obtained from Van-Gevaş. For this purpose, some plant growth parameters, total phenol, total antioxidant capacity, root colonization, and disease severity parameters were investigated. Fm and ERS significantly increased plant morphological parameters and reduced disease severity in both bean genotypes (V29 and T71) despite the *R. solani* inoculated pathogen. Furthermore, Fm was shown to be the most effective in plant growth among AMF treatments. ERS was the most effective treatment in total phenol concentration, antioxidant activity, and AMF root colonization. In general, it was observed that genotype T71 had a different effect on plant growth parameters and total antioxidant activity than V29. The study's results show that AMF, a sustainable agricultural technique, enhanced plant growth and reduced the damage caused by the important *Rhizoctonia* root rot disease in beans, regardless of genotype diversity. This study can be positively evaluated in the context of investigating sustainable agricultural measures against this disease in the field.

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Van Gölü Havzası Yerel Fasulye Genotiplerinde *Rhizoctonia* Kök Çürüklüğünün Kontrolünde Arbusküler Mikorizal Fungus (AMF) un Rolü

ÖZET

Van Gölü Havzası'nda yaygın olan ana sebzelerden biri olan fasulye (*Phaseolus vulgaris* L.), bölgede yerel genotiplerle sıklıkla yetiştirilmektedir. Özellikle bu bölgede fasulyeyi olumsuz etkileyen toprak kökenli önemli bir hastalık olan *Rhizoctonia solani* kök çürüklüğü, ülkemizde ve dünya genelinde ekonomik kayıplara neden olmaktadır. Bu nedenle, bu tür önemli toprak kaynaklı bitki hastalıklarının tedavisinde kimyasal kontrole uygun ve çevre dostu bir alternatif, Arbusküler Mikorizal Fungusların (AMF) uygulanmasıdır. Bu çalışmada, Van-Gevaş'tan temin edilen V29 ve TR 50763 (T71) fasulye genotiplerinde *R. solani* (Rs) kök çürüklüğü hastalığına karşı ticari AMF (ERS) ve *Funneliformis mosseae* (Fm)'nin etkilerinin araştırılması amaçlanmıştır. Bu amaçla, bazı bitki büyüme parametreleri, toplam fenol, toplam antioksidan kapasite, kök kolonizasyonu ve hastalık şiddeti parametreleri araştırılmıştır. Fm ve ERS, *R. solani* ile inokule patojene rağmen her iki fasulye genotipinde (V29 ve T71) bitki morfolojik parametrelerini önemli ölçüde artırmış ve hastalık şiddetini azaltmıştır.

Bitki Koruma

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Fasulye

Rhizoctonia solani

Arbusküler Mikorizal Fungus

Bitki büyüme parametreleri

Alternatif mücadele

Ayrıca, Fm'nin AMF uygulamaları arasında bitki büyümesinde en etkili olduğu gösterilmiştir. ERS, toplam fenol konsantrasyonu, antioksidan aktivite ve AMF kök kolonizasyonunda en etkili uygulama olmuştur. Genel olarak, T71 genotipinin bitki büyüme parametreleri ve toplam antioksidan aktivitesi üzerinde V29'dan farklı bir etkiye sahip olduğu gözlemlenmiştir. Çalışmanın bulguları, sürdürülebilir bir tarım tekniği olan AMF'nin bitki büyümesini artırdığını ve genotip çeşitliliğinden bağımsız olarak fasulyede önemli *Rhizoctonia* kök çürüklüğü hastalığının neden olduğu zararı azalttığını göstermektedir. Bu çalışma, sahada bu hastalığa karşı sürdürülebilir tarımsal önlemlerin araştırılması bağlamında olumlu olarak değerlendirilebilir.

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is a leguminous plant widely consumed in Turkey and worldwide. It plays an important role in both local and international agriculture. Beans are essential for human nutrition as they are rich in protein, minerals, and vitamins (Ekincialp & Şensoy, 2018; Gavilanes et al., 2020; Durak et al., 2024). Turkey ranks fourth with 519.713 tons of beans produced worldwide, or about 2.2% of the total. China is the world leader in bean production (Food and Agriculture Organization of the United Nations, 2022). Many biotic (fungal and bacterial, etc.) and abiotic variables negatively affect the productivity and quality of this significant legume crop for the national economy (Costa-Coelho et al., 2014).

Common bean production, which is significant worldwide, is susceptible to various diseases at different stages of the plant life cycle (Meziadi et al., 2016; Martins et al., 2018). With a wide range of hosts, *Rhizoctonia solani* Kühn is a soil-borne plant pathogen that causes the serious disease known as root rot in common bean production areas worldwide. Due to its facultative parasitic capacity, it can survive as a saprotroph in soil (Otten et al., 2001; Zhao et al., 2005). Symptoms of *R. solani* include plant wilt and necrotic lesions on leaves (Ghini & Zaroni, 2001). This disease agent can reduce bean production by up to 90% (Palacioglu et al., 2019). They can also cause early infections that kill plants and occur during the seedling stage or later in growth. This pathogen has been found to cause root and grain rot and net blight in the final stages of plant development (Valentín Torres et al., 2016).

Bean cultivation in the Van Lake Basin is a common practice involving both local and commercial species. The use of native species is particularly important for resource conservation initiatives that seek to reduce damage to plant populations. Adoption of some varieties is still limited, especially in areas affected by widespread diseases (Durak et al., 2024).

In recent years, many microbial biocontrol agents have been shown to reduce soil-borne diseases (Soylu et al., 2005; Sharifi & Ryu, 2017; Soylu et al., 2020; Soylu et al., 2021). Particularly *R. solani* and *Fusarium* spp., the Arbuscular Mycorrhizal Fungi (AMF) have a significant potential for suppressing soil-borne fungi (Akköprü & Demir, 2005; Sohrabi et al., 2015; Güneş et al., 2025). The AMF species *Rhizophagus intraradices*, in addition to improving growth and yield indices, substantially reduces the adverse effects of *R. solani* when colonizing bean roots (Abdel-Fattah et al., 2011; Nasir Hussein et al., 2018). Many studies have examined the basis of these interactions, including the induction of Mycorrhizal Induced Resistance (MIR), which is characterized by the induction of systemic resistance through the stimulation of defense molecules such as morphological changes in host root tissues, infection sites, and competition for nutrients (Dalpé, 2005; Saldajeno et al., 2008). It was also emphasized that when applied in communities with the additional beneficial effect of each AMF species, the observed good yields could be a result of positive interrelationships between AMF, increasing plant diversity, and production (Singh, 2015; Eke et al., 2016). A substantial body of research, conducted with a variety of plant species, has demonstrated that different genotypes may exhibit disparate responses to mycorrhizal growth (Sensoy et al., 2007; Erdinç et al., 2017; Berger & Gutjahr, 2021; Felföldi et al., 2022).

Considering the above-mentioned statements, finding a suitable combination of AMF can be beneficial in applying this technology to increase crop yields. The present study aimed to evaluate the effects of commercial AMF and *F. mosseae* on plant growth parameters in two different bean genotypes of the pathogen *R. solani*. Furthermore, to analyze the effects of AMF on both pathogen severity and root rot and plant wilt disease. The hypothesis that

different AMF inoculations increase the severity of *R. solani* disease and positively influence colonization and the effect between genotypes was tested.

MATERIAL and METHOD

Materials

The study employed two distinct genotypes of green beans (The reason for using these genotypes in the study should be stated.). TR 50763 (T71) is one of the collections of the Aegean Agricultural Research Institute genebank, and genotype V29 was obtained from the Van-Gevaş region. Two AMF inoculums suitable for plant growth and resistance were used in this study (Gunes et al., 2023). The first was Endo Roots Soluble (ERS)-a Bioglobal company commercial AMF inoculum containing 25 spores per gram. The second was *F. mosseae* (Fm) (T.H. Nicolson and Gerd.) Schüßler & Walker, 2010). *F. mosseae* inoculum was obtained from the stocks of the Mycology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University. *R. solani* AG-4 (Rs, code 18) pathogen (70% virulent), which was isolated from beans in previous studies (Durak et al., 2024), was obtained from the culture stocks of Van Yuzuncu Yil University, Faculty of Agriculture, Mycology Laboratory.

Sampling Method

Design of experiment

Bean seedlings were grown in a fluorescently illuminated growth chamber with a temperature of 23±2 °C, 60-70% relative humidity, and 16:8 light: dark period conditions according to a randomized plot experimental design with four replicates. Treatments are controlled, V29 genotype only, T71 genotype only, ERS only, Fm only, Rs only, V29 + ERS, V29 + Fm, V29 + Rs, T71 + ERS, T71 + Fm, T71 + Rs. Twelve different treatments, four repetitions of each treatment, and one plant in each repetition were used in a randomized experimental design.

The containers were sterilized by soaking them in a 5% sodium hypochlorite (NaClO) solution for 2 minutes and then washing them in tap water. Sterile 3 kg pots filled with sterile peat-perlite (2:1 ratio) were used to grow the bean seeds. Before planting, was infected with 10 g of *F. mosseae* and ERS AMF isolates (150 spores per g soil). Sterile sand was used instead of AMF isolates in the seedling beds of control treatments. *R. solani* was applied to bean seedlings three weeks after (when the first true leaves appeared) sowing. Therefore, wheat grains were used as an inoculum medium for *Rs* isolates. Using the wheat wrapping method, grains soaked in distilled water were placed in petri dishes (9 cm) and autoclaved at 121°C for one hour for two days. Then, sterile wheat grains were infected with three mycelial fragments obtained from a one-week-old immature *Rs* isolate. Petri dishes were then incubated at 25°C for four weeks. Fifteen wheat grains infected with mycelia were added to each pot (Ichielevich-Auster et al., 1985; Botha et al., 2003; Sharon et al., 2007). The pots of the control group contained 5 sterile wheat grains (Erper et al., 2011). The experiment was terminated after eight weeks when the plants showed symptoms of *R. solani*.

Laboratory Analysis

Evaluation of the characteristics related to plant growth

Assessment of the plant growth parameters after harvest, various morphological and biochemical parameters of the plant were measured, including shoot length (SL-cm), root length (RL-cm), stem diameter (SD-mm), TP (total phenol), TAC (total antioxidant capacity), shoot fresh weight (SFW-g), root fresh weight (RFW-g), shoot dry weight (SDW-g), root dry weight (RDW-g), AMF root colonization (%), relative mycorrhizal dependency (RMD) and *Rhizoctonia* disease 0-4 scale. The lengths of plants were determined with a ruler; total fresh weights were determined by weighing; total dry weights were measured by weighing dried at 70 °C for 48 h (Kacar, 1984). The shoot diameter was measured with a caliper (digital caliper, Insize, Chinese).

Total antioxidant capacity (TAC) and total phenol (TP)

The total phenol content was determined by the methodology established by Swain & Hillis (1959), while the total antioxidant content was quantified through the application of the ferric reducing antioxidant power (FRAP) method, as originally proposed by Benzie & Strain (1996).

A total of 150 µl of the extract was taken, 2400 µl of distilled water was added, and the solution was vortexed for 3-4 seconds. Subsequently, 300 µl of 20% sodium carbonate was added, and the mixture was kept at room temperature in the dark for 60 minutes. Finally, the solution was read at a wavelength of 760 nm in a spectrophotometer (Thermo Scientific Genesys 10S Model UV-VIS spectrophotometer Waltham, MA, USA.). In the calculation, the equivalent of gallic acid was employed, and the results were expressed as GA 100 mg FW⁻¹.

To ascertain the antioxidant activity of the extract, 150 µl of the extract was combined with 2850 µl of FRAP solution, comprising acetate buffer, TPTZ (TPTZ should be written clearly), and ferric chloride. The solution was transferred to a 10 ml glass tube and incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at a wavelength of 593 nm using a spectrophotometer. The results were calculated according to the Trolox equivalent and are expressed as µmol TE (TE should be written clearly) 100 mg FW⁻¹.

Evaluation of the severity of the disease

R. solani infected plants were evaluated on a 0-4 scale (0: healthy seedling, 1: very small brown superficial lesions on roots or stem, 2: deep and extensive lesions on roots or stem, retarded root growth, 3: severe root rot, deep lesions surrounding the main root or stem, significantly reduced root length, 4: dead plant) (Muyolo et al., 1993). Following the scale-based assessment, the Townsend & Heuberger (1943) formula (Equation 1) was used to determine the severity of disease (DS).

$$DS (\%) = [\sum(S \times L) / (M \times S_{max})] \times 100 \quad (1)$$

where M is the total number of plants (plant leaves), L is the number of plants (plant leaves) evaluated on the scale, S_{max} is the highest scale value, and S is the scale value.

AMF colonization rate (% AC) in roots was determined according to Equation 2 (Giovannetti & Mosse, 1980).

$$AC \% = ACR/R \times 100 \quad (2)$$

where R is the total number of roots and ACR is the number of roots colonized by AMF.

$$RMD (\%) = \left[\frac{A-B}{A} \right] \times 100 \quad (3)$$

where A: Dry weight of the mycorrhizal plant, B: Dry weight of the nonmycorrhizal plant.

Statistical Analysis

The data obtained in the study were subjected to variance analysis using the SPSS statistical program, in accordance with the randomized experimental design. In the analysis of the data, statistically significant averages were grouped according to the Duncan Multiple Comparison Test. A principal component analysis (PCA) was performed using the XLSTAT statistical program to define the dependent variable parameters corresponding to the independent variables (genotype and AMF) under *Rhizoctonia* and non-*Rhizoctonia* circumstances (Vidal et al., 2020). Additionally, a heat map cluster (ClustVis) and correlation analysis were accomplished through the utilization of the PAST 4 package program (Metsalu & Vilo, 2015).

RESULTS

Table 1 describes the effect of two different AMF treatments on SL, RL, SD, TP, and TAC in two different bean genotypes treated with *R. solani*. In general, the difference between the two bean genotypes and AMF species was statistically significant ($p \leq 0.05$). Especially in SL, the V29 control group was higher than T71 in both *Rhizoctonia* (+) and *Rhizoctonia* (-), and the difference between them was statistically significant ($p \leq 0.05$). In addition, AMF treatments increased SL despite the pathogen, especially in V29, where the Fm+Rs value increased by about 110% compared to the T71 Fm+Rs treatment (Table 1). At the same time, when the difference in shoot length between AMF types was statistically analyzed ($p \leq 0.05$), it was determined that Fm had a greater effect on the increase than ERS.

In root length, ERS in V29 was higher than in the control group despite the Rs, but about 58% lower than in Fm (Table 1). In the genotype T71, root length was higher in the control group than in the no-treatment group despite Rs. However, regarding AMF, the effect of ERS on root length in this genotype under Rs conditions was 40% higher than Fm (Table 1). It was also determined that there was no statistically significant difference between Rs (-) and Rs (+) ($p \leq 0.05$).

In shoot diameter, one of the plant growth parameters, the difference between the genotypes, AMF treatments, and pathogens was found to be statistically insignificant ($p \leq 0.05$) (Table 1). However, the difference between the AMF x genotype and AMF x Rs was found statistically significant ($p \leq 0.05$) (Table 1).

In total phenolic concentration, AMF treatments were higher than the control groups according to both genotypes (V29 and T71), and the total phenolic value of ERS was higher (42%) among AMF treatments than Fm. The difference between them was statistically significant ($p \leq 0.05$). In terms of the pathogen, the highest value was observed in the ERS+Rs (171.57 mg GAE 100 g dry weight) treatment in the T71 genotype, while the lowest total phenolic value was observed in the control + Rs (89.73 mg GAE 100 g dry weight) treatment in the V29 genotype and the difference between them was 91%. The difference between AMF treatments was statistically significant ($p \leq 0.05$), but there was no statistically significant difference between genotypes in this parameter ($p \leq 0.05$). At the same time, there was a statistically significant ($p \leq 0.05$) difference between treatments with and without Rs (Table 1).

In total antioxidant capacity, there was no statistical difference between genotypes ($p > 0.05$), while the difference between AMF treatments was statistically significant ($p < 0.05$). The difference between Rs treatments was also significant ($p \leq 0.05$). The highest total antioxidant capacity value was in V29 genotype ERS + Rhz (+) (77.60 Trolox μ mol TE 100 g dry weight) combination, while the lowest value was in V29 Fm + Rs (-) (26.49 Trolox μ mol TE 100 g dry weight) treatment (Table 1). The highest total antioxidant capacity value among AMF treatments was higher in ERS than Fm in both genotypes (V29 and T71). Similarly, the total antioxidant capacity of the pathogen-treated groups in both genotypes in Rs was higher than that of the untreated groups (Table 1).

Table 1. Effects of two different AMF treatments on shoot length (SL), root length (RL), stem diameter (SD), total phenolic concentration (TP), and total antioxidant activity (TAC) in two different bean genotypes treated with *Rhizoctonia solani* (mean $\pm$ standard deviation)

Çizelge 1. *Rhizoctonia solani* ile muamele edilen iki farklı fasulye genotipinde iki farklı AMF uygulamasının sürgün uzunluğu (SL), kök uzunluğu (RL), gövde çapı (SD), toplam fenolik konsantrasyon (TP) ve toplam antioksidan aktivite (TAC) üzerine etkileri (ortalama $\pm$ standart sapma)

Genotype	AMF	<i>Rs</i>	SL (cm)	RL (cm)	SD (mm)	TP (mg GAE / 100 g FW)	TAC (Trolox μmol TE 100 g FW)
V29	Control	(-)	52.28±6.94	27.13±3.23	3.80±0.33	100.29±7.70	31.20±3.20
		(+)	55.45±13.99	24.15±3.89	4.28±0.15	89.73±4.66	32.04±7.98
	ERS	(-)	32.70±2.00	46.50±2.00	5.60±1.00	180.07±2.00	39.55±2.00
		(+)	57.40±7.10	30.75±7.75	4.20±0.20	151.73±23.33	77.60±10.25
	Fm	(-)	57.17±16.24	27.13±5.16	3.90±0.49	94.07±14.10	26.49±9.63
		(+)	88.37±5.75	48.43±11.11	4.30±0.39	130.73±0.66	34.39±9.47
T71	Control	(-)	42.38±7.02	24.57±2.90	4.53±0.69	94.29±23.51	31.76±11.79
		(+)	43.88±5.16	27.90±6.05	4.50±0.46	99.51±8.05	34.79±4.30
	ERS	(-)	53.30±9.90	35.10±2.10	4.45±0.35	123.32±11.75	37.58±3.80
		(+)	47.30±12.21	45.80±13.50	4.07±0.20	171.57±12.83	58.55±11.81
	Fm	(-)	51.53±5.38	29.57±7.78	4.40±0.45	97.40±0.67	47.96±6.44
		(+)	42.00±7.17	32.63±5.48	4.68±0.45	120.07±24.00	47.05±7.40
Genotype (G)							
V29			56.89±18.14 A	33.50±11.27	4.29±0.70	124.44±35.50	38.74±18.02
T71			46.40±8.11 B	32.59±9.31	4.45±0.46	117.69±30.37	42.03±10.99
AMF (A)							
Control			48.49±9.81 B	25.80±3.98 B	4.27±0.50	95.96±12.12 C	32.33±6.57 B
ERS			47.68±12.26 B	39.54±9.79 A	4.59±0.81	156.67±25.97 A	52.84±18.76 A
Fm			57.91±19.20 A	34.44±10.91 A	4.31±0.49	110.57±19.95 B	38.01±12.11 B
<i>Rhizoctonia</i> (Rs)							
(-)			48.29±10.94 B	31.67±8.44	4.39±0.77	114.91±33.42 B	35.06±9.37 B
(+)			54.50±17.30 A	34.38±11.75	4.35±0.36	127.22±31.76 A	45.93±18.38 A
p values (≤0.05)							
Genotype			0.001	0.529	0.554	0.159	0.296
AMF			0.002	<0.001	0.263	<0.001	<0.001
<i>Rs</i>			0.011	0.154	0.472	0.014	<0.001
GxA			0.001	0.265	0.012	0.202	0.001
GxRs			<0.001	0.287	0.668	0.010	0.136
AxRs			0.394	0.030	0.007	0.029	<0.001
GxAxRs			0.015	0.002	0.147	0.002	0.323

There is no statistically significant difference between the same letters ($p \leq 0.05$) according to Duncan's test findings.

Mean (M): Mean is the sum of all data points in our dataset divided by the total number of data points, Standard deviation (SD): It is a method that measures closeness and agreement of observations in a dataset. The p-value is a function used to measure how extreme the observed sample results are, conditional on a statistical model.

ERS: Commercial AMF, Fm: *F. mosseae*, Rs: *Rhizoctonia solani*, TP: Total phenolic concentration, TAC: Total Antioxidant Capacity

The percentage distribution of AMF treatments and Rs combination treatments in V29 and T71 genotypes is given in Figure 1. Accordingly, ERS and Fm % values were close to each other in both V29 and T71 genotypes, and the highest mycorrhizal dependence value was observed in Fm. In the V29 + Rs combination, it was determined that the Fm value was around 40% while ERS was around 0% (Figure 1). In the T71 + Rs combination, while the Fm value was around 25%, ERS was determined to be in the opposite direction with negative (-25%) values (Figure 1).

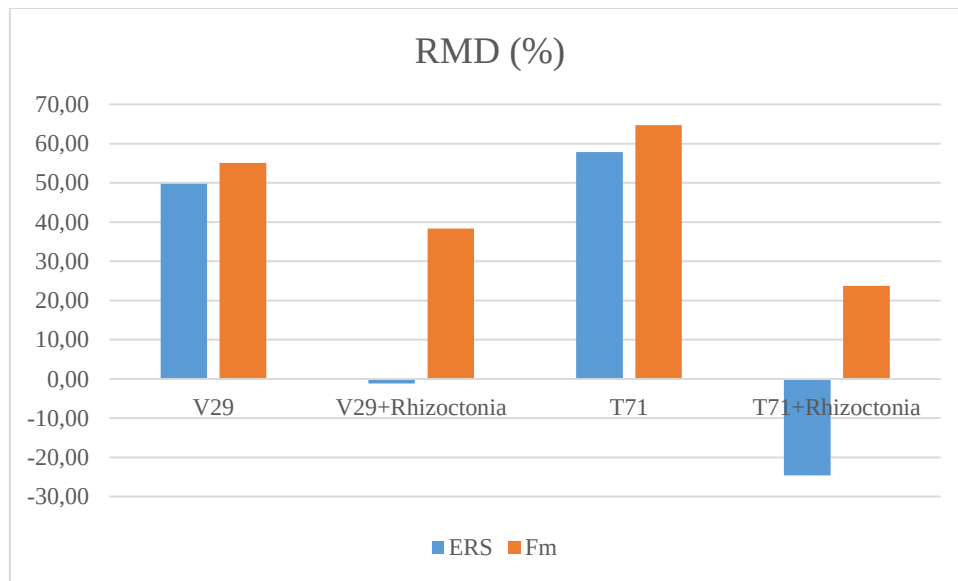


Figure 1. Percentage relative mycorrhizal dependency distribution of the combination of commercial AMF (ERS) and *F. mosseae* treatments with Rs in two different bean plant genotypes (V29 and T71)

Şekil 1. Ticari AMF (ERS) ve *F. mosseae* uygulamalarının Rs ile kombinasyonunun iki farklı fasulye bitkisi genotipinde (V29 ve T71) yüzde nispi mikorizal bağımlılık dağılımı

Table 2 describes the effect of two different AMF treatments on shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), AMF root colonization (%), and *Rhizoctonia* disease scale in two different bean genotypes treated with *R. solani*. Accordingly, in general, the difference between genotypes and pathogen treatments in the colonization parameter or between genotypes in the scale parameter was found statistically insignificant ($p \leq 0.05$). In all other parameters, the difference between treatments was statistically significant ($p < 0.05$). In addition, it was determined that the T71 genotype had a greater effect than the V29 genotype in all plant growth parameters except root colonization and pathogen scale. Similarly, it was determined that Fm AMF treatment had a greater effect than ERS treatment on all plant growth parameters except root colonization and pathogen scale. ERS was more effective than Fm in root colonization and scale parameters (Table 2).

In the shoot fresh weight parameter, the highest value (55.05 g) was observed in the Fm+ Rs (+) combination in the T71 genotype, while the lowest value (22.59 g) was determined in control+ Rs (-) treatment in the V29 genotype. There was a significant difference of about 144% (Table 2). It is noteworthy that both AMF treatments had higher shoot fresh weight values than the control group, despite the Rs pathogen, especially in the V29 genotypes ($p \leq 0.05$) (Table 2). However, it was determined that the shoot fresh weight value of control+ Rs (+) was higher than control+ Rs (-) in the T71 genotype ($p \leq 0.05$) (Table 2).

The difference between AMF treatments in the root fresh weight parameter was found statistically insignificant ($p \leq 0.05$). The highest root fresh weight value (13.58 g) was observed in the ERS + Rs (-) treatment in genotype V29, while the lowest value (2.43 g) was observed in the control + Rs (-) treatment in the same genotype. A difference of approximately 459% was observed between them (Table 2). It was determined that the root fresh weight value of the non-Rs (-) group was higher than the Rs (+) group, and the difference between them was statistically significant ($p \leq 0.05$) (Table 2).

In the shoot dry weight parameter, AMF treatments were higher than the control groups, despite the *Rs* pathogen in both bean genotypes. When AMF treatments were compared, it was determined that the compatibility with the T71 genotype was better, and Fm was higher than ERS (Table 2). It was determined that the highest shoot dry weight value (9.16 g) was in the Fm + Rs (+) combination in the T71 genotype, and the lowest value (3.56 g) was in the control + Rs (-) treatment in V29 genotype, with a difference of approximately 157% (Table 2).

In the root dry weight parameter, the highest value (1.27 g) was in the Fm + Rs (+) combination in the T71 genotype, the lowest value (0.21 g) was in the ERS + Rs (+) treatment in the V29 genotype and a difference of 505% was determined between them. The highest value in this parameter was in the T71 genotype, Fm, and Rs (+) treatments (Table 2). In genotype V29, there was a 92% difference between ERS + Rs (-) treatment (0.48 g) and Fm + Rs (-) (0.25), while there was a 48% difference between ERS + Rs (+) (0.21 g) and Fm + Rs (+) (0.31 g). In the T71 genotype, there was a 176% difference between ERS + Rs (+) treatment (0.46 g) and Fm + Rs (+) (1.27 g), and the difference between them was found to be statistically significant ($p \leq 0.05$) (Table 2).

In the root colonization parameter, ERS (63.31%) was about 15% higher than Fm (54.94%) for both genotypes, and the difference between them was statistically significant ($p \leq 0.05$) (Table 2). The highest root colonization % value was observed in both the V29 genotype ERS+ Rs (-) (65.25%) and T71 ERS+ Rs (+) combination. The lowest value was observed in the T71 Fm+ Rs (-) (52.75%) treatment, and the difference between them was approximately 24% and statistically significant ($p \leq 0.05$). In V29, when AMF treatments (ERS and Fm) were compared with Rs treatments (+ and -), the colonization value decreased in Rs (+), and the opposite was the case in the T71 genotype, and the root colonization value of AMF treatments increased in combination with Rs (+).

The highest pathogen scale value (69.00) was observed in the control+Rs (+) combination of genotype V29, while the lowest value (17.67) was observed in the ERS+Rs (+) treatment of the same genotype. Thus, the ERS reduced the value of the disease scale by almost 74% (Table 2). However, AMF had a significant effect on the pathogen scale value, but the difference between them was statistically insignificant ($p \leq 0.05$). In addition, while the difference in scale value between V29 and T71 genotypes was insignificant ($p \leq 0.05$), the T71 control+Rs (+) group was lower than the V29+Rs (+) combination, and a difference of approximately 21% was determined between them. However, the *R. solani* scale value decreased by approximately 57% with the *F. mosseae* T71 genotype (Table 2).

PCA, Correlation, and Heatmap Analysis

To determine the impact of AMF species (commercial AMF and *F. mosseae*) on *R. solani* in two different bean genotypes (V29 and T71), a Principal Component Analysis (PCA) was used to identify the traits contributing to the significant variation in the observed factors. The eigenvalues, variances, and contributions (PCA loadings) of the traits that differed between treatments were determined using the previously mentioned methods.

In *R. solani*, four principal components (PC) loadings were found when principal component analysis (PCA) was performed on twelve additional disease-related traits, as shown in Table 3. As indicated in Table 3, the initial component (PC1) is responsible for 35.89% of the variation in *Rhizoctonia* disease. SD, SFW, RFW, SDW, and RDW are the most effective explanatory traits for variation in *Rhizoctonia*. The second component (PC2) was found to account for 23.50% of the variation in *Rhizoctonia* disease. The most important contributors to this component are RL, TP, and Colonization. PC3 was found to explain 15.11% of the variation in *Rhizoctonia* with TAC, Scale, and RMD. The fourth component (PC4) accounted for 11.48% of the variation observed in *Rhizoctonia*, with only SL as the most significant contributing trait.

The effects of PCA1 and PCA2 components Fm and ERS on *R. solani* disease stress in two different bean genotypes were generated on a scorecard shown in Figure 2. Unstressed V29+ERS, T71+ERS, T71+Fm, and T71+ERS+Rs treatments are close to each other and in PCA1 and PCA2 positive regions. T71+Fm+Rs and T71+Rs treatments are in PCA1-positive and PCA2-negative regions. However, V29, T71, and V29+Rs treatments are in both PCA1 and PCA2 negative regions. V29+ERS+Rs, V29+Fm+Rs, and V29+Fm treatments were in the PCA1 negative PCA2 positive region. This was found to be associated with AMF interaction with bean genotypes. Therefore, the T71 bean genotype was more effective in reducing *Rs* biotic stress damage with Fm AMF treatment (Figure 2).

The correlation between the variables examined to determine the efficacy of ERS and Fm AMF treatments against *Rs* disease in V29 and T71 bean genotypes is shown in Figure 3. The data show a positive correlation between TP-Colonization, TAC-TP, SDW-RDW, SFW-RDW, SFW-SDW, SD-RFW, RL-TP, and RL-Colonization against *Rs* disease. Conversely, the correlation between SD-SL, RFW-SL, RDW-SL, and RMD scale was found to be negative (Figure 3).

Heat map clustering of traits of bean genotypes V29 and T71 with commercial AMF and *F. mosseae* is given in Figure 4. Red indicates an increase in the attribute in question, while blue indicates a decrease. In the heat maps showing the relationship between the T71 genotype and V29, especially concerning Fm, the predominance of the increase in most traits is striking in red color. The genotype-AMF heat map revealed two distinct clusters.

Table 2. Effects of two different AMF treatments on shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), AMF root colonization (%), and *Rhizoctonia* disease scale in two different bean genotypes treated with *Rhizoctonia solani* (mean $\pm$ standard deviation)

Çizelge 2. İki farklı AMF uygulamasının *Rhizoctonia solani* ile muamele edilen iki farklı fasulye genotipinde iki farklı AMF uygulamasının sürgün taze ağırlığı (SFW), kök taze ağırlığı (RFW), sürgün kuru ağırlığı (SDW), kök kuru ağırlığı (RDW), AMF kök kolonizasyonu (%) ve *Rhizoctonia* hastalık skalası üzerine etkileri (ortalama $\pm$ standart sapma)

Genotype	AMF	<i>Rs</i>	SFW (g)	RFW (g)	SDW (g)	RDW (g)	Colonization (%)	Scale
V29	Control	(-)	22.59 $\pm$ 2.95	2.43 $\pm$ 1.07	3.56 $\pm$ 0.91	0.23 $\pm$ 0.13	-	-
		(+)	32.28 $\pm$ 3.09	4.41 $\pm$ 1.29	4.06 $\pm$ 1.03	0.30 $\pm$ 0.04	-	69.00 $\pm$ 6.68 A
	ERS	(-)	36.47 $\pm$ 2.00	13.58 $\pm$ 2.00	5.08 $\pm$ 1.00	0.48 $\pm$ 0.10	65.25 $\pm$ 7.13	-
		(+)	24.38 $\pm$ 2.20	4.24 $\pm$ 1.14	3.81 $\pm$ 0.50	0.21 $\pm$ 0.02	61.75 $\pm$ 6.55	17.67 $\pm$ 6.42 ^D
	Fm	(-)	29.96 $\pm$ 0.62	5.74 $\pm$ 0.78	5.25 $\pm$ 0.10	0.25 $\pm$ 0.16	56.00 $\pm$ 7.39	-
		(+)	33.07 $\pm$ 5.20	6.19 $\pm$ 0.15	5.36 $\pm$ 0.07	0.31 $\pm$ 0.10	55.00 $\pm$ 8.52	27.75 $\pm$ 6.39 CD
T71	Control	(-)	28.14 $\pm$ 6.10	7.02 $\pm$ 0.56	3.87 $\pm$ 1.09	0.60 $\pm$ 0.19	-	-
		(+)	43.27 $\pm$ 5.55	10.39 $\pm$ 0.81	7.39 $\pm$ 0.12	0.94 $\pm$ 0.18	-	57.25 $\pm$ 3.77 B
	ERS	(-)	35.20 $\pm$ 3.50	7.92 $\pm$ 0.90	5.69 $\pm$ 1.18	0.60 $\pm$ 0.12	61.00 $\pm$ 16.12	-
		(+)	37.61 $\pm$ 8.60	4.65 $\pm$ 1.68	5.59 $\pm$ 1.22	0.46 $\pm$ 0.19	65.25 $\pm$ 10.24	32.00 $\pm$ 14.93 C
	Fm	(-)	35.70 $\pm$ 3.18	7.56 $\pm$ 1.21	5.99 $\pm$ 0.43	0.58 $\pm$ 0.11	52.75 $\pm$ 10.59	-
		(+)	55.05 $\pm$ 7.71	11.09 $\pm$ 3.57	9.16 $\pm$ 1.98	1.27 $\pm$ 0.07	56.00 $\pm$ 6.16	24.75 $\pm$ 5.25 CD
Genotype (G)								
V29			29.41 $\pm$ 5.74 B	6.09 $\pm$ 3.79 B	4.52 $\pm$ 0.96 B	0.29 $\pm$ 0.13 B	59.50 $\pm$ 7.94	40.00 $\pm$ 24.08
T71			39.38 $\pm$ 9.88 A	8.10 $\pm$ 2.66 A	6.28 $\pm$ 1.95 A	0.75 $\pm$ 0.31 A	58.75 $\pm$ 11.28	38.55 $\pm$ 16.91
AMF (A)								
Control			31.76 $\pm$ 9.27 B	6.06 $\pm$ 3.22 B	4.72 $\pm$ 1.78 B	0.55 $\pm$ 0.33 A	-	63.13 $\pm$ 8.04 A
ERS			33.41 $\pm$ 6.90 B	7.59 $\pm$ 4.10 A	5.04 $\pm$ 1.16 B	0.44 $\pm$ 0.18 B	63.31 $\pm$ 9.78 A	24.83 $\pm$ 12.93 B
Fm			38.45 $\pm$ 11.06 A	7.64 $\pm$ 2.74 A	6.44 $\pm$ 1.87 A	0.55 $\pm$ 0.42 A	54.94 $\pm$ 7.57 B	26.25 $\pm$ 5.65 B
<i>Rs</i>								
(-)			30.88 $\pm$ 6.11 B	7.37 $\pm$ 3.55 A	4.91 $\pm$ 1.18 B	0.45 $\pm$ 0.20 B	58.75 $\pm$ 10.94	-
(+)			37.91 $\pm$ 10.93 A	6.83 $\pm$ 3.29 B	5.89 $\pm$ 2.12 A	0.58 $\pm$ 0.40 A	59.50 $\pm$ 8.39	-
p values ($\leq$ 0.05)								
Genotype			<0.001	0.001	<0.001	<0.001	0.827	0.966
AMF			0.004	0.027	0.001	0.019	0.021	<0.001
<i>Rs</i>			<0.001	0.292	0.006	0.009	0.827	-
GxA			0.136	<0.001	0.412	0.001	0.913	0.006
GxRs			0.001	0.002	0.001	0.001	0.385	-
AxRs			<0.001	<0.001	0.005	<0.001	0.913	-
GxAxRs			0.319	0.183	0.410	0.052	0.799	-

Capital letters indicate the comparisons of the means of the applications and small letters indicate the interaction GxA for the scale. There is no statistically significant difference between the same letters ($p \leq 0.05$) according to Duncan's test findings.

Mean (M): Mean is the sum of all data points in our dataset divided by the total number of data points, Standard deviation (SD): It is a method that measures the closeness and agreement of observations in a dataset. The p-value is a function used to measure how extreme the observed sample results are, conditional on a statistical model.

ERS: Commercial AMF, Fm: *F. mosseae*, Rs: *Rhizoctonia solani*

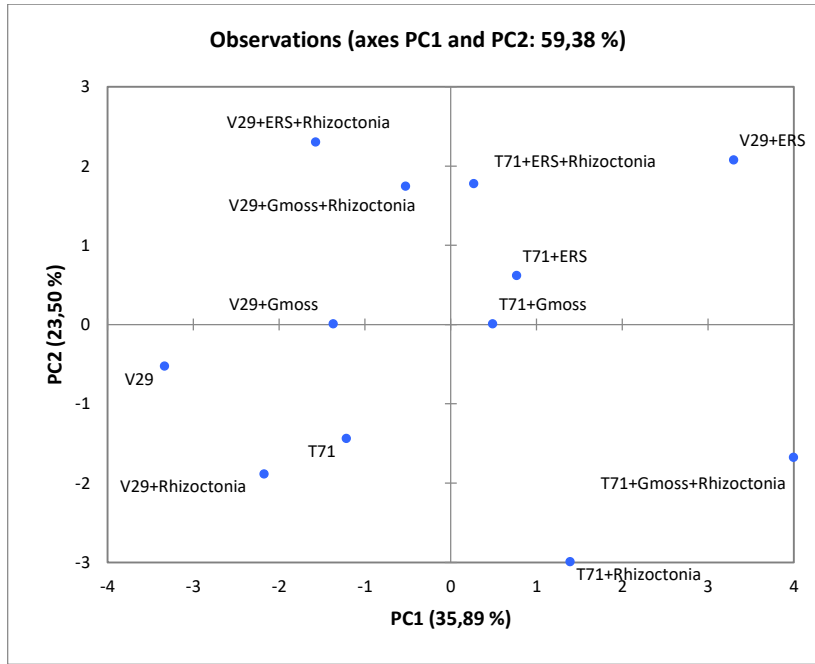


Figure 2. Plot of Principal Component Analysis (PCA) scores of the first two main components.
Şekil 2. İlk iki ana bileşenin Temel Bileşen Analizi (PCA) puanlarının grafiği.

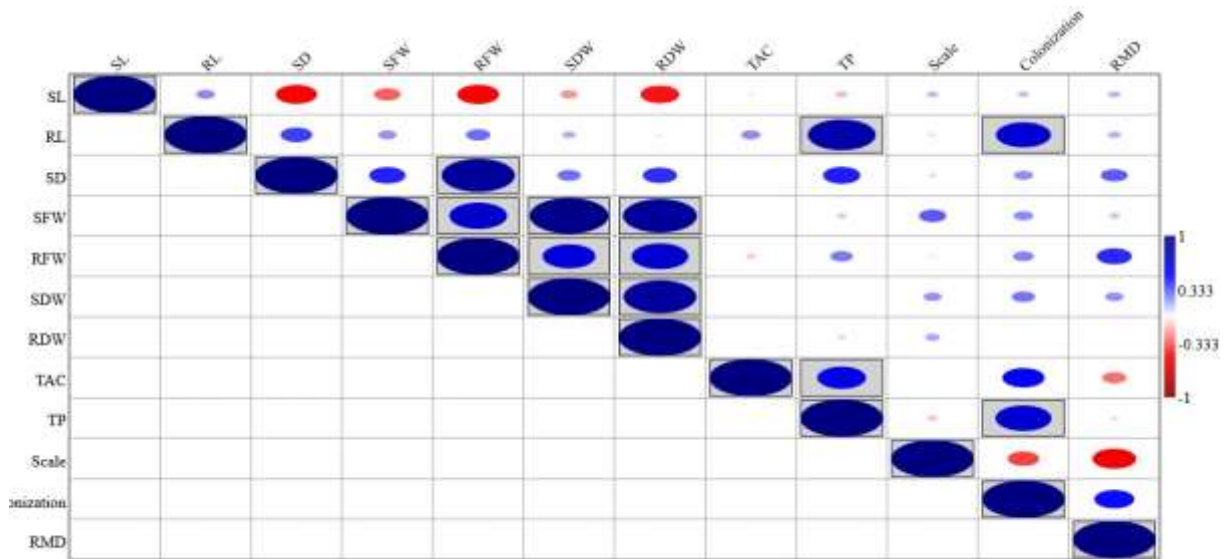


Figure 3. *Rhizoctonia solani* pathogen correlation between the traits used in the study; values in the bold frame are different from 0 and the significance level is alpha: 0,05.

Şekil 3. Çalışmada kullanılan özellikler arasındaki *Rhizoctonia solani* patojen korelasyonu; kalın çerçevedeki değerler 0'dan farklıdır ve anlamlılık düzeyi alfa'dır: 0,05.

Heat map clustering of traits of *R. solani* inoculation of bean genotypes V29 and T71 with commercial AMF and *F. mosseae* is given in Figure 5. Notably, despite disease stress, heat maps showing the relationship between traits in both genotypes and AMF treatments show a dominant decrease in most of the variables (blue color). The highest increase (red color) is between the V29+*Rs* application and the scale.

However, when *F. mosseae* was combined with the same treatment (V29+*Rs*+*Fm*), the heat map value decreased, and the color became blue. It was concluded that *Fm* was significantly affected, especially the scale parameter in the V29 genotype. In other words, AMF treatments increase plant growth characteristics by reducing the pathogenic effect.

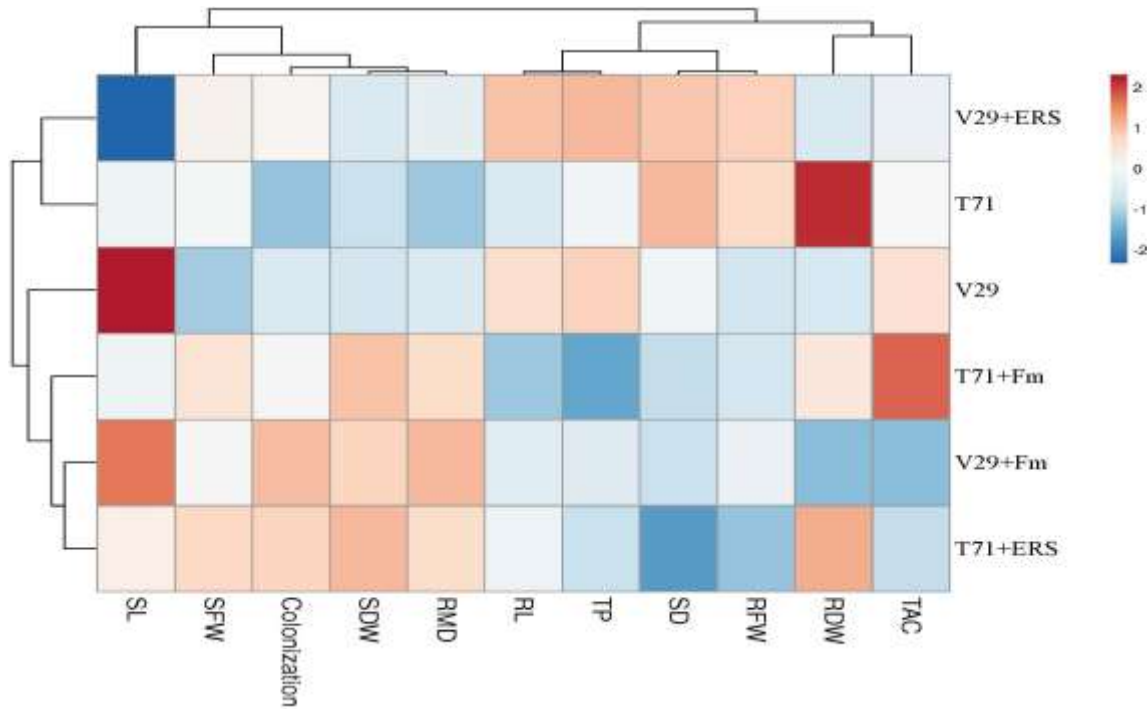


Figure 4. Heat map clustering of traits of bean genotypes V29 and T71 with commercial AMF and *Funneliformis mosseae*

Şekil 4. Fasulye genotiplerinin Ticari AMF ve *Funneliformis mosseae* ile V29 ve T71 fasulye genotiplerinin özelliklerinin ısı haritası kümelenmesi

Another noteworthy point in Figure 5 is that T71+*Rs* and T71+Fm+*Rs* treatments turned slightly pink in plant growth parameters (except SL and RL). In V29+Fm+*Rs* treatment, SL, RMD, and RL parameters, and V29+ERS+*Rs* treatment, TAC, TP, and colonization map temperatures were close to pink and increased. The heat map of *R. solani* disease revealed that genotypes V29 and T71 formed two distinct clusters, grouped with ERS and Fm. Heat maps propagating along the studied features and grouping ERS and Fm without exceedance showed the improvements made with AMF systems. This result was also correlated with genotype differences.

Table 3. Principal component analysis results for the characters of the study.

Çizelge 3. Çalışmanın karakterleri için temel bileşen analizi sonuçları.

	PC1	PC2	PC3	PC4
Eigenvalue	4.306	2.820	1.814	1.377
Variability (%)	35.885	23.496	15.114	11.476
Cumulative %	35.885	59.381	74.495	85.971
SL	-0.218	0.173	0.018	-0.649
RL	0.211	0.427	-0.105	-0.169
SD	0.365	0.061	0.143	0.360
SFW	0.415	-0.202	-0.154	-0.239
RFW	0.440	-0.071	0.174	0.149
SDW	0.387	-0.199	-0.087	-0.355
RDW	0.372	-0.321	-0.090	-0.056
TAC	0.064	0.304	-0.444	0.102
TP	0.204	0.465	-0.259	0.196
Scale	-0.005	-0.252	-0.506	-0.208
Colonization	0.224	0.456	0.043	-0.233
RMD	0.158	0.124	0.614	-0.254

*Values marked in yellow are different from 0, and the significance level is alpha: 0.05

** SL: Shoot Length, RL: Root Length, SD: Shoot Diameter, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, TAC: Total Antioxidant Concentration, TP: Total Phenolic, Scale: *R. solani* 0–4 scale, Colonization: AMF Root Colonization, RMD: Percentage Distribution

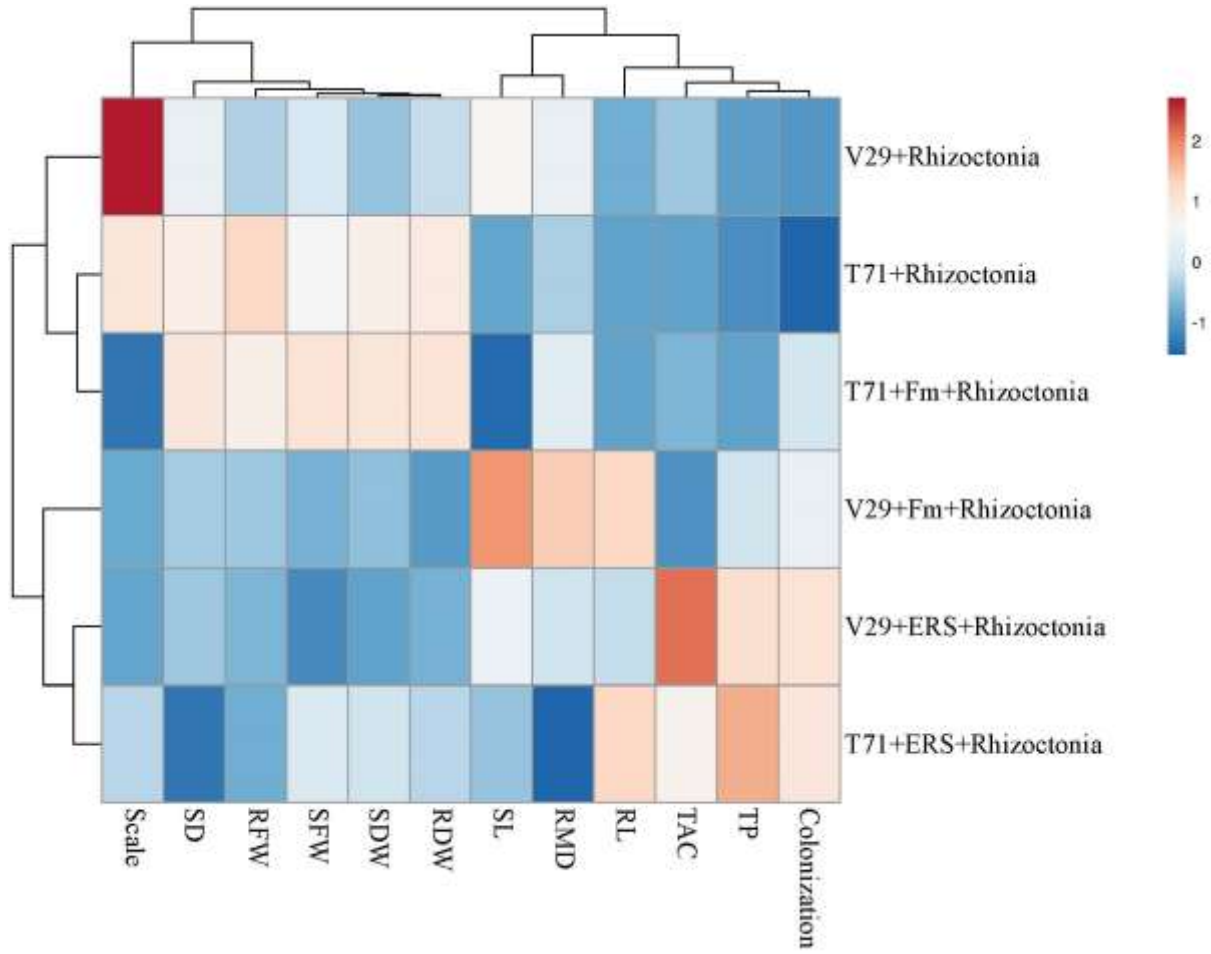


Figure 5. Heat map clustering of traits with commercial AMF and *F. mosseae* in *R. solani* inoculation of bean genotypes V29 and T71

Şekil 5. V29 ve T71 fasulye genotiplerinin *R. solani* inokulasyonunda ticari AMF ve *F. mosseae* ile özelliklerin ısı haritası kümelenmesi

DISCUSSION

In this work, we investigated the effects of commercial AMF and *F. mosseae* applications against the *R. solani* pathogen in two different bean genotypes (V29 and T71) (Table 1). Several studies have shown that *R. solani* root rot infects bean genotypes with different severity. For example, when Durak et al. (2024) investigated the anastomosis groups and pathogenicity of *Rhizoctonia* spp. Isolated from various bean genotypes grown in the Van Lake Basin, the most virulent group was AG-4. Isolate number 19 in genotype A64, isolate number 2 in TR68557, and isolate number 18 in cv. Gina was shown to be the most pathogenic isolate in *in vivo* studies.

Regardless of genotype, we found that AMF applications elevated plant growth metrics despite the *R. solani* pathogen (except RDW in ERS). According to a study by Moarrefzadeh et al. (2023), the first four of the six AMF species (*F. mosseae*, *Glomus claroideum*, *G. etunicatum*, *G. margarita*, *G. caledonium*, and *G. versiform*) significantly improved root length, shoot length, fresh-dry weight, and reduced disease incidence compared to the infected control. The highest root colonization was found in *Glomus fasciculatum*. ERS commercial AMF (mixture) showed the highest root colonization in our study (Table 2).

Furthermore, according to Moarrefzadeh et al. (2023), *F. mosseae* and *G. claroideum* had the lowest disease severity and incidence, respectively. In contrast, in our study, there was no statistically significant difference between AMF species in this respect, while ERS had the lowest value (Table 2). Beneficial microbes, such as arbuscular mycorrhizal fungi, offer a way to replace or reduce the use of fungicides in agriculture (Alabouvette et al., 2006).

AMF is useful in the control of plant diseases and improves ecosystem sustainability and productivity (Gianinazzi et al., 2010). The damage caused by *R. solani* to both bean genotypes was reduced by the use of mycorrhizal plants in this study. ERS and *F. mosseae* were found to reduce the disease scale value, and this effect was explained by

the disease severity in mycorrhizal plants. Previous reports on common beans or other plants have documented the anti-*R. solani* effect of some AMF species.

According to Aljawasim et al. (2020), disease severity induced by *R. solani* in cucumber plants decreased from 65% to 21% when *F. mosseae* mycorrhized. Furthermore, to improve growth parameters and reduce the severity and incidence of *R. solani* in common beans, Hafez et al. (2013) investigated the effect of a mixture containing five AMF species, *F. mosseae*, *Rhizophagus intraradices*, *G. clarum*, *Gigaspora margarita*, and *Gigaspora gigantea*, and found it to be significantly effective. According to Bagy et al. (2019), AMF primarily reduces the infection of plant roots by various soil pathogens, which in turn reduces disease incidence and severity index.

AMF has been proposed to work in several ways to strengthen plant defenses against soil-borne plant diseases (Kareem & Hassan, 2014). AMF is involved in the absorption and transport of nutrients to the roots, which reduces root damage (Liu et al., 2019). It also enhances plant growth by expanding their outer mycelium and activates plant defense mechanisms against soil pathogens through biochemical, physiological, and structural changes in the plant. These mechanisms include competition with pathogens in plant roots in the rhizosphere for host photosynthetic products and increased resistance/tolerance of AMF-inoculated plants to invasion by some plant pathogens (Amer & Abou El, 2008).

In the present study, *R. solani* did not show variable results between both bean genotypes (V29 and T71) or between both AMF treatments (ERS and *F. mosseae*), but ERS reduced the disease scale value by 74% in the V29 genotype and about 57% in the Fm T71 genotype. Moarrefzadeh et al. (2023) reported that AMF species *F. mosseae* and *G. claroideum* caused more than a 40% reduction in disease severity in beans compared to *R. solani*-infected control, which is in agreement with our findings. The fact that the biocontrol effect of AMF depends on several variables, including substrate, host plant, and even variety, may help explain these variations (Yao et al., 2002; Moarrefzadeh et al., 2022).

CONCLUSION

The results of the study suggest that AMF has a strong chance of biologically controlling *R. solani* in bean genotypes V29 and T71. As a result, AMF species had different beneficial effects. For example, plants inoculated with ERS had lower disease severity than plants inoculated with Fm, while *F. mosseae* exhibited the greatest positive effect on plant growth. Thus, regardless of the bean genotype, inoculation with appropriate AMF species can help plants compensate for the damage caused by *R. solani* and reduce the harmful effects of the pathogen. In conclusion, the mycorrhizal treatments used in this research can be considered viable, economical, and ecologically sound biological control strategies to deal with this soil-borne disease and as long-term biostimulants to promote bean plant growth. Further studies are needed to determine how well these AMF applications also work in the field to suppress this pathogen in different bean genotypes and how effective they are in combination with additional biostimulants.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

Research involving human participants and/or animals is not applicable

Neither humans nor animals were used in the study.

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Some Macrofungi Determined in Oltu and Narman (Erzurum-Türkiye) Region

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ABSTRACT

This study reports on macrofungus samples collected within the borders of Oltu and Narman (Erzurum) districts in 2018 and 2019. As a result of field and laboratory studies, 198 species were identified. The species identified in the region are distributed in 5 classes, 12 orders, 52 families, and 103 genera. 187 of the identified species are new to the research area. The species are listed with their habitat, locality, and edibility status.

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ÖZET

Bu çalışma, 2018 ve 2019 yıllarında Oltu ve Narman (Erzurum) ilçe sınırları içerisinde toplanan makrofungus örneklerini rapor etmektedir. Arazi ve laboratuvar çalışmaları sonucunda 198 tür belirlenmiştir. Bölgede tespit edilen türler 5 sınıf, 12 takım, 52 familya ve 103 cins içerisinde dağılım göstermektedir. Belirlenen türlerin 187 tanesi araştırma alanı için yenidir. Türler habitat, lokalite ve yenilebilirlik durumları ile birlikte liste halinde sunulmuştur.

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INTRODUCTION

Oltu district is located within Erzurum province's borders between 40° 33' 0" north latitude and 41° 58' 2" east longitude. The total surface area of the district is approximately 1380.40 km², the altitude of the district centre is 1275 m, and the distance to the city center is 129 km (Külekçi and Bulut, 2013). Narman District is located between 40°22'0" N latitudes and 41°55'59" E longitudes, lying at a distance of 96 km from 2 Erzurum province, covering an area of 1275 km and at an altitude of 1640 m (Külekçi and Koç, 2020). Both districts within the borders of Erzurum province provide a suitable habitat for macrofungus development due to their rich vegetation and forested areas.

The effects of climatic factors such as temperature, humidity, and precipitation on fungal development are important. According to the meteorological data obtained from Erzurum Meteorology Station in 2019, the average temperature, relative humidity, and precipitation amounts in Oltu and Narman districts, which have extremely cold winters and semi-arid Mediterranean climates, are 9.8 °C, 9.2 °C; 59.5%, 56.7%; 32.53 mm, 28.92 mm, respectively.

Fungi are immensely diverse, with 144,000 species named and classified at a current rate of around 2,000 per year. However, it is estimated that the vast majority (over 93%) of fungal species are currently unknown to science. The latest best estimate suggests that the total number of fungal species on Earth is somewhere between 2.2 and 3.8 million, exceeding the estimated number of plants by more than 6 times (Willis, 2018; Hawksworth & Lücking,

2017). Although studies on macrofungus systematics have increased in recent years, both in our country and worldwide, it is believed that this rate should be further increased to address such a significant deficiency.

The current checklist (Sesli et al., 2020; Solak ve Türkoğlu, 2022) and the latest studies (Acar et al., 2020; Acar et al., 2021; Çetinkaya & Uzun, 2021; Çetinkaya et al., 2021; Çevik et al., 2021; Doğan et al., 2021; Keleş & Kaya, 2021; Kesici & Uzun, 2021; Oruç et al., 2021; Şelem et al., 2021; Berber et al., 2022; Kaplan et al., 2023; Keleş & Kaya, 2023; Akata et al., 2024a; 2024b; 2024 c; 2024d; 2024e; Dalkıran et al., 2024; Doğan et al., 2024; Kaygusuz et al., 2024; Keleş et al., 2024; Kesici et al., 2024; Uzun et al., 2024) indicated that there isn't a specific work on the macrofungal biodiversity of Oltu and Narman districts.

The study aims to determine the macrofungal composition of Oltu and Narman districts, and to make a contribution to the mycobiota of Türkiye.

MATERIAL and METHOD

The research materials were collected from 47 suitable habitats in the Oltu and Narman districts of Erzurum province between 2018 and 2019 (Table 1).

Table 1. Locality information of the determined species

Çizelge 1. Tespit edilen türlerin lokalite bilgileri

No.	Lokalite	Koordinat	Altitude
1	Erzurum, Oltu, Southern slope of İnciköy pass	40° 34.050'K, 41° 50.455'D	2016 m
2	Erzurum, Oltu, Southwestern slope of İnciköy pass	40° 34.162'K, 41° 50.195'D	2046 m
3	Erzurum, Oltu, İnciköy pass Western slope	40° 34.342'K, 41° 49.592'D	2182 m
4	Erzurum, Oltu, İnciköy pass peak point	40° 34.430'K, 41° 50.036'D	2173 m
5	Erzurum, Oltu, İnciköy pass North-west slope 1	40° 34.566'K, 41° 49.890'D	2165 m
6	Erzurum, Oltu, İnciköy pass North west slope 2	40° 34.657'K, 41° 50.064'D	2092 m
7	Erzurum, Oltu, İnciköy recreation area	40° 34.773'K, 41° 50.064'D	2104 m
8	Erzurum, Oltu, On the İnciköy Esenyamaç village road	40° 36.012'K, 41° 50.749'D	1790 m
9	Erzurum, On the Oltu-Narman road, near the brick factory	40° 29.221'K, 41° 57.603'D	1369 m
10	Erzurum, On the Oltu-Narman road, around Ünlükaya village	40° 25.468'K, 41° 57.453'D	1455 m
11	Erzurum, On the Narman-Köprükoy road, around the Narman junction	40° 18.680'K, 41° 53.508'D	1578 m
12	Erzurum, Narman, Opposite Göllü village 1	40° 13.828'K, 41° 52.075'D	1863 m
13	Erzurum, Narman, Opposite Göllü village 2	40° 13.649'K, 41° 52.138'D	1937 m
14	Erzurum, Narman, Yanıktaş Village	40° 16.469'K, 41° 51.745'D	1637 m
15	Erzurum, Narman, Around Telli village	40° 13.219'K, 41° 48.362'D	1854 m
16	Erzurum, Narman, Around Yoldere village	40° 17.474'K, 41° 52.555'D	1607 m
17	Erzurum, Oltu, Obayayla village, recreation area vicinity 1	40° 30.547'K, 42° 3.990'D	1941 m
18	Erzurum, Oltu, Obayayla village, recreation area vicinity 2	40° 30.509'K, 42° 4.092'D	1984 m
19	Erzurum, Oltu, Obayayla village, recreation area vicinity 3	40° 30.519'K, 42° 4.367'D	1946 m
20	Erzurum, Oltu, Bahçelikişla village	40° 35.107'K, 42° 9.318'D	1501 m
21	Erzurum, Oltu, İnancı village	40° 28.719'K, 41° 41.974'D	2037 m
22	Erzurum, Narman, Around Telli village 1	40° 12.775'K, 41° 47.324'D	1950 m
23	Erzurum, Narman, Around Telli village 2	40° 12.617'K, 41° 47.796'D	2045 m
24	Erzurum, Narman, Around Telli village 3	40° 12.433'K, 41° 48.257'D	2104 m
25	Erzurum, Narman, Around Çamlıyayla village	40° 11.938'K, 41° 49.861'D	2067 m
26	Erzurum, Narman, Around Mercimekli village	40° 12.766'K, 41° 50.090'D	1940 m
27	Erzurum, Oltu, Çamlıbel village	40° 28.931'K, 41° 46.099'D	1720 m
28	Erzurum, Oltu, Derebasi village passage	40° 38.182'K, 41° 55.547'D	2066 m
29	Erzurum, Oltu, Around Derebaşı village	40° 38.266'K, 41° 54.616'D	1750 m
30	Erzurum, Oltu, Uzunoluk recreation area	40° 37.884'K, 41° 56.473'D	1903 m
31	Erzurum, Oltu, Around Gökçedere village	40° 36.689'K, 41° 58.117'D	1710 m
32	Erzurum, Narman, Surroundings of Göllü village, recreation area 1	40° 13.289'K, 41° 52.463'D	1972 m
33	Erzurum, Narman, Surroundings of Göllü village, recreation area 2	40° 13.142'K, 41° 52.659'D	2010 m
34	Erzurum, Narman, Around Mercimekli village	40° 12.634'K, 41° 50.178'D	1961 m
35	Erzurum, Narman, Around Çamlıyayla village	40° 11.383'K, 41° 49.424'D	2210 m
36	Erzurum, Narman, opposite Göllü village	40° 13.584'K, 41° 52.391'D	1907 m
37	Erzurum, Narman, Around İlca village	40° 12.995'K, 41° 52.716'D	2058 m
38	Erzurum, Oltu, Obayayla village recreation area	40° 30.392'K, 42° 4.198'D	1990 m
39	Erzurum, Oltu, Around Obayayla village 1	40° 29.885'K, 42° 4.403'D	1976 m
40	Erzurum, Oltu, Around Obayayla village 2	40° 29.887'K, 42° 4.915'D	2171 m
41	Erzurum, Oltu, Around Aşağıçam village	40° 32.521'K, 42° 4.899'D	1691 m
42	Erzurum, Oltu, On the road to Gökçedere village Uzunoluk Recreation area 1	40° 36.811'K, 41° 58.656'D	1575 m
43	Erzurum, Oltu, On the road to Gökçedere village Uzunoluk Recreation area 2	40° 36.754'K, 41° 58.596'D	1612 m
44	Erzurum, Narman, On the Yanıktaş-Telli village road	40° 13.251'K, 41° 48.445'D	1833 m
45	Erzurum, Oltu, Gökçedere-Derebaşı village road	40° 37.694'K, 41° 55.085'D	1963 m
46	Erzurum, Oltu, Gökçedere village Uzunoluk recreation area vicinity	40° 37.825'K, 41° 56.639'D	1871 m
47	Erzurum, Oltu, Gökçedere-Uzunoluk recreation area road	40° 36.800'K, 41° 57.956'D	1650 m

The locations of the collected fungal samples were transferred to the numerical environment using the GIS (Geographic Information Systems) technique (Fig. 1). Digital maps were then obtained with the ArcMap 10.2 program.

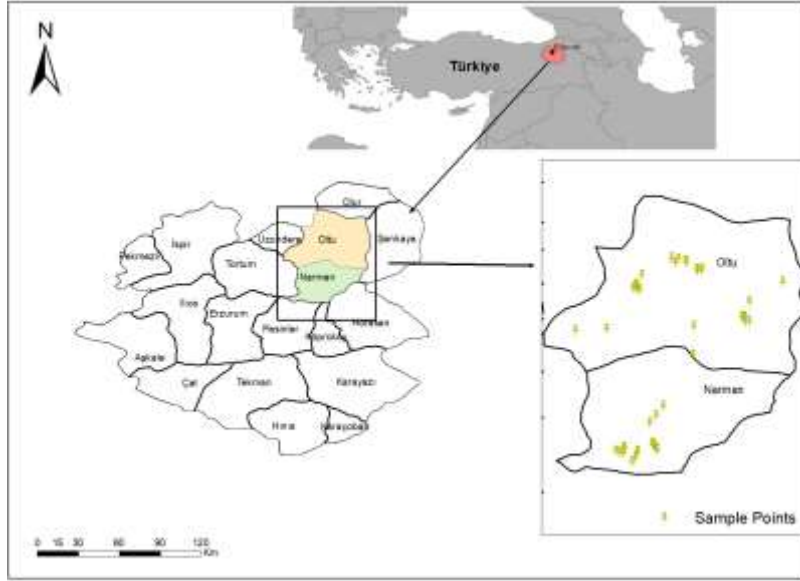


Figure 1. The map showing the district borders of Oltu and Narman, where macrufungi were collected
Şekil 1. Makrofungusların toplandığı Oltu ve Narman ilçe sınırlarını gösteren harita

During field studies, fruit bodies were photographed in their natural habitats, and their characteristics related to morphology and ecology were noted. Then the fungal samples were taken to the laboratory for further investigations. After spore prints were taken, fresh samples were dried. Macroscopic and microscopic analyses, as well as micro-chemical reactions, were performed using dried samples. Reagents such as Melzer's reagent, 5% KOH, H₂O, H₂SO₄, congo red, and cotton blue, etc., were used to make microscopic characters (ascus, basidium, spores, etc.) more clearly visible and distinct. Microscopic investigations were made using a light microscope (Leica DM500) and measurements with the Leica Application Suite (version 3.2.0) software. The identification of the materials was conducted based on the relevant literature (Breitenbach and Kränzlin 1984, 1986, 1991, 1995, 2000, Bas et al. 1988, 1990, 1995, Hansen and Knudsen 1992, 1997, 2000, Ryvarden and Gilbertson 1993, Pegler et al. 1997, Noordeloos et al. 2001, Jordan 2004, Kränzlin 2005, Medardi 2006, Knudsen and Versterholt 2008). The study materials were kept in the fungarium of Yüzüncü Yıl University (VANF).

RESULTS

The species determined in the region were alphabetically listed along with notes on the habitats, collection dates, and accession numbers (Cemil SADULLAHOĞLU: CS). Turkish names of taxa are given according to Sesli et al. (2020) and Anonymous 2025b. The systematic position of each taxa was given considering the Index Fungorum (Anonymous, 2025a).

Ascomycota Whittaker

Leotiomyces O.E. Erikss. & Winka

Helotiales Nannf.

Helotiaceae Rehm

1. *Cyathicula cyathoidea* (Bull.) Thüm. / Cıblı dikenli kadeh: On decaying plant residues, 06.06.2018, unedible, [8], CS. 119.
2. *Hymenoscyphus fructigenus* (Bull.) Gray / Meyve mırı: On fallen oak branch bark, unedible, 02.11.2018, [44], CS. 786.

Lachnaceae Raitv.

3. *Capitotricha bicolor* (Bull.) Baral / Papatya mantarı: On plant residue, unedible, 06.06.2018, [6], CS. 73.
4. *Lachnum brevopilosum* Baral / Kılıklı akçanak: On cone residue, unedible, 06.06.2018, [6, 8, 17], CS. 74, 113, 249.

Mollisiaceae Rehm

5. *Mollisia cinerea* (Batsch) P. Karst. / Boz tepsi: On poplar branch residue, unedible, 19.06.2019, [13], CS. 806.

Rutstroemiaceae Holst-Jensen, L.M. Kohn & T. Schumach.

6. **Rutstroemia firma** (Pers.) P. Karst. / Kuş düdüğü: On wood residue, unedible, 21.09.2019, [30], CS. 980.

Pezizomycetes O.E. Erikss. & Winka

Pezizales J. Schröt.

Discinaceae Benedix

7. **Discina ancilis** (Pers.) Sacc. / Dibi buruşuk: Under conifer trees, edible, 06.06.2018, [8], CS. 110.

Helvellaceae Fr.

8. **Dissingia leucomelaena** (Pers.) K. Hansen & X.H. Wang / Çukur semer mantarı: Under conifer trees, edible, 07.06.2018, [23], CS. 405.

9. **Helvella acetabulum** (L.) Quél. Kuzu kulağı mantarı: Under conifer trees, edible, 05-06.06.2018, [1, 5, 8], CS. 10, 70, 130.

10. **Helvella fibrosa** (Wallr.) Korf / Tüylü semer mantarı: On conifer leaf residues, unknown, 30.06.2018, [20], CS. 350.

11. **Helvella lacunosa** Afzel. / Bet semer mantarı: Mixed forest, edible, 07.06.2018, [9], CS. 141.

12. **Helvella latispора** Boud. / Kertik semer mantarı: Among conifer leaf residues, unedible, 06.06.2018, [20], CS. 351.

Morchellaceae Rchb.

13. **Morchella angusticeps** Peck / Köşeli göbek: Under conifer trees, edible, 06-07.06.2018, [7, 16], CS. 101, 103, 220, 242.

14. **Morchella esculenta** (L.) Pers. / Kuzu göbeği: Mixed forest, edible, 07.06.2018, [16], CS. 243.

15. **Morchella populiphila** M. Kuo, M.C. Carter & J.D. Moore / Kavak kuzu göbeği: Mixed forest, edible, 07.06.2018, [16], CS. 232, 234.

16. **Morchella semilibera** DC. / Hacı takkesi: Under poplar, edible, 07.06.2018, [16], CS. 238, 239, 240.

17. **Verpa bohemica** (Krombh.) J. Schröt. / Çan mantarı: Mixed forest, edible, 07.06.2018, [14], CS. 193.

Otideaaceae Eckblad

18. **Otidea bufonia** (Pers.) Boud. / Sık yer kulak: Mixed forest, unedible, 02.11.2018, [47], CS. 780.

Pezizaceae Dumort.

19. **Marcelleina atrovioleacea** Brumm. / Mor buztutan: On moss-covered conifer residues, unedible, 21.09.2019.2018, [42], CS. 952.

20. **Peziza fimeti** (Fuckel) E.C. Hansen / Ezik çanak: On cow manure, unedible, 01.07.2018, [35], CS. 425.

21. **Peziza repanda** Wahlenb. / Battal çanak: On wood residue, unedible, 07.06.2018, [15], CS. 210.

22. **Peziza violacea** (Bull.) Relhan / Pembe çanak: On burnt soil, unedible, 06.06.2018, [4], CS. 824.

Pyronemataceae Corda

23. **Anthracobia macrocystis** (Cooke) Boud. / Zerzenne: On burnt soil, unedible, 02.11.2018, [42], CS. 745.

24. **Geopora sepulta** (Fr.) Korf & Burds. / Düz dalga topu: Meadow, unedible, 07.06.2018, [15], CS. 213.

25. **Scutellinia scutellata** (L.) Lambotte / Dikenli tabak: By the stream, on soil, unedible, 05.07.2019, [45], CS. 830.

26. **Sepultariella semiimmersa** (P. Karst.) Van Vooren, U. Lindem. & Healy / Torba deren: Among mosses, under conifer trees, unedible, 28.09.2018, [34], CS. 613.

Sordariomycetes O.E. Erikss. & Winka

Xylariales Nannf.

Xylariaceae Tul. & C. Tul.

27. **Xylaria hypoxylon** (L.) Grev. / Güdük ölü parmak: On the base of moss-covered log, unedible, 02.11.2018, [44], CS. 772.

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Agaricales Underw.

Agaricaceae Chevall.

28. **Agaricus arvensis** Schaeff. / At mantarı: Meadow, edible, 07.06.2018, [13], CS. 181.

29. **Agaricus bresadolanus** Bohus / Halkalı kızıl: Meadow, unedible, 07.06.2018, [9], CS. 137.

30. **Agaricus campestris** L. / İçi kızıl: Meadow, edible, 07.06.2018, [11], CS. 156.

31. **Agaricus macrocarpus** F.H. Möller / Çayır mantarı: Mixed forest, edible, 07.06.2018, [14], CS. 194.

32. **Agaricus subperonatus** (J.E. Lange) Singer / Kestane mantarı: Among conifer leaves, edible, 06.06.2018, [8], CS. 109.

33. **Agaricus sylvaticus** Schaeff. / Orman mantarı: Under conifer trees, edible, 30.06.2018, [20], CS. 333.

34. **Agaricus sylvicola** (Vittad.) Peck / Boylu kızıl: Under conifer trees, edible, 06.06.2018, CS. [8], 115.

35. **Coprinus comatus** (O.F. Müll.) Pers. / Söbelen: Meadow, edible, 06.06.2018; 28.09.2018; 21.09.2019, [10, 15, 26], CS. 154, 204, 635, 1001.

36. *Lepiota clypeolaria* (Bull.) P. Kumm. / Ladin pullusu: Mixed forest, unedible, 05-30.06.2018, [2, 3, 17, 18, 20], CS.20, 35, 278, 303, 354.
37. *Macrolepiota procera* (Scop.) Singer / Kartal ayağı: Under conifer trees, edible, 20.09.2019, [42], CS. 914.
38. *Tulostoma brumale* Pers. / Gözenek mantarı: Mixed forest, unedible, 05.06.2018, [2], CS. 22.
- Amanitaceae** E.-J. Gilbert
39. *Amanita eliae* Quél. / El kesesi: Mixed forest, unedible, 30.06.2018, [17], CS. 292.
40. *Amanita fulva* Fr. / Parlak kese: Under conifer trees, edible, 30.06.2018, [21], CS. 367.
41. *Amanita gemmata* (Fr.) Bertill. / Kaba kese: Under conifer trees, unedible, 30.06.2018, [18, 19], CS. 301, 311, 316, 326.
42. *Amanita pantherina* (DC.) Krombh. / Panter mantarı: Under conifer trees, poisonous, 30.06.2018, [17, 18, 20] CS. 272, 298, 317, 340, 357.
43. *Amanita vaginata* (Bull.) Lam. / Yarıklı kese: Under conifer trees, edible, 01.07.2018, [22], CS. 383.
44. *Saproamanita vittadinii* (Moretti) Redhead, Vizzini, Drehmel & Contu / Duvakluca: Meadow, edible, 07.06.2018, [11], CS. 157.
- Bolbitiaceae** Singer
45. *Conocybe apala* (Fr.) Arnolds / Ak yalın etek: Meadow, unedible, 07.06.2018, [11], CS. 158.
46. *Conocybe blattaria* (Fr.) Kühner / Er yalın etek: Mixed forest, unedible, 02.11.2018, [44], CS. 787.
47. *Conocybe rickenii* (Jul. Schäff.) Kühner / El yalın etek: Meadow, unedible, 07.06.2018, [15], CS. 207.
48. *Conocybe tenera* (Schaeff.) Fayod / Daz yalın etek: Meadow, unedible, 07.06.2018, [12], CS. 170.
- Clavariaceae** Chevall.
49. *Clavaria acuta* Sowerby / Sivri çomak: Mixed forest, edible 30.06.2018, [20], CS. 352.
- Clitocybaceae** Vizzini, Consiglio & M. Marchetti
50. *Clitocybe bresadolana* Singer / El huni mantarı: Mixed forest, unedible, 05.06.2018, [2], CS. 23.
51. *Clitocybe catinus* (Fr.) Quél. / Beyaz huni mantarı: Under conifer trees, unedible, 30.06.2018, [17], CS. 255.
52. *Clitocybe dealbata* (Sowerby) P. Kumm. / Ağulu huni mantar: Under conifer trees, poisonous, 07.06.2018, [13], CS. 177.
53. *Collybia phyllophila* (Pers.) Z.M. He & Zhu L. Yang / Dallı huni mantar: Under conifer trees, poisonous, 30.06.2018, [17], CS. 288.
54. *Collybia nuda* (Bull.) Z.M. He & Zhu L. Yang / Mavi cincile: Mixed forest, edible, 06-07.06.2018; 30.06.2018; 27.09.2018; 05.07.2019, [8, 14, 20, 34, 46], CS. 114, 189, 337, 611, 845.
- Cortinariaceae** R. Heim ex Pouzar
55. *Calonarius elegantissimus* (Rob. Henry) Niskanen & Liimat. / Kel örümcek mantarı: Mixed forest, unedible, 24.08.2018, [28], CS. 480.
56. *Cortinarius decipiens* (Pers.) Fr. / Som örümcek mantarı: Mixed forest, unedible, 07.06.2018, [16], CS. 225.
57. *Cortinarius orellanus* Fr. / Ağulu örümcek mantarı: Mixed forest, poisonous, 07.06.2018, [13], CS. 187.
58. *Cortinarius pulchripes* J. Favre / Kır örümcek mantarı: Under willow, unedible, 07.06.2018, [9], CS. 142.
59. *Cortinarius vernus* H. Lindstr. & Melot / Sidikli örümcek mantarı: Under conifer trees, unedible, 30.06.2018, [21], CS. 373.
60. *Meotomycetes dissimulans* (Berk. & Broome) Vizzini: Mixed forest, unknown, 07.06.2018, [16], CS. 235, 236.
61. *Thaxterogaster vibratilis* (Fr.) Niskanen & Liimat. / Titrek örümcek mantarı: Under conifer trees, unedible, 30.06.2018, [17], CS. 269.
- Entolomataceae** Kotl. & Pouzar
62. *Entoloma lampropus* (Fr.) Hesler: Under conifer trees, unknown, 01.07.2018, [25], CS. 428.
63. *Entoloma longistriatum* (Peck) Noordel. / Uzun kıvrıkbaş: On the conifer residue, poisonous, 30.06.2018; 01.07.2018, [17, 23], CS. 262, 398
64. *Entoloma rusticoides* (Gillet) Noordel. / Pash kıvrıkbaş: Meadow, unedible, 07.06.2018, [12], CS. 172.
65. *Entoloma undatum* (Gillet) M.M. Moser / Kesik kıvrıkbaş: Mixed forest, unedible, 05.06.2018, [4], CS. 42.
66. *Rhodophana nitellina* (Fr.) Papetti / Kızıl kafa: Mixed forest, unedible, 05.06.2018, [43], CS. 43.
- Galeropsidaceae** Singer
67. *Panaeolus fimicola* (Fr.) Quél. / Yoz ters çanı: On manure, poisonous, 07.06.2018; 21.09.2019, [14, 40], CS. 195, 999
68. *Panaeolus semiovatus* (Sowerby) S. Lundell & Nannf. / Kel ters çanı: On bovine manure, unedible, 06.06.2018, [21], CS. 379.
- Hygrophoraceae** Lotsy
69. *Arrhenia rickenii* (Hora) Watling / Narin yosun mantarı: Meadow, unedible, 07.06.2018, [15], CS.215.
70. *Hygrocybe acutoconica* (Clem.) Singer / Has mum mantarı: Under conifer trees, unedible, 01.07.2018, [27], CS. 450.
71. *Hygrophorus ligatus* (Fr.) Fr. / Engin gaypaşuk: Under conifer trees, unedible, 30.06.2018, [17], CS. 290.

Hymenogasteraceae Vittad.

72. *Galerina marginata* (Batsch) Kühner / Kenar galerina: On conifer wood residues, poisonous, 30.06.2018; 02.11.2018, [20, 43], CS. 358, 747.
73. *Hebeloma birrus* (Fr.) Gillet / Yünlü turp kokan: Under conifer trees, unedible, 06.06.2018, [6, 7], CS. 84, 100.
74. *Hebeloma crustuliniforme* (Bull.) Quél. / Benekli turp kokan: Under conifer trees, poisonous, 06.06.2018; 30.06.2018; 01.07.2018, [8, 17, 19, 20, 22], CS. 132, 263, 324, 356, 387
75. *Hebeloma laterinum* (Batsch) Vesterh. / Yatık turp kokan: Under conifer trees, unedible, 06.06.2018, [8], CS. 131.
76. *Hebeloma mesophaeum* (Pers.) Quél. / Ala turp kokan: Under conifer trees, poisonous, 07.06.2018, [13], CS. 178.
77. *Hebeloma populinum* Romagn. / Kavak turp kokan: Mixed forest, unedible, 07.06.2018, [13], CS. 176, 186.
78. *Hebeloma sinapizans* (Paulet) Gillet: Under conifer trees, poisonous, 06.06.2018, [7], CS. 99.
79. *Psilocybe coronilla* (Bull.) Noordel. / Kefgarik: Meadow, edible, 07.06.2018, [15], CS. 205.
80. *Psilocybe subcoprophila* (Britzelm.) Sacc. / Ters kefgarik: On horse manure, poisonous, 06.06.2018, [16], CS. 228.

Incerta Sedis

81. *Crucibulum laeve* (Huds.) Kambly / Kuş yuvası: On conifer branch residues, unedible, 06.06.2018; 30.06.2018; 30.10.2018, [5, 6, 8, 17, 18, 37], CS. 68, 81, 112, 248, 287, 318, 666.
82. *Cyathus olla* (Batsch) Pers. / Çizgili yuva: On soil, unedible, 21.09.2019, [42], CS. 954.
83. *Cystoderma carcharias* (Pers.) Fayod / Gamsız nar mantarı: Under conifer trees, unedible, 30.10.2018, [37], CS. 653.
84. *Cystodermella cinnabarina* (Alb. & Schwein.) Harmaja / Nazlı tozlu deri: Under conifer trees, unedible, 06.06.2018; 30.06.2018, [5, 6, 20] CS. 71, 85, 338.
85. *Cystodermella granulosa* (Batsch) Harmaja / Tozlu deri: Under conifer trees, unedible, 01.11.2018, [41], CS. 724.
86. *Infundibulicybe geotropa* (Bull.) Harmaja/ Etçe: Under conifer trees, edible, 06.06.2018, [6], CS. 90.
87. *Infundibulicybe gibba* (Pers.) Harmaja / Koca huni mantar: Under conifer trees, edible, 30.06.2018, [17], CS. 284.
88. *Lepista irina* (Fr.) H.E. Bigelow / Süslü cincile: Meadow, edible, 07.06.2018; 20.09.2019, [11, 42], CS. 161, 933
89. *Lepista personata* (Fr.) Cooke / Diken mantarı: Under poplar, edible, 07.06.2018, [14, 15], CS. 196, 197, 214.
90. *Melanoleuca cognata* (Fr.) Konrad & Maubl. / Kahve yılan mantarı: Under conifer trees, edible, 07.06.2018, [15], CS. 217.
91. *Melanoleuca graminicola* (Velen.) Kühner & Maire / Top yılan mantarı: Under conifer trees, edible, 06.06.2018, [5, 6], CS. 69, 88.
92. *Melanoleuca microcephala* (P. Karst.) Singer / Küçük yılan mantarı: Under conifer trees, edible, 07.06.2018, [13], CS. 179.
93. *Melanoleuca stridula* (Fr.) Singer / Ağu yılan mantarı: Under conifer trees, edible, 06.06.2018, [5, 6, 7], CS. 60, 83, 93.
94. *Panaeolina foenisecii* (Pers.) Maire / Dön şapka: Meadow, poisonous, 07.06.2018, [10], CS. 148.
95. *Tricholomopsis rutilans* (Schaeff.) Singer / Mor sarı: On the conifer log, unedible, 30.06.2018, [17], CS. 252.

Inocybaceae Jülich

96. *Inocybe dulcamara* (Pers.) P. Kumm. / Hanım kümbet: Under conifer trees, poisonous, 06.06.2018; 30.06.2018; 01.07.2018, [7, 17, 20, 22] CS. 106, 254, 329, 382
97. *Inocybe flocculosa* Sacc. / Ay kümbet: Under conifer trees, poisonous, 06.06.2018, [17, 18], CS. 246, 304.
98. *Inocybe fuscidula* Velen. / Bulanık kümbet: Under conifer trees, poisonous, 30.06.2018, [17], CS. 273.
99. *Inocybe hirtella* Bres. / Uzak kümbet: Under poplar trees, poisonous, 07.06.2018, [10], CS. 151.
100. *Inocybe mixtilis* (Britzelm.) Sacc. / Ters kümbet: Under conifer trees, poisonous, 30.06.2018, [19], CS. 319.
101. *Inocybe nitidiuscula* (Britzelm.) Lapl. / Koz kümbet: Under conifer trees, poisonous, 30.06.2018, [17], CS. 253.
102. *Inocybe pallida* Velen. / Saplı kümbet: Under conifer trees, poisonous, 30.06.2018, [20], CS. 344.
103. *Inocybe sindonia* (Fr.) P. Karst. / Oymalı kümbet: Under conifer trees, poisonous, 30.06.2018, [20], CS. 348.
104. *Inocybe splendens* R. Heim / Has kümbet: Under conifer trees, poisonous, 30.06.2018, [20], CS. 353.
105. *Inocybe vaccina* Kühner / Oyuk kümbet: Under conifer trees, poisonous, 06.06.2018, [6, 8], CS. 75, 126.
106. *Inosperma bongardii* (Weinm.) Matheny & Esteve-Rav. / Gâvur kümbet: Under conifer trees, poisonous, 02.11.2018, [44], CS. 783.
107. *Inosperma cookei* (Bres.) Matheny & Esteve-Rav. / Dik kümbet: Under conifer trees, poisonous, 01.07.2018, [24], CS. 415.

108. *Inosperma quietiodor* (Bon) Matheny & Esteve-Rav. / Yoğun kümbet: Mixed forest, poisonous, 01.07.2018, [23], CS. 401.
109. *Mallochybe gymnocarpa* (Kühner) Matheny & Esteve-Rav. / Engin kümbet: Under conifer trees, poisonous, 30.06.2018, [22], CS. 381
110. *Mallochybe leucoblema* (Kühner) Matheny & Esteve-Rav. / Öz kümbet: Under conifer trees, poisonous, 06.06.2018, [17], CS. 274.
111. *Pseudosperma rimosum* (Bull.) Matheny & Esteve-Rav. / Uysal kümbet: Mixed forest, poisonous, 0507.06.2018; 30.06.2018, [2, 5, 11], CS.31, 57, 58, 163.
- Lycoperdaceae** F. Bercht. & J. Presl
112. *Lycoperdon marginatum* Vittad. / Kenar poslak: Under conifer trees, edible, 06.06.2018; 01.07.2018, [12, 22], CS. 174, 385.
113. *Lycoperdon perlatum* Pers. Fıssa kuri: Under conifer trees, edible, 30.06.2018, [18, 21], CS. 309, 375.
114. *Utraria mollis* (Pers.) R.L. Zhao & J.X. Li / Zayıf poslak: Under conifer trees, edible, 05-06.06.2018; 30.06.2018, [1, 7, 18], CS.14, 96, 305.
- Lyophyllaceae** Jülich
115. *Calocybe chrysenteron* (Bull.) Singer / Çatık çoban damı: Under conifer trees, unedible, 05.06.2018, [2], CS. 29.
116. *Calocybe gambosa* (Fr.) Donk / Gugule: Under conifer trees, edible, 06.06.2018 [6], CS. 76.
117. *Calocybe onychina* (Fr.) Donk / Kızıl karadönek: Under conifer trees, unedible, 06.06.2018, [5, 6], CS. 64, 87.
118. *Lyophyllum decastes* (Fr.) Singer / Küme karadönek: Under conifer trees, edible, 05.07.2019, [46], CS. 848.
- Macrocystidiaceae** Kühner
119. *Macrocystidia cucumis* (Pers.) Joss. / Hıyar kokar: Meadow, unedible, 07.06.2018, [15], CS. 211.
- Marasmiaceae** Roze ex Kühner
120. *Marasmius oreades* (Bolton) Fr. / Mıh başı: Meadow, edible, 07.06.2018, [11, 12], CS.159, 168.
- Mycenaceae** Roze
121. *Mycena abramsii* (Murrill) Murrill / Sil kukulcuk: On the remnants of burnt wood, unedible, 02.11.2018, [42], CS. 732.
122. *Mycena acicula* (Schaeff.) P. Kumm. / Sivri kukulcuk: Among the remnants of oak leaves, unedible, 05-07.06.2018, [1, 16], CS. 12, 221.
123. *Mycena aetites* (Fr.) Quél. / Orak kukulcuk: Mixed forest, unedible, 06.06.2018, [5], CS. 65.
124. *Mycena epipterygia* (Scop.) Gray / Yel kukulcuk: On the conifer log, unedible 25.08.2018, [31], CS. 554.
125. *Mycena latifolia* (Peck) A.H. Sm. / Geniş kukulcuk: Under conifer trees, unknown, 01.11.2018, [41], CS. 720.
126. *Mycena pura* (Pers.) P. Kumm. / Mor kukulcuk: Under conifer and oak trees, edible, 06.06.2018; 30.06.2018; 27.09.2018; 02.11.2019, [6, 7, 21, 32, 43], CS. 77, 79, 108, 362, 587, 749.
127. *Xeromphalina campanella* (Batsch) Kühner & Maire / Göbekli çan: Under conifer trees, unedible, 06.06.2018, [6], CS. 78.
128. *Xeromphalina caudicinalis* (Fr.) Kühner & Maire / Sarı göbekli çan: Among mosses, under conifer trees, unedible, 07-30.06.2018, [36, 17, 18], CS. 180, 258, 259, 260, 313.
- Omphalotaceae** Bresinsky
129. *Collybiopsis confluens* (Pers.) R.H. Petersen / Ak çırpı bacak: Under conifer trees, unedible, 01.07.2018, [25], CS. 438.
130. *Gymnopus alkalivirens* (Singer) Halling / Kara çırpı bacak: Under conifer trees, unedible, 06.06.2018, [5], CS. 63.
131. *Gymnopus androsaceus* (L.) Della Magg. & Trassin. / Çırpı bacak: On the cone, unedible, 06.06.2018, [8] CS. 124.
132. *Gymnopus dryophilus* (Bull.) Murrill / Sarı çırpı bacak: Mixed forest, edible, 05.06.2018; 06.06.2018; 30.06.2018; 07.07.2018; 02.11.2018, [1, 2, 3, 4, 8, 13, 38, 22, 43], CS. 8, 17, 27, 37, 53, 133, 183, 299, 388, 753.
133. *Rhodocollybia butyracea* (Bull.) Lennox / Göverték: Among the remnants of conifer leaves, edible 06.06.2018, [8], CS. 134.
- Physalacriaceae** Corner
134. *Flammulina ononidis* Arnolds / Kirli tüylü bacak: Among mosses, mixed forest, unedible, 02.11.2018, [44], CS. 792.
135. *Flammulina velutipes* (Curtis) Singer / Tüylü bacak: Under poplar log, edible, 02.11.2018, [44], CS. 798.
136. *Strobilurus tenacellus* (Pers.) Singer / Kozalak ebesi: On the remnants of a cone, unedible, 06.06.2018, [8], CS. 111.
- Pleurotaceae** Kühner

137. *Pleurotus ostreatus* (Jacq.) P. Kumm. / İstiridye mantarı: On the poplar log, edible, 07.06.2018, [15, 16], CS. 200, 230.
138. *Pleurotus populinus* O. Hilber & O.K. Mill. / Kavak mantarı: On the poplar log, edible, 07.06.2018, [14, 16], CS. 190, 231.
- Pluteaceae** Kotl. & Pouzar
139. *Pluteus podospileus* Sacc. & Cub. / Yüksük çıtkırdı: On the conifer log, unedible, 05.06.2018, [4], CS. 44.
140. *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo / Kakilvik: Meadow, edible, 07.06.2018, [12], CS. 169.
- Psathyrellaceae** Vilgalys, Moncalvo & Redhead
141. *Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer (Fr.) Maire / Güzel pulcuklu: Under poplar, edible, 07.06.2018, [9, 16], CS. 138, 145, 241.
142. *Coprinellus disseminatus* (Pers.) J.E. Lange / Minik mürekkep: On the poplar log, unedible, 07.06.2018, [10], CS. 152.
143. *Tulosesus ephemerus* (Bull.) D. Wächt. & A. Melzer / Ehrami mürekkep: On the cow manure, unedible, 02.11.2018, [42], CS. 741.
144. *Tulosesus impatiens* (Fr.) D. Wächt. & A. Melzer Cam mürekkebi: Under oak trees, unedible, 07.06.2018; 02.11.2018, [15, 43], CS. 201, 765.
145. *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson / Pullu mürekkep: Under poplar trees, edible, 06.06.2018, [10], CS. 147.
146. *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo / Kütük döbeleni: Under conifer trees, poisonous, 21.09.2019, [30], CS. 961.
147. *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo / Ak döbelen: On manure, unedible, 01.07.2018, [22], CS. 384.
148. *Parasola kuehneri* (Ulje & Bas) Redhead, Vilgalys & Hopple / Doruk sevelen: Under conifer trees, unedible, 06.06.2018, [8], CS. 127.
149. *Psathyrella longipes* (Peck) A.H. Sm. / Uzun pulcuklu: Mixed forest, edible, 07.06.2018, [16], CS. 233.
150. *Psathyrella spadiceogrisea* (Schaeff.) Maire / Yol pulcuklu: Under poplar, unedible, 07.06.2018, [11, 14], CS. 165, 191.
151. *Psathyrella tephrophylla* (Romagn.) Bon / Zarif sevelen: Meadow, unedible, 07.06.2018, [15], CS. 206, 216.
- Pseudoclitocybaceae** Vizzini, Consiglio, P.-A. Moreau & P. Alvarado
152. *Pseudoclitocybe expallens* (Pers.) M.M. Moser / Yayvan peslivan: Under conifer trees, edible, 06.06.2018, [5], CS. 55.
- Schizophyllaceae** Quél.
153. *Schizophyllum commune* Fr. / Kımuk: On remnants of the oak branch, unedible, 24.08.2018, [29], CS. 482.
- Strophariaceae** Singer & A.H. Sm.
154. *Agrocybe dura* (Bolton) Singer / Yaz meteliği: Near the conifer forest, edible, 05.-07.06.2018, [1, 12], CS. 5, 166, 171.
155. *Agrocybe paludosa* (J.E. Lange) Kühner & Romagn. ex Bon / Yaş metelik: Meadow unedible, 05.06.2018, [2], CS. 24.
156. *Agrocybe splendida* Cléménçon / Hoş metelik: Meadow, unedible, 07.06.2018, [9], CS. 136.
157. *Deconica montana* (Pers.) P.D. Orton / Kafa bulduran: Mixed forest, poisonous, 06.06.2018, [5], CS. 59.
158. *Hypholoma fasciculare* (Huds.) P. Kumm. / Ağulu sarıpapak: On the conifer log, poisonous, 07.06.2018; 27.09.2018, [22, 32], CS. 395, 591.
159. *Pholiota aurivella* (Batsch) P. Kumm. / Sarı pulbaş: On the willow trunk, edible, 01.11.2018, [39], CS. 698.
160. *Pholiota populnea* (Pers.) Kuyper & Tjall.-Beuk. / Kavak yamağı: On the poplar trunk, unedible, 01.11.2018, [40], CS. 689.
- Tricholomataceae** Lotsy
161. *Leucopaxillus gentianeus* (Quél.) Kotl. / Boz huni şapka: Under conifer trees, unedible, 06-30.06.2018, [7, 21], CS. 107, 372.
162. *Tricholoma fracticum* (Britzelm.) Kreisel / Halkalı karakız: Under conifer trees, unedible, 30.06.2018; 25.08.2018, [17, 31], CS. 294, 562.
163. *Tricholoma sciodes* (Pers.) C. Martín / Zarif karakız: Mixed forest, unedible, 01.07.2018, [27], CS. 443.
164. *Tricholoma sejunctum* (Sowerby) Quél. / Telli karakız: Under conifer trees, unedible, 07.06.2018; 25.08.2018, [16, 31], CS. 226, 227, 526, 527.
165. *Tricholoma terreum* (Schaeff.) P. Kumm. / Karakız mantarı: Under conifer trees and mixed forest, edible, 05-06-07-30.06.2018; 27.09.2018, [1, 2, 4, 6, 13, 21, 33], CS. 7, 19, 39, 47, 80, 182, 361, 596.
166. *Tricholoma virgatum* (Fr.) P. Kumm. / Sivri karakız: Under conifer trees, unedible, 30.06.2018, [17], CS. 286.

Tubariaceae Vizzini

167. *Tubaria conspersa* (Pers.) Fayod / Ökse tubarya: On remnants of leaf and branch, unedible 07.06.2018, [13], CS. 185.
168. *Tubaria furfuracea* (Pers.) Gillet / Fırfırlı tubarya: Among poplar leaves, unedible, 07.06.2018, [11], CS. 162.
169. *Tubaria romagnesiana* Arnolds / Borazan tubarya: Mixed foresti among leaves, unedible, 07.06.2018, [16], CS. 237.

Boletales E.-J. Gilbert

Boletaceae Chevall.

170. *Xerocomellus chrysenteron* (Bull.) Šutara / Pöslen: Under conifer trees, edible, 30.06.2018; 05.07.2019, [20, 21, 46], CS. 359, 371, 846.

Diplocystidiaceae Kreisel

171. *Astraeus hygrometricus* (Pers.) Morgan / Dişli yıldız: Mixed forest, unedible, 05.06.2018, [5], CS. 40.

Gomphidiaceae Maire ex Jülich

172. *Chroogomphus rutilus* (Schaeff.) O.K. Mill / Geyik mantarı: Under conifer trees, edible, 01.07.2018; 27.09.2018, [26, 34], CS. 445, 620.

Suillaceae Besl & Bresinsky

173. *Suillus collinitus* (Fr.) Kuntze / Benekli sünger mantarı: Under conifer trees, edible, 06.06.2018, [7], CS. 94.
174. *Suillus luteus* (L.) Roussel / Sünger mantarı: Under conifer trees, edible, 05-06.06.2018; 05.07.2019, [1, 6, 46], CS. 2, 89, 856.

Cantharellales Gäum.

Clavariadelphaceae Corner

175. *Clavariadelphus ligula* (Schaeff.) Donk / Sarı topuz mantarı: Under conifer trees, edible, 30.06.2018, [21], CS. 370.

Hydnaceae Chevall.

176. *Clavulina cinerea* (Bull.) J. Schröt. / Gümüş tepeli mercan: Under conifer trees, unedible, 06.06.2018, [31], CS. 547.
177. *Clavulina coralloides* (L.) J. Schröt. / Tepeli mercan: Under conifer trees, unedible, 30.06.2018, 01.07.2018, [20, 23], CS. 341 402.

Tapinellaceae C. Hahn

178. *Tapinella atrotomentosa* (Batsch) Šutara / Kadife sap: Under conifer trees, unedible, 25.08.2018, [31], CS. 543, 560.
179. *Tapinella panuoides* (Fr.) E.-J. Gilbert / Çam yelpazesi: Under conifer trees, unedible, 30.06.2018, [20], CS. 342.

Gastrales K. Hosaka & Castellano

Gastraceae Corda

180. *Geastrum pectinatum* Pers./ Alımlı yer yıldızı: Under conifer trees, unedible, 20.09.2019, [34], CS. 883.
181. *Geastrum triplex* Jungh. / Yakalı yer yıldızı: Under conifer trees, edible, 01.11.2018, [36], CS. 674.

Hymenochaetales Oberw.

Hymenochaetaceae Donk

182. *Coltricia perennis* (L.) Murrill / Kangal delikli huni: Under conifer trees, unedible, 01.07.2018, [23], CS. 399.

Polyporales Gäum.

Polyporaceae Fr. ex Corda

183. *Lentinus arcularius* (Batsch) Zmitr./ Delikli kaplan mantarı: On the remnant of the branch, unedible, 06.06.2018, [8], CS. 128.
184. *Lentinus tigrinus* (Bull.) Fr./ Kaplan mantarı: On the poplar log, edible, 07.06.2018, [16], CS. 219.
185. *Trametes cinnabarina* (Jacq.) Fr. / Kızıl kenet: On the remnants of the oak branch, unedible, 24.08.2018; 19.06.2019, [13, 29], CS. 484, 814.
186. *Trametes ochracea* (Pers.) Gilb. & Ryvarden / Limon hindi kuyruğu: On the poplar log, unedible, 07.06.2018, [9], CS. 143.
187. *Trametes trogii* Berk. / Som hindi kuyruğu: On the poplar log, unedible, 06.06.2018; 24.08.2018, [10, 28], CS. 149, 467.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

Auriscalpiaceae Maas Geest.

188. *Auriscalpium vulgare* Gray/ Sarkık diş: On the cone, unedible, 05.06.2018, [3, 4], CS. 36, 49, 50.

Russulaceae Lotsy

189. *Lactarius deliciosus* (L.) Gray / Kanlıca mantarı: Under conifer trees, edible, 05-06-30.06.2018; 27.09.2018, [2, 5, 17, 33], CS. 25, 66, 271, 600.
190. *Lactarius semisanguifluus* R. Heim & Leclair / Çıntar: Under conifer trees, edible, 01.11.2018, [41], CS. 723.
191. *Lactarius volemus* (Fr.) Fr. / Tirit: Mixed forest, edible, 30.06.2018 [19], CS. 321.
192. *Russula atropurpurea* (Krombh.) Britzelm. / Kızıl kirit: Mixed forest, edible, 30.06.2018, [17, 18, 20], CS. 261, 314, 335.
193. *Russula betularum* Hora / Huş kiriti: Under conifer trees, poisonous, 30.06.2018, [17], CS. 245.
194. *Russula delica* Fr. / Ak çıntar: Mixed forest, edible, 25.08.2018, [29], CS. 494.
195. *Russula roseipes* Secr. ex Bres. / Kavruk kirit: Under conifer trees, edible, 30.06.2018, [17], CS. 277.
196. *Russula vinosa* Lindblad / Allı kirit: Under conifer trees, edible, 06-30.06.2018, [7, 17], CS. 92, 285, *Thelephorales* Corner ex Oberw.
Thelephoraceae Chevall.
197. *Thelephora terrestris* Ehrh. / Kuru onparmak: Under conifer trees, inedible, 30.06.2018; 05.07.2019, [21, 34], CS. 360, 833.
- Dacrymycetes* Doweld
Dacrymycetales Henn.
Dacrymycetaceae J. Schröt.
198. *Calocera viscosa* (Pers.) Fr. / Yaş sarı boynuz: Under conifer trees, inedible, 25.08.2018, [31], CS. 539.

DISCUSSION and CONCLUSION

In the research area, 66 species (33%) were found to be edible, while 96 species (49%) were inedible due to factors like hard structure or unpleasant taste. Additionally, 32 species (16%) contain toxic substances that can cause poisoning, and the edibility of 4 species (2%) remains undetermined.

Although 64 edible species were identified in the research area, the local population only consumes certain species belonging to the genera "*Agaricus*," "*Morchella*," and "*Pleurotus*," which they are well acquainted with. It was found that they refer to "*Agaricus*" species as "çayır mantarı," "*Morchella*" species as "dana burnu," and "*Pleurotus*" species as "kavak mantarı."

The Turkish names of 196 identified mushroom species have been provided. However, since the names of *Meotomycetes dissimulans* (Berk. & Broome) Vizzin and *Entoloma lampropus* (Fr.) Hesler have not yet been finalized in the list of Turkish mushrooms, their local names have not been included.

When examining the monthly distribution of macrofungi samples collected from the research area, it is observed that the highest number of samples were collected in June, September, October, and November, respectively (Figure 2).

A hundred ninety eighth macrofungi species were determined within the boundaries of Oltu-Narman (Erzurum) districts. Twenty-seven (%14%) of them belong to *Ascomycota* and 171 (%86%) to *Basidiomycota*. 187 taxa are new for the region except. The taxa determined in the region are distributed in 5 classes (Fig. 3), 12 orders (Fig. 4), 52 families, and 103 genera.

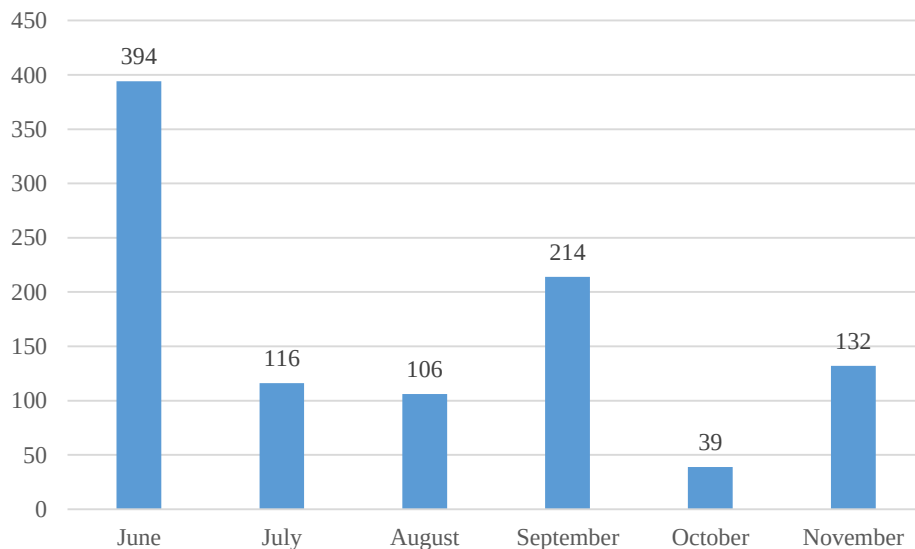


Figure 2. The monthly distribution of macrofungi samples collected from the research area.
Şekil 2. Araştırma alanından toplanan makrofungus örneklerinin aylık dağılımı

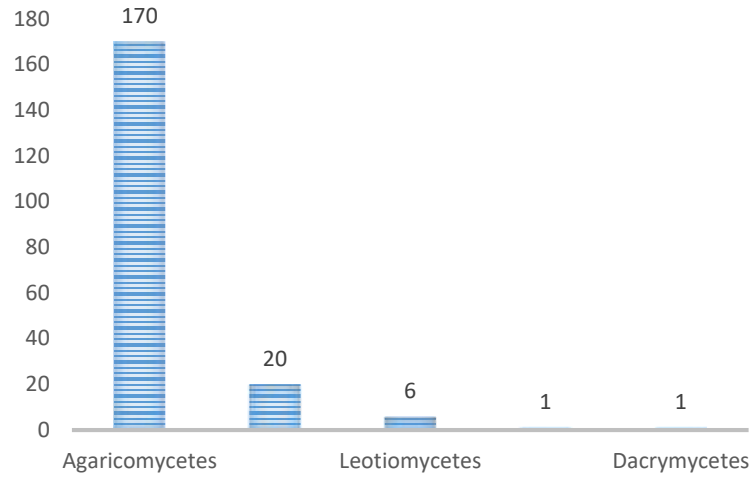


Figure 3. Class-wise distribution of the determined species.

Şekil 3. Tespit edilen türlerin sınıf bazında dağılımı

Inocybaceae (16), *Agaricaceae* (11), *Psathyrellaceae* (11), *Hymenogastraceae* (9), *Mycenaceae* (8), *Russulaceae* (8), *Cortinariaceae* (7) ve *Strophariaceae* (7) were found to be the most crowded 8 families.

The most crowded 6 genera were determined as *Inocybe*, *Agaricus*, *Hebeloma*, *Mycena*, *Amanita*, *Russula*, and *Tricholoma* with 10, 7, 6, 6, 5, 5, and 5 species, respectively.

The following 6 genera (*Conocybe*, *Cortinarius*, *Entoloma*, *Helvella*, and *Melanoleuca*) are represented with four taxa, The remaining genera are represented by 6 with 4 species, 11 with 3 species, 18 with 2 species, and 61 with 1 species.

The macrofungi species identified in the study area exhibit similarities with those reported in neighboring regions (Table 2). These similarities may be attributed to the shared vegetation structure and climate, while the observed differences could be due to various micro-climatic effects in the research area and the presence of distinct micro-habitats.

As shown in Table 2, the fungi identified in the study area were compared with similar studies conducted in nearby regions: Allahuekber Mountains National Park (Akçay, 2017) with 35.82% similarity, Erzincan (Keleş and Demirel, 2010) with 27.75%, Karagöl Sahara (Demirel et al., 2017) with 23.83%, Erzurum (Demirel et al., 2003) with 29.82%, Bingöl (Uzun et al., 2017b) with 24.1%, Kop Mountain (Polat, 2014) with 42.5%, Muş-Bitlis (Kaya, 1999) with 42.25%, Ağrı (Demirel et al., 2002) with 46.66%, and Hınıs and Karaçoban (Erzurum) (Öztürk et al., 2000) with 61.11% similarity. These similarities and differences are thought to be due to the unique climate and vegetation of the study area.

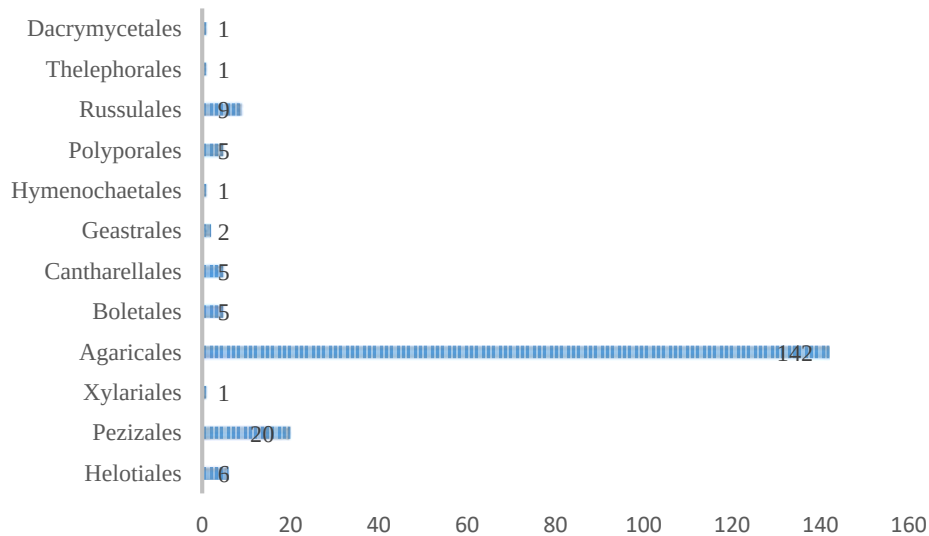


Figure 4. Order-wise distribution of the determined species

Şekil 4. Tespit edilen türlerin takım bazında dağılımı.

Table 2. The similarity percentages (in terms of taxa reported) with former studies around the study area
Çizelge 2. Çalışma alanı çevresinde gerçekleştirilmiş önceki çalışmalarla benzerlik yüzdeleri (rapor edilen taksonlar açısından)

Research area / Araştırma yören	Total species number Toplam tür sayısı	Common species number Ortak tür sayısı	Similarity (%) Benzerlik (%)
Allahuekber Dağları Milli Parkı (Şenkaya-Sarıkamış) (Akçay, 2017)	254	91	35.82
Erzincan (Keleş and Demirel, 2010)	191	53	27.75
Karagöl-Sahara (Artvin) (Demirel et al., 2017).	172	41	23.83
Erzurum (Demirel et al., 2003)	114	34	29.82
Bingöl (Uzun et al., 2017b)	112	27	24.1
Kop Dağı (Erzurum-Bayburt) (Polat, 2014)	80	34	42.5
Muş-Bitlis (Kaya, 1999)	71	30	42.25
Ağrı (Demirel et al., 2002)	45	21	46.66
Hinis and Karaçoban (Erzurum) (Öztürk et al., 2000)	18	11	61.11

Although fungi produce millions of spores for reproduction, it is a well-known fact that their presence in the environment is directly or indirectly dependent on plants or plant debris. Environmental factors such as climate change, human impact, and habitat loss, which affect forests and forest ecosystems, also leave deep marks on the ecological balance of macrofungi in the region. These factors narrow the habitats of macrofungi, reshaping their survival strategies and roles within the ecosystem. Consequently, these changes affect the biodiversity and ecosystem functions of macrofungi in complex and unpredictable ways.

As a result of this study, habitat and substrate preferences, edibility status, and seasonal distributions of macrofungus species growing in Oltu and Narman (Erzurum) districts were determined, and the basis for future taxonomic studies was provided.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

Authors have declared no conflict of interest.

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Bazı Yerel Ekmeklik Buğday Çeşitlerinin (*Triticum aestivum* L.) Sulu Koşullarda ve Geç Kuraklık Stresi Oluşturulan Koşullarda Bazı Kalite Parametrelerinin Belirlenmesi

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ÖZET

Buğday üretiminde biyotik ve abiyotik stres faktörlerinin etkileri sonucu verim ve kalitede büyük oranda değişimler olmaktadır. Buğdayda kurağa toleranslı çeşitlerin geliştirilmesinde genetik kaynak olarak kullanılan kültür çeşitlerinin ve yerel çeşitlerin kuraklık stresine karşı tepkilerinin iyi bilinmesi gerekmektedir. Araştırma, 22 adet yerel ekmeklik buğday çeşidi ve beş adet tescilli ekmeklik buğday çeşidi (Gün 91, Müfitbey, Dağdaş 94, Bezostaja 1, Aldane) ile sulu ve geç kuraklık stresi oluşturulan koşullar altında 2017-2018 ve 2018-2019 yetiştirme dönemlerinde Tokat-Kazova koşullarında yürütülmüştür. Çalışma iki ayrı deneme halinde (sulu koşullar ve geç kuraklık stresi oluşturulan koşullar) Tesadüf Blokları Deneme Desenine göre 3 tekerrürlü olarak kurulmuştur. Çalışmada, kalite özellikleri olarak bin tane ağırlığı, hektolitreye ağırlığı, protein oranı, Zeleny sedimantasyon değeri, bayrak yaprak ve tane kül oranları incelenmiştir. Çalışmada, incelenen özellikler bakımından hem sulu koşullarda hem de geç kuraklık stresi koşullarında ekmeklik buğday çeşitleri arasında önemli farklılıklar saptanmıştır. Uygulanan geç kuraklık stresinin sulu koşullara göre bin tane ağırlığını ve hektolitreye ağırlığını azalttığı; protein oranını, bayrak yaprak ve tane kül oranını ve Zeleny sedimantasyon değerini artırdığı belirlenmiştir. Yılların ortalamalarına bakıldığında hem sulu koşullarda hem de geç kuraklık stresi oluşturulan koşullarda, yerel çeşitlerde bin tane ağırlığı, hektolitreye ağırlığı, Zeleny sedimantasyon değeri ve bayrak yaprak kül oranı tescilli çeşitlere göre daha düşük, protein oranı ve tane kül oranı ise daha yüksek bulunmuştur. İncelenen özellikler bakımından yerel çeşitler arasında önemli varyasyonlar elde edilmiş, çeşitlerin farklı yıl ve uygulamalara tepkileri değişmiştir.

Tarla Bitkileri

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Anahtar Kelimeler

Kuraklık

Bin tane ağırlığı

Hektolitreye ağırlığı

Protein oranı

Kül oranı

Determination of Some Quality Parameters of Some Bread Wheat (*Triticum aestivum* L.) Landraces under Irrigated Conditions and Late Drought Stressed Conditions

ABSTRACT

There are significant changes in yield and quality as a result of the effects of biotic and abiotic stress factors in wheat production. In developing drought-tolerant cultivars of wheat, the responses of cultivated cultivars and landraces used as genetic resources to drought stress should be well known. The research was carried out in Tokat-Kazova conditions with 22 landraces and five registered bread wheat cultivars Gün 91, Müfitbey, Dağdaş 94, Bezostaja 1, Aldane) under irrigated and late drought stress conditions in the 2017-2018 and 2018-2019 growing periods. The study was established in two separate trials (irrigated conditions and late drought stress conditions) according to the Randomized Block Trial Design with 3 replications. In the study, thousand-grain weight, hectoliter weight, protein rate, Zeleny sedimentation value, flag leaf, and grain ash rate were examined as quality characteristics. In the study, significant differences were obtained among bread wheat cultivars in terms of the

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investigated traits both under irrigated conditions and under late drought stress conditions. It was determined that late drought stress applied reduced thousand grain weight and hectoliter weight compared to irrigated conditions; it was determined that increased the protein ratio, flag leaf and grain ash ratio and Zeleny sedimentation value. Considering the averages of the years, both in irrigated conditions and late drought stress conditions, thousand grain weight, hectoliter weight, Zeleny sedimentation value and flag leaf ash ratio was found to be lower in landraces compared to registered cultivars, while protein ratio and grain ash ratio were higher. Significant variations were obtained among the landraces in the traits examined; the responses of the cultivars to different years and applications changed.

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GİRİŞ

Buğday ekim, bakım, hasat, taşıma, depolama gibi işlemlerinin kolay olması bakımından Dünya’da ve Türkiye’de geniş alanlarda yetiştirilmektedir. Ayrıca insan beslenmesinde önemli bir yere sahip olması da buğday yetiştiriciliğine olan talebi artırmaktadır. Bu yüzden buğdayın birim alandan elde edilen verim miktarının, tane kalitesinin ve olumsuz koşullara toleransının artırılması önem arz eden bir husustur.

Türkiye, hem farklı ekolojik bölgelere sahip olması hem de coğrafi ve iklimsel açıdan elverişli bir konumda bulunması nedeniyle hayvan ve bitki çeşitliliği açısından zengindir. Buğdayın ilk kültüre alındığı ve en önemli gen merkezlerinden biri olan Türkiye, buğday ıslah çalışmalarında doğrudan veya dolaylı olarak kullanılabilecek zenginlik ve çeşitlilikte yerel buğdaylara sahiptir. Yerel çeşitler (köy çeşitleri) doğal ve yapay seleksiyon baskısı altında hayatta kalan, yerel damak zevkine uygun, çoğunlukla popülasyon yapısında olan dış görünüşleri benzer bireyler topluluğu olup geniş bir genetik temele sahiptirler (Özberk ve ark., 2016).

Buğday yetiştirme sezonu içerisindeki yağışın yetersiz ve dağılımın düzensiz olması verimde azalmalara neden olmaktadır (Öztürk ve Korkut, 2018). Kuraklık, üretimi genellikle kuru tarım alanlarında yapılmakta olan buğdayın tane verimini sınırlandıran ve dünyanın pek çok yerinde ciddi problemlere sebep olan bir abiyotik stres faktörüdür. Kuraklık, tahıllarda vejetatif dönem, çiçeklenme öncesi dönem (kardeşlenme ve başaklanmadan tam çiçeklenmeye kadar) ve çiçeklenme sonrası dönem (çiçeklenmeden sonra ve olgunlaşmaya kadar) olmak üzere 3 ana dönemde bitki gelişimini etkilemektedir (Shavrukov ve ark., 2017).

Buğday tanesinin tohumluk ve un verimi hakkında bilgi veren bin tane ağırlığının (Aydoğan & Soylu, 2017), genetik yapıya, ekolojik faktörlere (Bulut, 2015; Ghaffar ve ark., 2018), bitkideki ana sap ve kardeşlere, metrekaresindeki başak sayısına, başaktaki tane sayısına ve uygulanan kültürel işlemlere göre (Abbas, 2017) değişiklik gösterdiği bildirilmiştir. Un verimin göstergesi olarak kabul edilen ve buğday standartlarında kalite unsuru olarak dikkate alınan hektolitreye ağırlığı (Bulut, 2015) genotipik özelliğe, kültürel uygulamalara, yatma, hastalık ve zararlı gibi faktörlere (Taghouti ve ark., 2010; Kendal, 2013), tanenin dolgunluğuna, yoğunluğuna, şekline, büyüklüğüne, homojenliğine, tanedeki kavuz oranına, endosperm yapısına (Kılıç ve ark., 2010; Kendal, 2013; Arlotti & Silvestri, 2019), ekim zamanına, ekolojik koşullara (Elgün ve ark., 1999) bağlı olarak değişmektedir. Ticarete, tane ve unun ekmekçilik değeri açısından sınıflandırılmasında protein miktarının makul bir kriter olarak kabul edildiği, protein miktarının genotipe, yetiştirme şartlarına, ekim zamanına ve iklim koşullarına göre değişiklik gösterdiği belirtilmiştir (Elgün ve ark., 2012). Zeleny sedimantasyon testi buğdayın hem protein miktarı hem de kalitesi hakkında fikir veren kolay ve hızlı bir metot olup (Elgün ve ark., 2012), sedimantasyon değerinin genotipik özelliğe bağlı olmakla birlikte iklim faktörlerinin etkisine (Aydoğan ve ark., 2010), ekim zamanına (Caglar ve ark., 2011) göre de değişiklik gösterdiği belirtilmiştir. Kül birikim mekanizması; vejetatif dokularda minerallerin ksilem yoluyla pasif olarak taşınmasıyla tanede ise floem yoluyla gerçekleşmektedir. Vejetatif kül içeriği fotosentetik ve transpiratif gaz alışverişi aktivitesi hakkında bilgi verirken, tane kül içeriği tane doldurma dönemi boyunca fotosentetik ve translokasyon sürecinin birlikte performansı hakkında bilgi vermektedir (Bogale & Tesfaye, 2011). Kül içeriğinin; çevre şartlarına (Araus ve ark., 2001; Misra ve ark., 2006), örnek alınan bitki kısmına, örnekleme zamanına (Misra ve ark., 2006) göre değişiklik gösterdiği ileri sürülmüştür. Bunun yanında, kuru tarım alanlarında yetiştirilen buğdaylarda geç kuraklık stresinin tane

kalitesini önemli bir şekilde etkilediği bildirilmiştir (Panozzo & Eagles, 2000; Öztürk & Aydın, 2004; Ayrancı, 2012; Sakin ve ark., 2017; Zhang ve ark., 2019).

Yapılan bu çalışmada; Tokat, Amasya ve Çorum illerinden toplanan bazı yerel ekmeklik buğday çeşitlerinin Tokat-Kazova şartlarında sulu ve geç kuraklık stresi oluşturulan koşullarda bazı kalite özellikleri bakımından arazi şartlarında değerlendirilmesi, bu çeşitlerin kuraklığa tepkilerinin belirlenerek öne çıkan çeşitlerin ileride yapılacak olan ıslah çalışmalarında değerlendirilme imkanlarının ortaya konulması amaçlanmıştır.

MATERYAL ve METOD

Denemeler, 2017-2018 ve 2018-2019 yetiştirme dönemlerinde Orta Karadeniz Bölgesinde Tokat-Kazova koşullarında (40° 18' kuzey enlemi ile 36° 34' doğu boylamı, 623 m rakım) Tokat Gaziosmanpaşa Üniversitesi Tarımsal Uygulama ve Araştırma Merkezi deneme alanlarında yürütülmüştür. Deneme alanının uzun yıllar ve araştırma yıllarına ait bazı iklim verileri Çizelge 1'de, deneme alanlarının toprak analiz sonuçları ise Çizelge 2'de verilmiştir. Araştırmada materyal olarak Tokat, Amasya ve Çorum illerinden toplanarak gen bankalarında muhafaza edilen ve Tokat'taki çiftçilerden temin edilen 22 adet yerel ekmeklik buğday çeşidi ile kontrol olarak da Öztürk ve Aydın (2014)'ın ekmeklik buğday çeşitleri ile yürüttükleri çalışmalarından elde edilen sonuçlara göre geç gelişme dönemlerindeki kuraklığa tolerans gösteren Gün 91, Müfitbey ve Dağdaş 94, kurağa duyarlılığı belirlenen Bezostaja 1 ve Aldane tescilli ekmeklik buğday çeşitleri kullanılmıştır (Çizelge 3).

Çizelge 1. Deneme alanının iklim faktörleri*

Table 1. Climatic factors of the trial area*

İklim faktörleri <i>Climatic factors</i> Aylar <i>Months</i>	Toplam yağış (mm) <i>Total precipitation (mm)</i>			Ortalama sıcaklık (°C) <i>Average temperature (°C)</i>			Ortalama nispi nem (%) <i>Average relative humidity (%)</i>		
	2017-18	2018-19	Uzun yıllar <i>Long terms</i>	2017-18	2018-19	Uzun yıllar <i>Long terms</i>	2017-18	2018-19	Uzun yıllar <i>Long terms</i>
Kasım <i>November</i>	42.6	8.2	44.1	7.7	9.2	7.9	68.0	73.3	70.1
Aralık <i>December</i>	49.3	49.4	46.6	5.5	4.9	3.9	72.8	80.7	71.3
Ocak <i>January</i>	33.4	71.6	40.3	5.1	2.2	1.8	66.8	76.5	69.1
Şubat <i>February</i>	15.4	14.7	34.0	8.3	5.9	3.5	62.0	67.0	64.7
Mart <i>March</i>	79.7	36.8	40.7	11.9	7.2	7.4	58.4	61.8	60.6
Nisan <i>April</i>	4.5	63.5	55.3	14.9	11.5	12.5	46.4	65.2	59.1
Mayıs <i>May</i>	59.1	49.1	58.5	18.5	19.1	16.5	60.9	59.7	60.7
Haziran <i>June</i>	41.5	26.2	38.3	22.0	23.1	19.9	55.0	63.4	59.0
Temmuz <i>July</i>	7.2	16.9	11.1	24.2	21.9	22.3	52.7	59.6	56.9
Ortalama <i>Average</i>	-	-	-	13.1	11.7	10.6	60.3	67.5	63.5
Toplam <i>Total</i>	332.7	336.4	368.9	-	-	-	-	-	-

*: Tokat Meteoroloji İstasyon Müdürlüğü (2019)

Araştırma sulu koşullar ve geç kuraklık stresi oluşturulan koşullar olmak üzere iki ayrı deneme halinde Tesadüf Blokları Deneme Desenine göre 3 tekerrürlü olarak kurulmuştur. Ekimler, 20 cm sıra arası mesafede elle yapılmış olup, ekim sıklığı, tohum canlılık testi sonuçlarına göre m²'de 500 canlı tohum olacak şekilde ayarlanmıştır. Her bir parsel 2 m uzunluğunda 2 sıradan oluşmuş ve aralarında boşluk bırakılmamıştır. Toprak analiz sonuçlarına göre denemelerde dekara 10 kg N ve 6 kg P₂O₅ olacak şekilde gübreleme yapılmıştır. Azotun yarısı ve fosforun ise

tamamı ekimle birlikte verilmiş, azotun geri kalan kısmı ise sapa kalkma döneminde verilmiştir. Tüm denemelerde yabancı ot kontrolü 2.4-D etkili madde içeren bir herbisit ile yapılmıştır. Ayrıca parsellerde külleme hastalığı başlangıcı görüldüğünde, Boscalid ve Kresoxim-methyl etkili maddeli fungusit ile ilaçlama yapılmıştır. Denemede toprağının 0-60 cm derinliği ortalama hacim ağırlığı ile tarla kapasitesi ve devamlı solma noktasında ağırlıkça tutabildikleri nem içerikleri saptanmıştır (Çizelge 2). Sapa kalkma başlangıcından başlayıp fizyolojik olgunluk tarihine kadar devam etmek üzere toprakların 60 cm derinliğindeki nem içerikleri gravimetrik yöntemle belirlenmiştir (Cömert & Öztekin, 2016). Deneme alanlarının toprak nem içerikleri Şekil 1 ve Şekil 2’de verilmiştir.

Çizelge 2. Deneme alanları topraklarına ait fiziksel ve kimyasal özellikler

Table 2. Physical and chemical properties of trial areas soils

Yıllar Years	Tekstür sınıfı Texture class	Organik madde Organic matter (%)	Toplam tuz Total salt (%)	Toprak reaksiyonu Soil reaction (pH)	Kireç Lime (CaCO ₃) (%)	Fosfor P ₂ O ₅ Phosphorus (kg da ⁻¹)	Potasyum K ₂ O Potassium (kg da ⁻¹)
2017-18*	Killi-Tınlı Clay-loam	1.00	0.022	8.1	20.60	5.68	157.70
2018-19**	Killi-Tınlı Clay-loam	1.73	0.015	8.5	7.47	6.98	41.08
2018-19**							
Derinlik Depth (cm)	Tarla kapasitesi Field capacity(%)	Solma noktası Wilting point (%)	Tarla kapasitesi Field capacity(%)	Solma noktası Wilting point (%)			
0-30	20.97	10.39	23.45	13.36			
30-60	21.35	11.34	23.32	14.18			

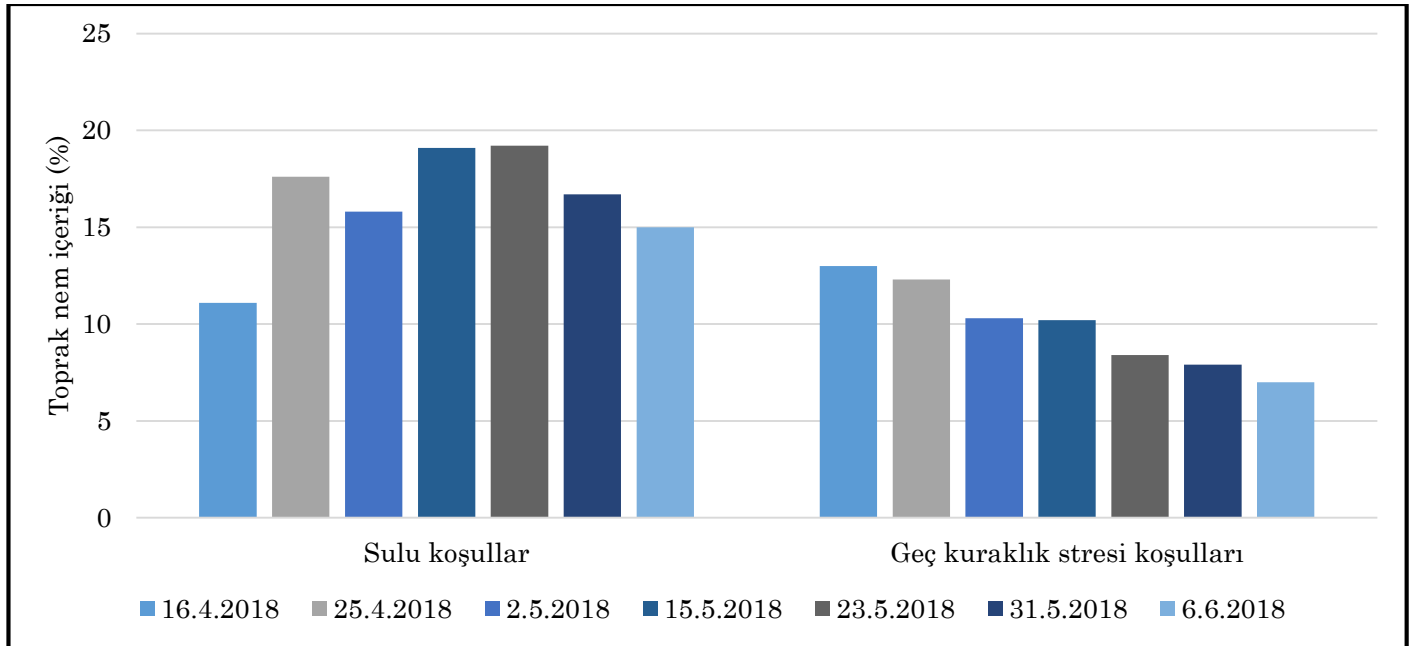
*: Orta Karadeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsü Müdürlüğü, Tokat (2017)

** : Tokat Gaziosmanpaşa Üniversitesi Bilimsel Araştırma Projeleri Birimi (2018)

Çizelge 3. Denemelerde kullanılan çeşitlerin botanik adları, toplandıkları iller ve temin edildikleri kuruluşlar

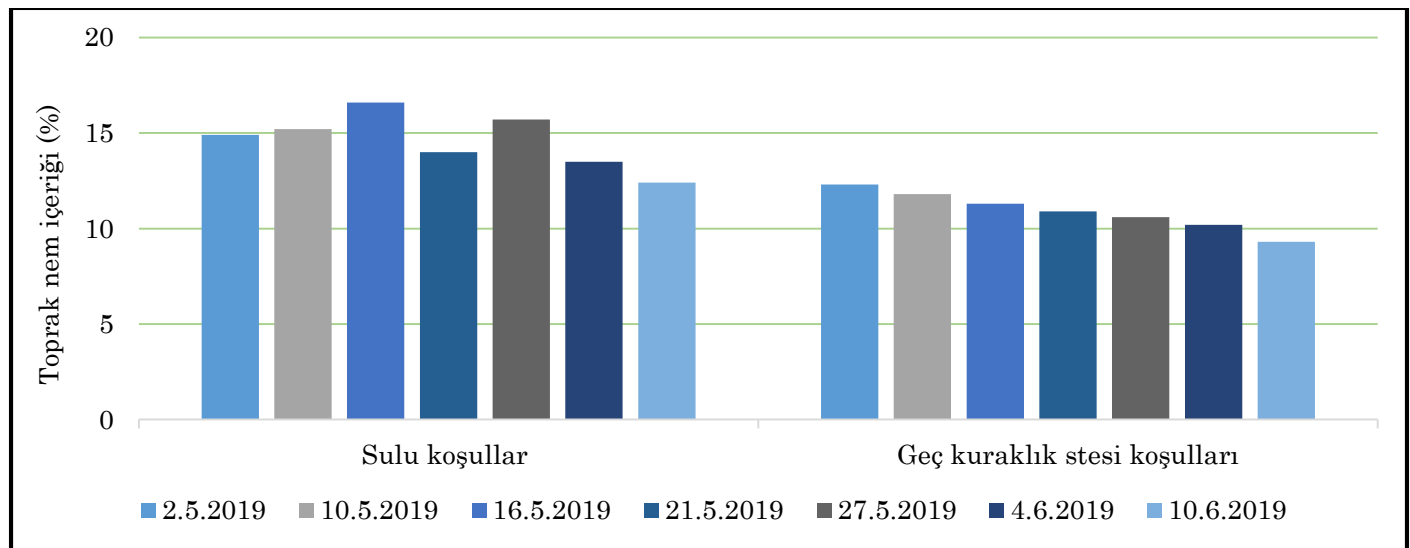
Table 3. Botanical names of the cultivars used in the experiments, the provinces where they were collected and the organizations from which they were supplied

Çeşit Cultivar	Botanik adı Botanical name	İli Provinces	Temin edildiği kuruluş Organizations from which it is supplied
TGB 003247	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 000543	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 003249	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 000526	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 003232	<i>Triticum aestivum</i> L.	Tokat	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 000534	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 000521	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 003246	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TGB 003248	<i>Triticum aestivum</i> L.	Çorum	Tarla Bitkileri Merkez Araştırma Ens. Müd.
TR 48371	<i>Triticum aestivum</i> subsp. <i>aestivum</i>	Tokat	Ege Tarımsal Araştırma Ens. Müd.
TR 37373	<i>Triticum aestivum</i> subsp. <i>aestivum</i>	Çorum	Ege Tarımsal Araştırma Ens. Müd.
TR 63501	<i>Triticum aestivum</i>	Tokat	Ege Tarımsal Araştırma Ens. Müd.
TR 44433	<i>Triticum aestivum</i> subsp. <i>aestivum</i>	Tokat	Ege Tarımsal Araştırma Ens. Müd.
TR 63497	<i>Triticum aestivum</i>	Tokat	Ege Tarımsal Araştırma Ens. Müd.
TR 63581	<i>Triticum aestivum</i>	Amasya	Ege Tarımsal Araştırma Ens. Müd.
TR 63575	<i>Triticum aestivum</i>	Amasya	Ege Tarımsal Araştırma Ens. Müd.
Ak Sunteri	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Dimenit	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Örmece	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Çam Buğdayı	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Zerun	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Çalı Basıran	<i>Triticum aestivum</i>	Tokat	Tokat İl Tarım ve Orman Müdürlüğü
Aldane	<i>Triticum aestivum</i>	-	Trakya Tarımsal Araştırma Ens. Müd.
Bezostaja 1	<i>Triticum aestivum</i>	-	Geçit Kuşağı Tarımsal Araştırma Ens. Müd.
Müfitbey	<i>Triticum aestivum</i>	-	Geçit Kuşağı Tarımsal Araştırma Ens. Müd.
Gün 91	<i>Triticum aestivum</i>	-	Tarla Bitkileri Merkez Araştırma Ens. Müd.
Dağdaş 94	<i>Triticum aestivum</i>	-	Bahri Dağdaş Uluslararası Tarımsal Araştırma Ens. Müd.



Şekil 1. 2017-2018 yetiştirme dönemine ait sulu koşullar ve geç kuraklık stresi koşulları oluşturulan deneme alanlarının toprak nem içerikleri (%)

Figure 1. Soil moisture contents of the trial areas under irrigated and late drought stress conditions in the 2017-2018 growing seasons (%)



Şekil 2. 2018-2019 yetiştirme dönemine ait sulu koşullar ve geç kuraklık stresi koşulları oluşturulan deneme alanlarının toprak nem içerikleri (%)

Figure 2. Soil moisture contents of the trial areas under irrigated and late drought stress conditions in the 2018-2019 growing seasons (%)

Sulu koşullarda yürütülen denemelerde, belirlenen nem içeriklerine göre topraktaki elverişli suyun (Güngör ve ark., 2012) yaklaşık %50'si tüketildiğinde bütün parseller damla sulama yöntemi ile sulanmıştır. Şekil 1 ve 2'de verilen tarihler toprak örneklerinin alındığı tarihler olup bu tarihlerden bir gün sonra hesaplanan toprak nem içeriği değerleri, sulamaya başlama değerinden düşük olduğunda sulama suyu miktarı Cömert ve Öztekin (2016)'e göre belirlenip sulama yapılmıştır. Geç kuraklık stresi oluşturulan koşullarda yürütülen denemelerde ise bitkiler, çeşitlerin %50'si gebecik dönemi başlangıcına (Feekes 10.00) ulaşana kadar doğal yağış koşullarında yetiştirilmiş daha sonra deneme alanlarının üzeri hasada kadar kalmak üzere yanları açık, şeffaf polietilen örtü ile kapatılmıştır (Öztürk & Aydın, 2014). Her iki yılda da kuraklık stresi oluşturulan denemelerde, yarlardan yağmur sularının gelmesinin engellenmesi için drenaj çukurları açılmıştır. Bitkiler hasat olgunluğuna geldiğinde tüm parseller başlarından kenar tesiri alınmadan çim biçme makasları ile elle hasat edilmiş ve buğday demetleri

tarlada kurutulduktan sonra makinayla harmanlanmıştır. Çalışmada bin tane ağırlığı, hektolitre ağırlığı (Kırtok ve ark., 1988), protein oranı (AACC, 2000; Elgün ve ark., 2012), Zeleny sedimentasyon değeri (ICC, 2002), bayrak yaprak ve tane kül oranları (AACC, 1995) gibi parametreler belirlenmiştir.

Deneme yıllarından elde edilen veriler homojenite testine (Bartlett, 1950) tabi tutulmuş, yıllar ayrı ayrı ve birlikte deneme planına uygun olarak MSTAT-C Version 2.10 paket programında varyans analizi yapılmış ve ortalamalar önemlilik düzeyine göre (0.05, 0.01) Duncan çoklu karşılaştırma testiyle karşılaştırılmıştır (Freed & Eisensmith, 1986).

BULGULAR ve TARTIŞMA

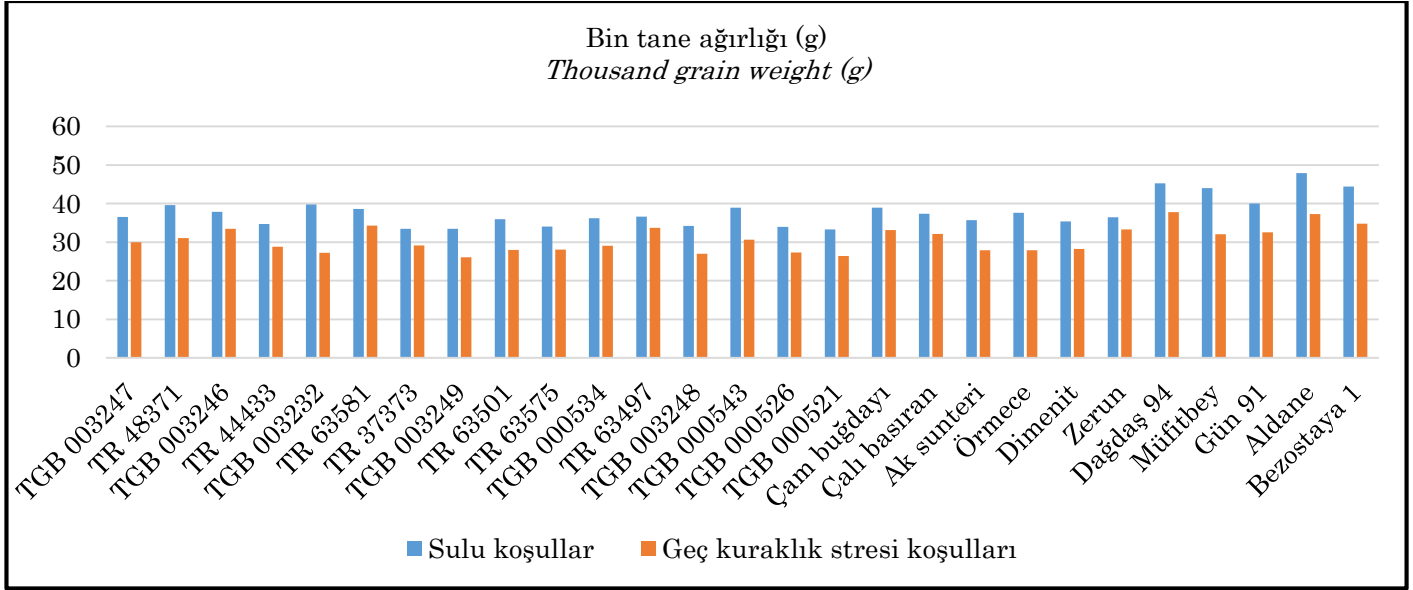
Bin Tane Ağırlığı (g)

Ekmeklik buğday çeşitlerinde sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda bin tane ağırlığı bakımından her iki deneme yılında ve yılların birlikte analiziyle, her iki koşulda da çeşitler arasında $p < .01$ düzeyinde önemli farklar bulunmuştur (Çizelge 4). Sulu koşullarda bin tane ağırlığı yerel çeşitlerde ilk yıl 31.53-41.12 g, ikinci yıl 33.92-42.33 g, kontrol çeşitlerinde ise ilk yıl 39.53-50.92 g, ikinci yıl 40.50-44.83 g olarak elde edilmiştir (Çizelge 4). Geç kuraklık stresi koşullarında ise bin tane ağırlığı yerel çeşitlerde ilk yıl 26.46-39.96 g, ikinci yıl 24.25-31.83 g, standart çeşitlerin ise ilk yıl 34.95-41.12 g, ikinci yıl 26.67-36.50 g arasında belirlenmiştir (Çizelge 4). Buğday tanesinin bin tane ağırlığı genetik yapıya, ekolojik faktörlere (Bulut, 2015; Ghaffar ve ark., 2018), bitkideki ana sap ve kardeşlere, metrekaresindeki başak sayısına, başaktaki tane sayısına ve uygulanan kültürel işlemlere (Abbas, 2017) göre değişiklik göstermektedir.

Çizelge 4. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin bin tane ağırlıkları (g)
Table 4. Thousand grain weights of bread wheat cultivars grown under irrigated and late drought stress conditions (g)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions					Geç kuraklık stresi koşulları Late drought stress conditions				
	2017-2018		2018-2019		Birleşik yıllar Combined years	2017-2018		2018-2019		Birleşik yıllar Combined years
TGB 003247	35.10±0.78	e-k**	37.92±0.98	d-h**	36.51±0.84	e-j**	32.36±0.46	fgh**	27.67±0.68	def**
TR 48371	41.12±1.08	c	38.17±0.92	d-g	39.64±0.91	cd	33.94±0.86	efg	28.25±1.13	de
TGB 003246	36.03±0.26	d-ı	39.67±0.85	c-f	37.85±0.90	c-h	35.09±0.39	def	31.83±1.42	bc
TR 44433	33.20±1.63	g-k	36.17±1.37	f-ı	34.68±1.16	ijkl	30.23±0.47	hi	27.42±0.96	d-g
TGB 003232	38.74±1.43	cde	40.75±0.43	bcd	39.74±0.81	cd	27.54±1.68	ij	26.92±1.08	efg
TR 63581	34.88±0.96	e-k	42.33±1.06	abc	38.60±1.79	c-g	38.41±1.74	a-d	30.25±0.58	cd
TR 37373	31.53±0.40	k	35.38±1.08	ghi	33.45±1.00	kl	32.47±0.83	fgh	25.83±0.33	efg
TGB 003249	32.95±0.86	h-k	33.92±0.42	ı	33.44±0.48	kl	26.46±1.34	j	25.67±0.36	efg
TR 63501	35.50±1.42	e-j	36.33±0.65	e-ı	35.92±0.72	h-k	29.71±0.12	hij	26.33±1.17	efg
TR 63575	33.71±0.72	g-k	34.42±0.67	hi	34.06±0.47	jkl	29.96±0.99	hij	26.17±0.33	efg
TGB 000534	35.59±0.74	e-j	36.75±0.95	e-ı	36.17±0.60	g-j	32.52±1.65	fgh	25.58±0.58	efg
TR 63497	37.14±0.27	d-g	36.17±1.34	f-ı	36.65±0.65	e-ı	39.96±0.77	ab	27.42±0.96	d-g
TGB 003248	32.14±0.71	ijk	36.25±0.95	f-ı	34.19±1.06	r-l	27.75±1.02	ij	26.25±0.95	efg
TGB 000543	36.71±1.18	d-h	41.17±0.67	bcd	38.94±1.17	c-f	35.24±0.68	def	26.13±0.22	efg
TGB 000526	32.81±0.26	h-k	35.08±0.96	ghi	33.95±0.68	jkl	28.08±0.44	ij	26.58±0.51	efg
TGB 000521	32.04±1.43	jk	34.50±0.29	hi	33.27±0.85	l	28.54±0.34	ij	24.25±0.76	g
Çam buğdayı	38.13±0.40	c-f	39.83±0.88	cde	38.98±0.58	cde	37.71±0.71	a-d	28.58±0.73	de
Çalı basıran	35.82±0.93	d-j	39.00±1.09	c-f	37.41±0.96	d-h	35.67±1.00	c-f	28.58±0.93	de
Ak sunteri	35.25±0.51	e-k	36.25±0.76	f-ı	35.75±0.47	h-l	28.60±0.75	ij	27.25±0.95	d-g
Örmece	38.46±0.97	c-f	36.83±0.22	e-ı	37.65±0.57	c-h	30.87±1.63	ghi	25.00±0.87	fg
Dimenit	34.18±0.94	g-k	36.58±1.46	e-ı	35.38±0.94	h-l	29.59±0.46	hij	26.83±0.44	efg
Zerun	34.69±0.48	f-k	38.17±1.06	d-g	36.43±0.94	f-j	38.96±0.30	abc	27.58±0.85	def
Ortalama Average	35.26		37.35		36.31		32.26		27.11	
Dağdaş 94	45.75±0.25	b	44.75±1.32	a	45.25±0.64	b	41.12±0.21	a	34.50±1.56	ab
Müfitbey	46.06±0.63	b	42.00±0.72	abc	44.03±1.00	b	37.37±1.06	b-e	26.67±1.26	efg
Gün 91	39.53±1.64	cd	40.50±0.80	cd	40.01±0.85	c	34.95±0.46	def	30.08±0.83	cd
Aldane	50.92±0.52	a	44.83±0.46	a	47.88±1.40	a	38.06±0.58	a-d	36.50±0.43	a
Bezostaja 1	44.88±1.34	b	44.00±0.63	ab	44.44±0.69	b	35.67±0.22	c-f	34.00±0.43	ab
Ortalama Average	45.43		43.22		44.33		37.43		32.35	
Genel ortalama General average	37.14		38.43		37.79		33.22 a**		28.08 b	
V.K. (%) C.V. (%)	4.16		3.62		3.89		4.26		4.37	

** : Aynı harf grubuna giren ortalamalar arasında $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation.



Şekil 3. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait bin tane ağırlıkları (g)

Figure 3. Thousand grain weights of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (g)

Çalışmada çeşitlerin ortalama bin tane ağırlıkları sulu denemede ilk yıl 37.14 g ikinci yıl 38.43 g, geç kuraklık stresi oluşturulan koşullarda ise ilk yıl 33.22 g ikinci yıl 28.08 g olarak belirlenmiş olup, geç kuraklık stresi oluşturulan koşullarda yıllar arasındaki fark önemli bulunmuştur (Çizelge 4). Her iki yılda da kuraklık stresi koşullarında bin tane ağırlığının azaldığı görülmektedir. Nitekim kuraklık stresinin bin tane ağırlığını önemli bir şekilde azalttığı bildirilmiştir (Chen ve ark., 2012; Nawaz ve ark., 2013; Öztürk & Aydın, 2014; Aghanejad ve ark., 2015; Zhang ve ark., 2019).

Şekil 3 incelendiğinde, iki yılın ortalamasına göre bin tane ağırlığı sulu koşullarda yerel çeşitlerde en yüksek 39.74 g ile TGB 003232, en düşük 33.27 g ile TGB 000521, tescilli çeşitlerde ise en yüksek 47.88 g ile Aldane, en düşük 40.01 g ile Gün 91 çeşitlerinden elde edilmiştir. Geç kuraklık stresi oluşturulan koşullarda ise yerel çeşitlerde en yüksek 34.33 g ile TR 63581 en düşük 26.06 g ile TGB 003249, tescilli çeşitlerde ise en yüksek 37.81 g ile Dağdaş 94 en düşük 32.02 g ile Müfitbey çeşitlerinden elde edilmiştir. Bin tane ağırlığı yüksek yerel çeşitlerin verimli ve kaliteli buğday çeşidi geliştirmede kullanılabileceği bildirilmiştir (Masood ve ark., 2005).

Hektolitre Ağırlığı (kg)

Hektolitre ağırlığı bakımından çeşitler arasındaki fark sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda her iki yılda ve birleştirilmiş yıllarda $p < .01$ düzeyinde önemli bulunmuştur (Çizelge 5). Birleştirilmiş yıllara bakıldığında; hektolitre ağırlığı değerlerinin sulu denemede yerel çeşitlerde 76.16-81.89 kg tescilli çeşitlerde 82.37-85.22 kg arasında değiştiği, geç kuraklık stresi oluşturulan denemede ise yerel çeşitlerde 74.15-81.94 kg tescilli çeşitlerde 79.10-82.10 kg arasında değiştiği görülmektedir (Çizelge 5). Hektolitre ağırlığı genotipik özelliğe, kültürel uygulamalara, yatma, hastalık ve zararlı gibi faktörlere (Taghouti ve ark., 2010; Kendal, 2013), tanenin dolgunluğuna, yoğunluğuna, şekline, büyüklüğüne, homojenliğine, tanedeki kavuz oranına, endosperm yapısına (Kılıç ve ark., 2010; Kendal, 2013; Arlotti & Silvestri, 2019), ekim zamanına ve ekolojik koşullara (Elgün ve ark., 1999) bağlı olarak değişmektedir.

Çalışmada hem sulu koşullarda hem de geç kuraklık stresi koşullarında her iki yılda da yerel genotiplerin ortalama hektolitre ağırlıklarının tescilli çeşitlerden düşük olduğu görülmektedir (Çizelge 5). Yerel genotiplerin düşük hektolitre ağırlığı potansiyeline sahip oldukları daha önce yapılan bir çalışmada da bildirilmiştir (Tekdal ve ark., 2018). Çalışmada, genel olarak yerel genotiplerin taneleri tescilli çeşitlere göre daha küçük ve cılız olarak gözlemlenmiştir. Bu durumun sonucu olarak yerel çeşitlerden daha düşük hektolitre ağırlığı değerleri elde edildiği düşünülmektedir. Nitekim uzun taneli buğdayların kısa taneli buğdaylara, küçük tanelilerin de iri tanelilere göre daha az hektolitre ağırlığına sahip oldukları ifade edilmiştir (Elgün ve ark., 2001).

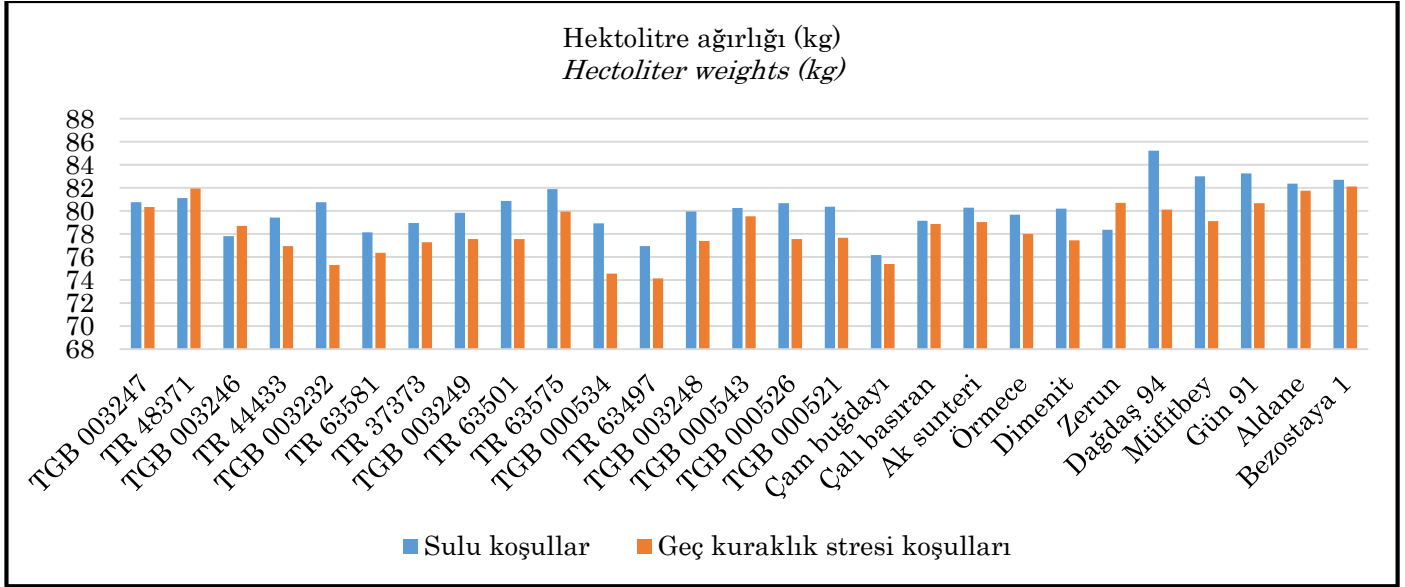
Çizelge 5 incelendiğinde, birleştirilmiş yıllarda çeşitlerin ortalama hektolitre ağırlığı sulu koşullarda 80.26 kg, geç kuraklık stresi oluşturulan koşullarda 78.37 kg olarak bulunmuştur. Sulu koşullarda geç kuraklık stresi oluşturulan koşullara göre TR 48371, TGB 003246 ve Zerin çeşitleri dışında diğer bütün çeşitlerden daha yüksek

hektolitre ağırlığı değerleri elde edilmiştir (Şekil 4). Konu ile ilgili yapılan çalışmalarda da sulamanın hektolitre ağırlığını artırdığı bildirilmiştir (Kendal, 2013; Sakin ve ark., 2017). Çalışmada hem sulu koşullarda hem de geç kuraklık stresi oluşturulan koşullarda hektolitre ağırlığı bakımından yıllar arasında önemli farklılıklar saptanmıştır (Çizelge 5). Sulu koşullarda hektolitre ağırlığının ikinci yıl ilk yıla göre daha yüksek olmasının, ikinci yıl düşen toplam yağışın fazla olmasından kaynaklandığı düşünülmektedir. Ayrıca geç kuraklık stresi oluşturulan koşullarda hektolitre ağırlığının ilk yıl ikinci yıla göre daha yüksek olmasında, denemelerin üzeri kapatılmadan önce (Nisan ayından önce) ilk yıl ikinci yıla göre daha fazla yağış miktarının düşmesinin (Çizelge 1) etkili olabileceği düşünülmektedir.

Çizelge 5. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin hektolitre ağırlıkları (kg)
Table 5. Hectoliter weights of bread wheat cultivars grown under irrigated and late drought stress conditions (kg)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions						Geç kuraklık stresi koşulları Late drought stress conditions					
	2017-2018		2018-2019		Birleşik yıllar Combined years		2017-2018		2018-2019		Birleşik yıllar Combined years	
TGB 003247	79.18±0.54	d-g**	82.35±0.16	a-e**	80.76±0.75	b-h**	77.89±0.30	ijk**	82.79±2.26	a**	80.34±1.50	a-d**
TR 48371	79.42±0.86	def	82.82±0.85	a-e	81.12±0.93	b-g	83.10±0.20	a	80.78±1.92	ab	81.94±1.01	a
TGB 003246	76.73±0.33	hi	78.88±2.28	def	77.80±1.14	jkl	80.13±0.53	efg	77.26±0.83	b-e	78.70±0.78	c-h
TR 44433	76.98±0.21	hi	81.83±0.85	a-e	79.41±1.15	f-k	76.37±0.41	kl	77.53±1.32	b-e	76.95±0.67	ghi
TGB 003232	80.70±0.57	bcd	80.82±1.34	b-e	80.76±0.65	b-h	77.83±0.34	ijk	72.78±1.32	ef	75.30±1.28	ij
TR 63581	78.37±0.65	e-h	77.94±0.26	ef	78.15±0.33	i-l	78.03±0.26	hij	74.71±1.36	c-f	76.37±0.97	hij
TR 37373	76.96±0.08	hi	80.91±1.79	b-e	78.94±1.19	g-k	78.41±0.63	hij	76.16±0.57	b-f	77.28±0.63	f-i
TGB 003249	77.20±0.37	hi	82.45±1.30	a-e	79.83±1.32	e-j	78.58±0.32	ghi	76.52±0.23	b-f	77.55±0.49	e-i
TR 63501	79.51±0.44	de	82.22±0.80	a-e	80.86±0.73	b-h	80.39±0.25	def	74.71±0.46	c-f	77.55±1.29	e-i
TR 63575	79.76±0.24	cde	84.02±1.02	abc	81.89±1.06	b-f	81.92±0.05	a-d	77.96±0.77	bcd	79.94±0.95	a-e
TGB 000534	78.30±0.66	e-h	79.56±0.48	c-f	78.93±0.46	g-k	76.84±0.05	jkl	72.30±0.70	f	74.57±1.06	j
TR 63497	78.39±0.39	e-h	75.49±0.84	f	76.94±0.77	kl	75.50±0.20	l	72.80±1.92	ef	74.15±1.05	j
TGB 003248	77.12±0.46	hi	82.79±0.65	a-e	79.95±1.32	e-j	78.62±0.43	ghi	76.13±0.56	b-f	77.38±0.64	f-i
TGB 000543	79.18±0.51	d-g	81.33±0.94	b-e	80.25±0.68	d-j	82.16±0.27	abc	76.91±0.48	b-f	79.53±1.20	b-f
TGB 000526	79.42±0.30	def	81.91±0.73	a-e	80.67±0.66	c-i	78.10±0.86	hij	77.05±0.36	b-f	77.57±0.48	e-i
TGB 000521	77.41±0.43	gh	83.29±1.52	a-d	80.35±1.49	d-j	79.63±0.06	fgh	75.69±0.85	c-f	77.66±0.96	e-i
Çam buğdayı	76.69±0.26	hi	75.63±1.73	f	76.16±0.82	l	77.18±0.27	ijk	73.60±1.01	def	75.39±0.93	ij
Çalı basıran	79.13±0.30	d-g	79.14±1.08	c-f	79.13±0.50	g-k	81.33±0.32	b-e	76.41±1.37	b-f	78.87±1.27	c-g
Ak sunteri	78.57±0.56	e-h	82.02±1.58	a-e	80.29±1.08	d-j	80.36±0.22	def	77.73±1.38	bcd	79.04±0.86	c-g
Örmece	77.55±0.69	fgh	81.79±0.07	a-e	79.67±1.00	f-j	81.09±0.12	c-f	74.93±0.54	c-f	78.01±1.40	d-h
Dimenit	77.97±0.05	e-h	82.42±1.34	a-e	80.19±1.16	d-j	80.42±0.23	def	74.47±0.13	c-f	77.44±1.34	f-i
Zerun	75.50±0.40	i	81.23±0.12	b-e	78.37±1.29	h-l	82.97±0.25	ab	78.43±1.23	abc	80.70±1.16	abc
Ortalama Average	78.18		80.95		79.57		79.40		76.26		77.83	
Dağdaş 94	83.97±0.24	a	86.47±1.72	a	85.22±0.96	a	79.61±0.26	fgh	80.62±1.60	ab	80.11±0.76	a-d
Müfitbey	82.35±0.50	ab	83.66±0.05	a-d	83.01±0.37	abc	81.75±0.52	a-e	76.45±1.34	b-f	79.10±1.35	c-g
Gün 91	81.63±0.42	b	84.86±0.32	ab	83.25±0.76	ab	82.82±0.35	ab	78.52±1.17	abc	80.67±1.11	abc
Aldane	81.27±0.25	bc	83.47±0.37	a-d	82.37±0.53	b-e	82.69±0.20	abc	80.79±1.67	ab	81.74±0.86	ab
Bezostaja 1	81.74±0.06	b	83.63±0.80	a-d	82.69±0.55	bcd	81.43±1.02	b-e	82.77±1.37	a	82.10±0.82	a
Ortalama Average	82.20		84.42		83.31		81.66		79.83		80.75	
Genel ortalama General average	78.93 b		81.59 a**		80.26		79.82 a**		76.92 b		78.37	
V.K. (%) C.V. (%)	0.95		2.32		1.80		0.83		2.40		1.77	

** : Aynı harf grubuna giren ortalamalar arasında p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation.



Şekil 4. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait hektolitre ağırlıkları (kg)

Figure 4. Hectoliter weights of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (kg)

Protein Oranı (%)

Sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda yetiştirilen ekmeklik buğday çeşitlerinde protein oranı bakımından her iki yılda ve birleştirilmiş yıllarda $p < .01$ düzeyinde önemli farklılıklar bulunmuştur (Çizelge 6). Sulu koşullarda protein oranı yerel çeşitlerde ilk yıl %13.30-17.20 ikinci yıl %14.27-17.80, standart çeşitlerde ise ilk yıl %14.20-16.17 ikinci yıl %12.57-16.67 arasında belirlenmiştir (Çizelge 6). Geç kuraklık stresi koşullarında ise protein oranı yerel çeşitlerde ilk yıl %16.73-19.47 ikinci yıl %15.70-19.10, standart çeşitlerin ise ilk yıl %15.77-17.37 ikinci yıl %13.10-17.57 arasında değişiklik göstermiştir (Çizelge 6). Buğdayda protein miktarı genotipe, yetiştirme şartlarına, ekim zamanına ve iklim koşullarına göre değişmektedir (Elgün ve ark., 2012). Çalışmada hem sulu koşullarda hem de kuraklık stresi koşullarında her iki yılda da yerel çeşitlerin ortalama protein oranları tescilli çeşitlerin ortalama protein oranlarından daha yüksek bulunmuştur. Akçura ve ark. (2011) da yürüttüğü çalışmasında, yerel buğday çeşitlerinin protein oranlarının modern çeşitlerden daha yüksek olduğunu bildirmişlerdir.

Birleştirilmiş yıllara bakıldığında ortalama protein oranı sulu koşullarda %15.61 geç kuraklık stresi oluşturulan koşullarda %17.61 olarak bulunmuş olup, kuraklık stresi protein oranını artırmıştır (Çizelge 6). Konu ile ilgili yapılan birçok çalışmada kuraklığın protein oranını artırdığı bildirilmiştir (Tosun ve ark., 2006; Partigöç, 2009; Ayrancı, 2012; Aydoğan, 2016). Şekil 5'te çeşitlerin dağılımına bakıldığında Gün 91 çeşidi dışındaki bütün çeşitlerde protein oranı geç kuraklık stresi koşullarında daha yüksek bulunmuştur.

Zeleny Sedimentasyon Değeri (ml)

Zeleny sedimentasyon değeri bakımından çeşitler arasındaki fark sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda her iki yılda ve birleştirilmiş yıllarda $p < .01$ düzeyinde önemli bulunmuştur (Çizelge 7). Birleştirilmiş yıllara bakıldığında, Zeleny sedimentasyon değerlerinin sulu denemede yerel çeşitlerde 20.50-35.33 ml tescilli çeşitlerde 27.00-41.17 ml arasında değiştiği, geç kuraklık stresi oluşturulan denemede ise yerel çeşitlerde 25.83-47.67 ml tescilli çeşitlerde 30.83-47.67 ml arasında değiştiği görülmektedir (Çizelge 7). Buğdayda sedimentasyon değeri genotipik özelliğe bağlı olmakla birlikte iklim faktörlerinin etkisine göre de değişiklik göstermektedir (Aydoğan ve ark., 2010). Çalışmada hem sulu hem de kuraklık stresi koşullarında her iki deneme yılında da ortalama Zeleny sedimentasyon değerleri yerel çeşitlerde tescilli çeşitlere göre daha düşük olarak belirlenmiştir. Nitekim Akçura ve ark. (2014) tarafından yürütülen çalışmada da tescilli çeşitlere göre yerel ekmeklik buğday çeşitlerinde Zeleny sedimentasyon değerinin daha düşük olduğu saptanmıştır.

Çalışmada çeşitlerin ortalama Zeleny sedimentasyon değerleri sulu koşullarda ilk yıl 24.9 ml ikinci yıl 33.4 ml, geç kuraklık stresi oluşturulan koşullarda ise ilk yıl 34.1 ml ikinci yıl 37.5 ml olarak belirlenmiş, yıllar arasındaki fark önemli bulunmuştur (Çizelge 7). Başaklanmadan tane doldurma dönemine kadar geçen sürede iklimin serin, yağışlı ve rutubetli geçmesi sedimentasyon değerlerinde düşüşe neden olmaktadır (Kılıç, 2003; Aksoy, 2012). Ayrıca, çeşitlerin ortalama Zeleny sedimentasyon değerleri kuraklık stresi koşullarında sulu koşullara göre her

iki deneme yılında da daha yüksek olarak bulunmuştur. Öztürk ve Aydın (2004) da sedimantasyon değerinin su stresi altında önemli ölçüde arttığını belirtmişlerdir. Çeşitlerin sulu koşullarda ve geç kuraklık stresi oluşturulan koşullardaki Zeleny sedimantasyon değerlerinin dağılımına bakıldığında TGB 000521 çeşidi dışında bütün çeşitlerde geç kuraklık stresi koşullarında daha yüksek değerler elde edilmiştir (Şekil 6).

Çizelge 6. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin protein oranları (%)
Table 6. Protein rates of bread wheat cultivars grown under irrigated and late drought stress conditions (%)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions						Geç kuraklık stresi koşulları Late drought stress conditions					
	2017-2018		2018-2019		Birleşik yıllar Combined years		2017-2018		2018-2019		Birleşik yıllar Combined years	
TGB 003247	15.50±0.10	b-g**	14.57±0.32	def**	15.03±0.26	fg**	18.33±0.09	bed**	15.70±0.44	d**	17.02±0.62	e-h**
TR 48371	14.97±0.26	e-ı	14.53±0.41	def	14.75±0.24	f-ı	19.13±0.15	ab	17.23±0.33	a-d	18.18±0.45	a-e
TGB 003246	15.17±0.19	e-h	16.33±0.38	a-e	15.75±0.32	b-f	17.93±0.09	cde	17.23±0.84	a-d	17.58±0.41	c-g
TR 44433	16.23±0.19	bcd	14.53±0.62	def	15.38±0.48	d-g	18.43±0.03	bcd	18.67±0.49	ab	18.55±0.23	abc
TGB 003232	13.30±0.10	j	14.27±0.70	ef	13.78±0.38	ı	18.37±0.19	bcd	15.90±0.60	cd	17.13±0.62	d-h
TR 63581	14.73±0.15	ghı	16.43±0.60	a-e	15.58±0.47	c-g	18.30±0.15	bcd	17.80±0.23	a-d	18.05±0.17	a-e
TR 37373	14.87±0.17	f-ı	15.60±0.64	a-e	15.23±0.34	efg	19.23±0.12	ab	17.67±0.96	a-d	18.45±0.56	a-d
TGB 003249	16.37±0.03	b	17.80±0.96	a	17.08±0.54	a	18.00±0.15	cde	17.77±0.56	a-d	17.88±0.27	a-f
TR 63501	15.87±0.19	b-e	16.00±1.12	a-e	15.93±0.51	a-f	19.10±0.12	ab	19.03±0.26	a	19.07±0.13	a
TR 63575	16.30±0.20	bc	14.60±0.17	def	15.45±0.40	c-g	16.73±0.27	fg	17.40±0.06	a-d	17.07±0.19	e-h
TGB 000534	15.53±0.27	b-g	16.33±0.20	a-e	15.93±0.23	a-f	18.43±0.24	bcd	16.97±0.46	a-d	17.70±0.40	b-g
TR 63497	14.23±0.20	ı	15.87±0.20	a-e	15.05±0.39	fg	19.47±0.28	a	18.47±0.72	abc	18.97±0.41	ab
TGB 003248	17.20±0.17	a	16.57±1.07	a-e	16.88±0.50	ab	18.30±0.21	bcd	17.70±0.82	a-d	18.00±0.40	a-e
TGB 000543	15.53±0.27	b-g	15.30±0.70	b-e	15.42±0.34	d-g	16.73±0.17	fg	16.17±0.74	bcd	16.45±0.36	gh
TGB 000526	15.03±0.17	e-ı	16.80±0.17	a-d	15.92±0.41	a-f	17.10±0.15	ef	16.87±0.43	a-d	16.98±0.21	e-h
TGB 000521	16.17±0.26	bcd	17.10±0.67	abc	16.63±0.38	abc	17.93±0.15	cde	17.23±1.05	a-d	17.58±0.50	c-g
Çam buğdayı	14.87±0.29	f-ı	16.20±0.35	a-e	15.53±0.36	c-g	17.37±0.09	ef	18.00±0.66	a-d	17.68±0.33	b-g
Çalı basıran	16.27±0.15	bcd	17.37±0.49	ab	16.82±0.34	ab	18.43±0.09	bcd	19.10±0.80	a	18.77±0.39	abc
Ak sunteri	15.43±0.15	c-g	16.40±0.68	a-e	15.92±0.38	a-f	18.43±0.27	bcd	17.30±0.42	a-d	17.87±0.34	a-f
Örmece	14.50±0.31	hı	15.53±1.04	a-e	15.02±0.54	fg	17.57±0.20	def	17.50±0.71	a-d	17.53±0.33	c-h
Dimenit	16.33±0.22	bc	16.30±0.58	a-e	16.32±0.28	a-e	17.77±0.18	de	18.30±0.17	abc	18.03±0.16	a-e
Zerun	15.73±0.45	b-f	17.17±0.98	abc	16.45±0.58	a-d	18.83±0.15	abc	17.80±0.85	a-d	18.32±0.45	a-e
Ortalama Average	15.46		15.98		15.72		18.18		17.54		17.86	
Dağdaş 94	15.10±0.21	e-ı	12.57±0.52	f	13.83±0.62	hı	17.37±0.12	ef	17.57±0.49	a-d	17.47±0.23	c-h
Müftibey	14.87±0.17	f-ı	14.97±0.29	cde	14.92±0.15	fgh	17.10±0.21	ef	16.90±0.82	a-d	17.00±0.38	e-h
Gün 91	16.17±0.15	bcd	16.67±0.41	a-d	16.42±0.22	a-e	17.33±0.30	ef	13.10±1.25	e	15.22±1.11	ı
Aldane	15.37±0.24	d-h	16.47±0.32	a-e	15.92±0.30	a-f	15.77±0.15	h	16.73±0.99	a-d	16.25±0.50	hı
Bezostaja 1	14.20±0.26	ı	14.63±0.52	def	14.42±0.28	ghı	16.07±0.64	gh	17.07±0.38	a-d	16.57±0.40	fgh
Ortalama Average	15.14		15.06		15.10		16.73		16.27		16.50	
Genel ortalama General average	15.40		15.81		15.61		17.91		17.30		17.61	
V.K. (%) C.V. (%)	2.36		5.55		4.31		2.08		5.69		4.23	

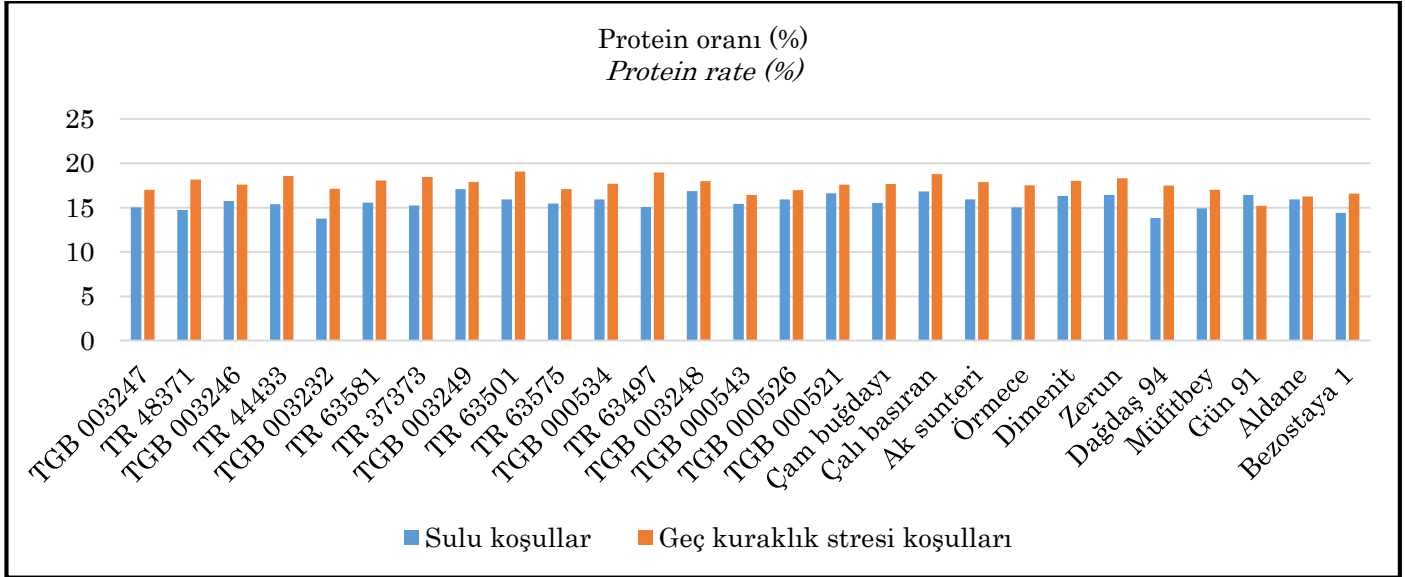
** : Aynı harf grubuna giren ortalamalar arasında $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation.

Bayrak Yaprak Kül Oranı (%)

Ekmeklik buğday çeşitlerinde bayrak yaprak kül oranı bakımından çeşitler arasındaki fark sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda her iki yılda ve birleştirilmiş yıllarda $p < .01$ düzeyinde önemli bulunmuştur (Çizelge 8). Sulu koşullarda bayrak yaprak kül oranları yerel çeşitlerde ilk yıl %7.95-12.09 ikinci yıl %6.61-12.19, standart çeşitlerde ise ilk yıl %11.26-18.29 ikinci yıl %10.04-17.37 olarak belirlenmiştir (Çizelge 8). Geç kuraklık stresi koşullarında ise bayrak yaprak kül oranları yerel çeşitlerde ilk yıl %5.84-10.27 ikinci yıl %9.79-12.75, standart çeşitlerde ise ilk yıl %7.53-15.84 ikinci yıl %12.18-17.48 arasında değişiklik göstermiştir (Çizelge 8). Kül içeriği çevre şartlarına (Araus ve ark., 2001; Misra ve ark., 2006), örnek alınan bitki kısmına, örnekleme zamanına (Misra ve ark., 2006) göre değişiklik gösteren bir karakterdir. Çizelge 8 incelendiğinde, hem sulu koşullarda hem de geç kuraklık stresi koşullarında her iki yılda da yerel çeşitlerin ortalama bayrak yaprak kül oranları tescilli çeşitlerden düşük bulunmuştur. Birleşik yılların sonuçlarına bakıldığında çeşitlerin ortalama bayrak yaprak kül oranı çoğunlukla sulu koşullarda geç kuraklık stresi koşullarına göre daha düşük bulunmuştur (Şekil 7).

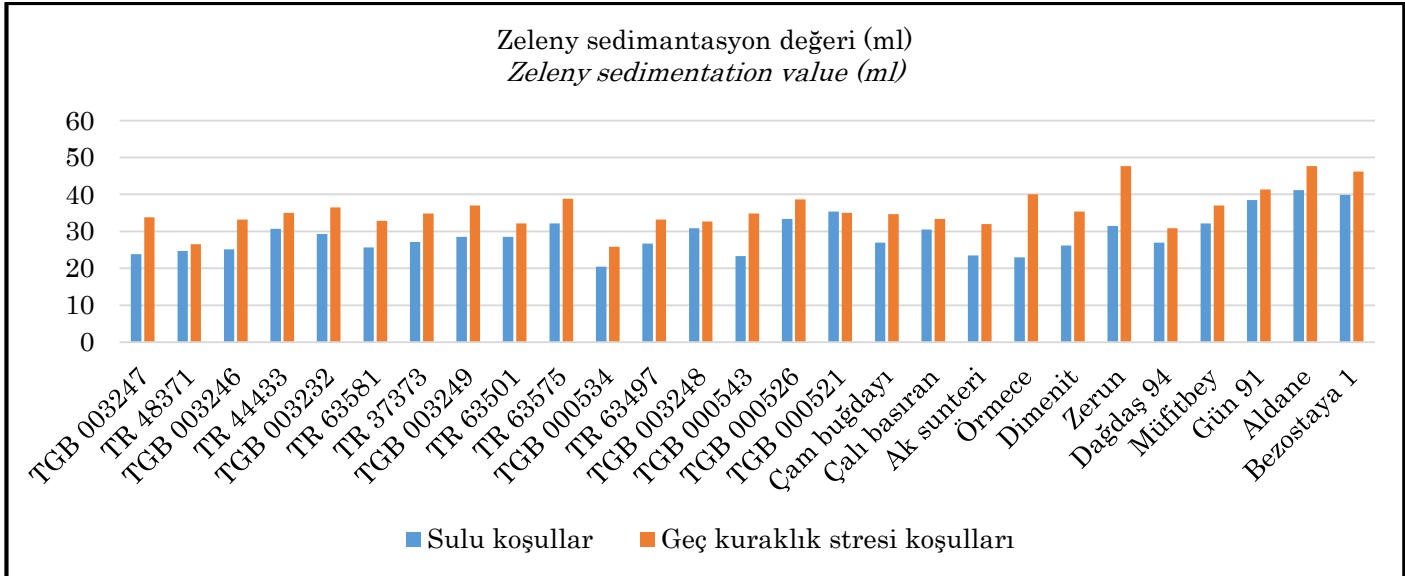
Çalışmada çeşitlerin ortalama bayrak yaprak kül oranları sulu denemede ilk yıl %10.39 ikinci yıl %9.30, geç kuraklık stresi oluşturulan koşullarda ise ilk yıl %8.49, ikinci yıl %12.07 olarak belirlenmiş, yıllar arasındaki fark önemli bulunmuştur (Çizelge 8). Sulu koşullara göre kuraklık stresi koşullarında bayrak yaprak kül oranının ilk yıl azaldığı, ikinci yıl ise arttığı görülmektedir. İkinci yıldaki bu artış yüksek toprak nem içeriğinden (Şekil 2)

kaynaklanmış olabilir (Öztürk & Aydın, 2014). Hem sulu koşullarda hem de kuraklık stresi koşullarında her iki yılda da çalışmada kurağa duyarlı Aldane ve Bezostaya 1 çeşitlerinin en yüksek yaprak kül oranı değerleri gösterdiği belirlenmiştir (Çizelge 8).



Şekil 5. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait protein oranları (%)

Figure 5. Protein rates of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (%)



Şekil 6. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait Zeleny sedimentasyon değerleri (ml)

Figure 6. Zeleny sedimentation values of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (ml)

Tane Kül Oranı (%)

Sulu koşullarda ve geç kuraklık stresi oluşturulan koşullarda yetiştirilen ekmeklik buğday çeşitlerinde tane kül oranı bakımından her iki yılda ve birleştirilmiş yıllarda $p < .01$ düzeyinde önemli farklılıklar bulunmuştur (Çizelge 9). Sulu koşullarda tane kül oranları yerel çeşitlerde ilk yıl %1.64-3.36 ikinci yıl %1.32-2.26, standart çeşitlerin ise ilk yıl %1.69-2.11 ikinci yıl %1.51-1.80 arasında değişiklik göstermiştir (Çizelge 9). Geç kuraklık stresi koşullarında ise tane kül oranları yerel çeşitlerde ilk yıl %1.92-2.55 ikinci yıl %1.76-2.28, standart çeşitlerin ise ilk yıl %1.72-1.93 ikinci yıl %1.73-2.39 arasında belirlenmiştir (Çizelge 9). Tane kül içeriğinin yaprak yaşlanma

başlangıcından sonra, bitkinin vejetatif organlarından taneye yeniden taşınan asimilat ve mineral miktarına bağlı olduğu belirtilmiştir (Merah ve ark., 2001; Zhu ve ark., 2008). Çizelge 9 incelendiğinde, hem sulu koşullarda hem de geç kuraklık stresi koşullarında her iki yılda da yerel çeşitlerin ortalama tane kül oranları tescilli çeşitlerden yüksek bulunmuştur. Tane dolum döneminde yüksek stomal iletkenlik ve transpirasyon oranlarını sürdüremeyen yerel çeşitler tane dolumu için esas olarak çiçeklenme öncesi sentezlenen asimilatları ve mineralleri kullandıklarından tescilli çeşitlere göre daha yüksek tane kül içeriğine sahiptirler (Merah, 2001). Kuraklık stresi koşullarında tane kül oranı bakımından yüksek gruplar içerisinde yer alan TR 63497, TGB 003248 ve TR 63581 çeşitlerinin bayrak yaprak kül içeriklerinin düşük gruplar içerisinde yer aldığı saptanmıştır. Kuraklık stresinin, vejetatif organlardaki minerallerin tanelere taşınmasını artırarak, olgunluk döneminde yapraklardaki kül içeriğinin azalmasına neden olduğu bildirilmiştir (Zhu ve ark., 2008).

Çizelge 7. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin Zeleny sedimentasyon değerleri (ml)

Table 7. Zeleny sedimentation values of bread wheat cultivars grown under irrigated and late drought stress conditions (ml)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions						Geç kuraklık stresi koşulları Late drought stress conditions					
	2017-2018		2018-2019		Birleşik yıllar Combined years		2017-2018		2018-2019		Birleşik yıllar Combined years	
TGB 003247	19.0±0.58	klm**	28.7±0.67	hij**	23.83±2.20	jk**	35.0±1.15	def**	32.7±2.03	fg**	33.83±1.17	e-h**
TR 48371	22.7±0.88	g-k	26.7±0.88	ij	24.67±1.05	ijk	26.7±1.20	hij	26.3±0.33	ı	26.50±0.56	ı
TGB 003246	19.3±0.33	klm	31.0±0.58	fgh	25.17±2.63	ijk	37.3±1.20	cde	29.0±1.00	ghı	33.17±1.99	fgh
TR 44433	27.7±0.88	c-f	33.7±0.67	d-g	30.67±1.43	def	28.7±1.20	g-j	41.3±0.67	b	35.00±2.90	efg
TGB 003232	24.0±2.08	f-j	34.7±0.33	def	29.33±2.56	efg	40.0±1.53	bc	33.0±0.58	fg	36.50±1.73	def
TR 63581	20.0±1.00	jkl	31.3±0.67	fgh	25.67±2.59	h-k	26.0±1.15	ij	39.7±1.45	b-e	32.83±3.17	gh
TR 37373	21.7±1.20	h-k	32.7±1.20	e-h	27.17±2.57	ghı	30.0±1.73	ghı	39.7±1.20	b-e	34.83±2.36	efg
TGB 003249	27.0±1.00	def	30.0±1.15	ghı	28.50±0.96	fgh	37.7±0.88	cde	36.3±0.88	c-f	37.00±0.63	cde
TR 63501	23.3±0.88	f-k	33.7±0.88	d-g	28.50±2.38	fgh	29.0±0.00	ghı	35.3±0.33	ef	32.17±1.42	gh
TR 63575	27.7±1.20	c-f	36.7±1.45	cd	32.17±2.18	de	33.7±0.33	efg	44.0±0.58	b	38.83±2.33	bcd
TGB 000534	15.7±0.33	m	25.3±0.67	j	20.50±2.19	l	24.0±1.15	j	27.7±0.33	hı	25.83±0.98	ı
TR 63497	22.3±1.20	h-k	31.0±0.58	fgh	26.67±2.03	ghı	30.0±1.00	ghı	36.3±1.20	c-f	33.17±1.58	fgh
TGB 003248	27.7±1.45	c-f	34.0±1.15	d-g	30.83±1.64	def	38.0±1.15	cde	27.3±1.45	hı	32.67±2.53	gh
TGB 000543	20.7±1.20	ı-l	26.0±1.53	j	23.33±1.48	jk	37.7±0.88	cde	32.0±0.58	fgh	34.83±1.35	efg
TGB 000526	27.7±1.20	c-f	39.0±1.15	bc	33.33±2.64	cd	41.0±0.58	bc	36.3±1.20	c-f	38.67±1.20	bcd
TGB 000521	30.7±0.88	cd	40.0±0.58	bc	35.33±2.14	c	39.7±1.33	bcd	30.3±1.45	ghı	35.00±2.27	efg
Çam buğdayı	25.3±0.67	e-h	28.7±1.45	hij	27.00±1.03	ghı	29.0±1.53	ghı	40.3±0.33	bcd	34.67±2.63	efg
Çalı basıran	29.0±1.53	cde	32.0±0.00	fgh	30.50±0.96	def	26.0±1.00	ij	40.7±0.88	bc	33.33±3.33	fgh
Ak sunteri	17.0±0.58	lm	30.0±1.53	ghı	23.50±3.00	jk	24.0±1.73	j	40.0±2.08	b-e	32.00±3.78	gh
Örmece	19.3±0.67	klm	26.7±1.45	ij	23.00±1.79	kl	40.3±0.67	bc	39.7±1.20	b-e	40.00±0.63	bc
Dimenit	21.3±0.33	h-k	31.0±0.58	fgh	26.17±2.18	hij	29.3±0.67	ghı	41.3±0.88	b	35.33±2.73	efg
Zerun	26.7±1.33	d-g	36.3±1.45	cde	31.50±2.33	de	43.7±0.33	ab	51.7±1.76	a	47.67±1.96	a
Ortalama (Average)	23.4		31.8		27.6		33.0		36.4		34.7	
Dağdaş 94	23.3±0.88	f-k	30.7±0.88	fgh	27.00±1.73	ghı	31.3±0.33	fgh	30.3±0.67	ghı	30.83±0.40	h
Müfitbey	24.7±0.67	e-ı	39.7±0.88	bc	32.17±3.39	de	30.7±0.88	fı	43.3±0.88	b	37.00±2.89	cde
Gün 91	35.0±0.00	b	42.0±1.00	b	38.50±1.63	b	47.0±1.53	a	35.7±1.45	def	41.33±2.70	b
Aldane	41.0±0.58	a	41.3±0.88	b	41.17±0.48	a	46.0±2.08	a	49.3±0.67	a	47.67±1.23	a
Bezostaja 1	31.7±2.03	bc	48.0±0.58	a	39.83±3.77	ab	39.7±1.20	bcd	52.7±0.88	a	46.17±2.98	a
Ortalama (Average)	31.1		40.3		35.7		38.9		42.3		40.6	
Genel ortalama General average	24.9 b		33.4 a**		29.2		34.1 b		37.5 a**		35.8	
V.K. (%) C.V. (%)	6.98		4.79		5.73		5.86		5.18		5.51	

** : Aynı harf grubuna giren ortalamalar arasında p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation.

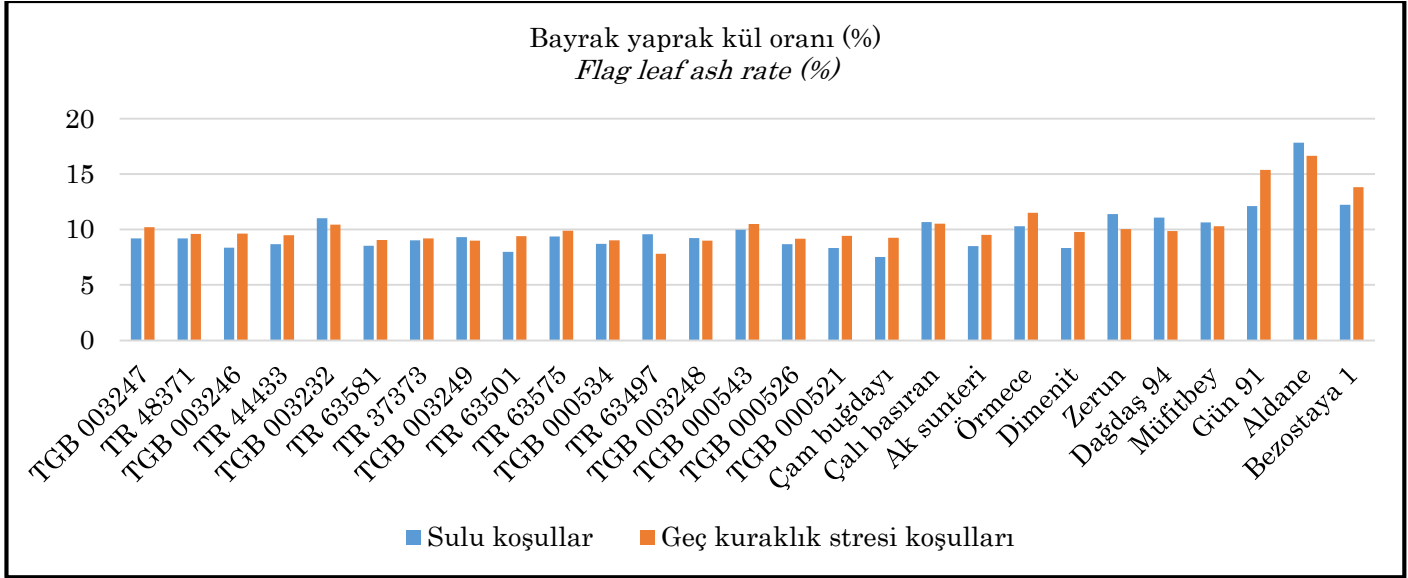
Çizelge 8. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin bayrak yaprak kül oranları (%)

Table 8. Flag leaf ash rates of bread wheat cultivars grown under irrigated and late drought stress conditions (%)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions						Geç kuraklık stresi koşulları Late drought stress conditions					
	2017-2018		2018-2019		Birleşik yıllar Combined years		2017-2018		2018-2019		Birleşik yıllar Combined years	
TGB 003247	8.98±0.27	h-l**	9.40±0.32	d-h**	9.19±0.21	ghi**	9.06±0.25	cde**	11.38±0.18	bcd**	10.22±0.54	e-h**
TR 48371	9.56±0.12	g-j	8.81±0.61	d-ı	9.19±0.32	ghi	7.21±0.17	fgh	11.99±0.53	bc	9.60±1.10	e-h
TGB 003246	8.26±0.12	kl	8.43±0.16	e-j	8.35±0.10	ijk	7.62±0.10	efg	11.63±0.40	bcd	9.63±0.91	e-h
TR 44433	8.66±0.07	jkl	8.70±0.26	e-ı	8.68±0.12	hij	7.51±0.24	efg	11.44±0.72	bcd	9.48±0.94	e-h
TGB 003232	9.83±0.09	f-ı	12.19±0.69	b	11.01±0.61	cd	8.21±0.26	d-g	12.68±0.56	b	10.45±1.04	def
TR 63581	8.83±0.40	ı-l	8.23±0.05	e-j	8.53±0.22	h-k	6.91±0.15	gh	11.18±0.13	bcd	9.05±0.96	gh
TR 37373	10.81±0.19	def	7.24±0.16	ıj	9.03±0.81	g-j	6.94±0.23	gh	11.43±0.34	bcd	9.19±1.02	fgh
TGB 003249	9.36±0.07	hij	9.27±0.57	d-h	9.32±0.26	f-ı	7.55±0.18	efg	10.44±0.35	cd	9.00±0.67	h
TR 63501	8.78±0.06	ı-l	7.18±0.50	ıj	7.98±0.42	jk	7.60±0.30	efg	11.17±0.47	bcd	9.39±0.84	e-h
TR 63575	10.62±0.19	d-g	8.12±0.30	f-j	9.37±0.58	f-ı	8.85±0.06	c-f	10.92±0.41	bcd	9.89±0.50	e-h
TGB 000534	9.63±0.45	g-j	7.76±0.12	g-j	8.70±0.47	hij	7.05±0.08	gh	11.02±0.36	bcd	9.03±0.90	gh
TR 63497	9.27±0.63	h-k	9.86±0.27	c-f	9.57±0.33	fgh	5.84±0.14	h	9.79±0.46	d	7.81±0.91	ı
TGB 003248	9.93±0.11	e-h	8.51±0.34	e-j	9.22±0.35	ghi	6.89±0.32	gh	11.13±0.76	bcd	9.01±1.02	h
TGB 000543	9.84±0.13	f-ı	10.11±0.16	cde	9.97±0.11	efg	9.50±0.28	cd	11.52±0.38	bcd	10.51±0.50	de
TGB 000526	10.63±0.36	d-g	6.71±0.54	j	8.67±0.92	hij	6.90±0.06	gh	11.44±0.64	bcd	9.17±1.06	fgh
TGB 000521	9.07±0.25	h-k	7.61±0.72	hij	8.34±0.47	ijk	7.92±0.05	d-g	10.92±0.10	bcd	9.42±0.67	e-h
Çam buğdayı	7.95±0.44	ı	7.12±0.11	ıj	7.54±0.27	k	7.22±0.16	fgh	11.32±0.32	bcd	9.27±0.93	e-h
Çalı basıran	11.31±0.15	cd	10.01±0.53	c-f	10.66±0.38	cde	8.96±0.11	cde	12.12±0.31	bc	10.54±0.72	de
Ak sunteri	9.07±0.06	h-k	7.92±0.81	g-j	8.50±0.44	h-k	7.54±0.29	efg	11.52±0.78	bcd	9.53±0.97	e-h
Örmece	10.95±0.10	de	9.68±0.02	d-g	10.31±0.29	def	10.27±0.18	bc	12.75±0.37	b	11.51±0.58	d
Dimenit	10.03±0.09	e-h	6.61±0.74	j	8.32±0.84	ijk	7.84±0.15	efg	11.72±0.60	bcd	9.78±0.91	e-h
Zerun	12.09±0.17	bc	10.68±0.40	bcd	11.39±0.37	bc	7.96±0.15	d-g	12.12±0.53	bc	10.04±0.96	e-h
Ortalama Average	9.70		8.64		9.17		7.79		11.44		9.62	
Dağdaş 94	12.12±0.13	bc	10.07±0.07	cde	11.09±0.46	cd	7.53±0.12	efg	12.18±0.14	bc	9.86±1.04	e-h
Müftibey	11.26±0.31	cd	10.04±1.14	c-f	10.65±0.59	cde	8.07±0.51	d-g	12.56±0.39	b	10.31±1.05	efg
Gün 91	12.47±0.09	b	11.75±0.71	bc	12.11±0.36	b	14.85±0.14	a	15.88±1.04	a	15.37±0.52	b
Aldane	18.29±0.19	a	17.37±0.34	a	17.83±0.27	a	15.84±0.07	a	17.48±1.13	a	16.66±0.62	a
Bezostaja 1	12.78±0.32	b	11.69±0.43	bc	12.24±0.34	b	11.62±1.67	b	16.02±0.26	a	13.82±1.24	c
Ortalama Average	13.38		12.18		12.78		11.58		14.82		13.20	
Genel ortalama General average	10.39 a*		9.30 b		9.85		8.49 b		12.07 a**		10.28	
V.K. (%) C.V. (%)	4.15		8.18		6.28		7.79		6.40		7.00	

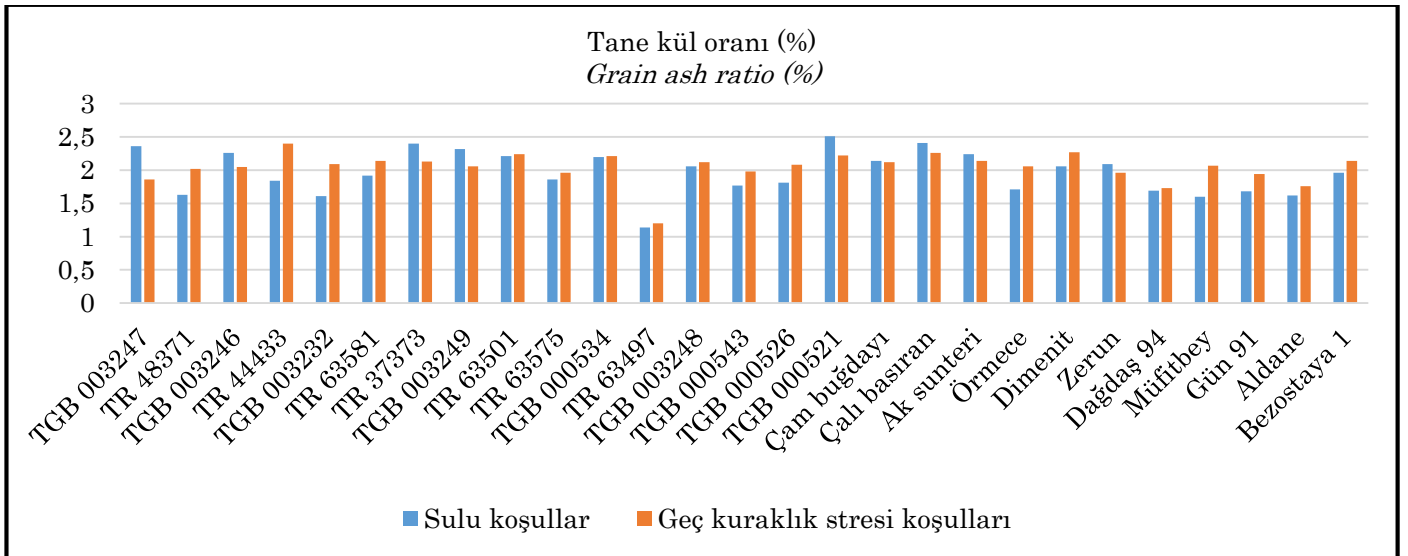
*, **: Aynı harf grubuna giren ortalamalar arasında sırasıyla p < .05 ve p < .01 önem düzeyine göre fark yoktur.
V.K: Varyasyon katsayısı, C.V: Coefficient of variation.

Çalışmada sulu koşullarda tane kül oranı ikinci yıl (%1.73) ilk yıla (%2.28) göre önemli ölçüde azalmıştır (Çizelge 9). Bunun durumun ilk yıl toprak nem içeriğinin (Şekil 1) daha yüksek olmasından kaynaklandığı düşünülmektedir. Birleştirilmiş yıllara bakıldığında tane kül oranı sulu koşullarda %1.60 ile 2.51 arasında, geç kuraklık stresi koşullarında %1.73 ile 2.40 arasında değişmiş (Şekil 8), ortalama tane kül oranı sulu koşullarda %2.01, geç kuraklık stresi oluşturulan koşullarda ise %2.08 olarak bulunmuş ve kuraklık stresi tane kül oranını artırmıştır (Çizelge 9). Konu ile ilgili yapılan araştırmalarda geç kuraklık stresinin tane kül içeriğini önemli derecede artırdığı belirlenmiştir (Bogale & Tesfaye, 2011; Öztürk & Aydın, 2014). Çizelge 9 incelendiğinde kontrol grubunda kurağa toleranslı olarak denemeye alınan Dağdaş 94 çeşidinin tane kül içeriğinin en düşük olduğu görülmektedir. Kuraklık stresi koşullarında tane kül oranı yüksek olan genotiplerin kurağa daha duyarlı oldukları bildirilmiştir (Bogale & Tesfaye, 2011).



Şekil 7. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait bayrak yaprak kül oranları (%)

Figure 7. Flag leaf ash rates of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (%)



Şekil 8. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin birleşik yıllara ait tane kül oranları (%)

Figure 8. Grain ash rates of bread wheat varieties grown under irrigated and late drought stress conditions for the combined years (%)

SONUÇ ve ÖNERİLER

İncelenen özellikler dikkate alındığında çeşitlerin genel ortalamasına göre, sulu koşullara göre geç kuraklık stresi koşullarında bin tane ağırlığı, hektolitre ağırlığı azalmış, protein oranı, Zeleny sedimantasyon değeri, bayrak yaprak kül oranı ve tane kül oranı değerleri artmıştır. Ayrıca hem sulu koşullarda hem de geç kuraklık stresi koşullarında yılların ortalamalarına bakıldığında kurağa duyarlı ve toleranslı olarak kullanılan tescilli çeşitlere göre yerel çeşitlerde bin tane ağırlığı, hektolitre ağırlığı, Zeleny sedimantasyon değeri, bayrak yaprak kül oranı daha düşük, protein oranı ve tane kül oranı ise daha yüksek bulunmuştur. Hem sulu koşullarda hem de geç kuraklık stresi koşullarında bin tane ağırlığı bakımından TGB 003246, TR 63581 ve Çam buğdayı, hektolitre ağırlığı bakımından TGB 003247, TR 48371 ve TR 63575, protein oranı bakımından TGB 003248, TGB 003249, TR 63501, Ak sunteri, Dimenit ve Zerun, Zeleny sedimantasyon değeri bakımından TR 63575 ve Zerun, bayrak yaprak kül oranı bakımından TGB 003232, Çalı basıran ve Zerun, tane kül oranı bakımından ise TGB 000521, Çalı basıran ve Ak sunteri yerel çeşitleri öne çıkmıştır. Elde edilen sonuçlara göre incelenen özellikler bakımından

yerel çeşitler arasında önemli varyasyonlar elde edilmiş, çeşitlerin farklı yıl ve uygulamalara tepkileri de değişmiştir. Dolayısıyla ıslahçıların yerel çeşitleri ıslah çalışmalarında gen kaynağı olarak kullanmaları önem göstermektedir.

Çizelge 9. Sulu ve geç kuraklık stresi koşullarında yetiştirilen ekmeklik buğday çeşitlerinin tane kül oranları (%)
Table 9. Grain ash ratios of bread wheat cultivars grown under irrigated and late drought stress conditions (%)

Çeşitler Cultivars	Sulu koşullar Irrigated conditions						Geç kuraklık stresi koşulları Late drought stress conditions					
	2017-2018		2018-2019		Birleşik yıllar Combined years		2017-2018		2018-2019		Birleşik yıllar Combined years	
TGB 003247	3.07±0.05	ab**	1.66±0.02	c-g**	2.36±0.32	abc**	1.96±0.01	c-g**	1.76±0.05	cd**	1.86±0.05	fgh**
TR 48371	1.94±0.04	fgh	1.32±0.01	h	1.63±0.14	j	1.95±0.03	c-g	2.09±0.04	a-d	2.02±0.04	b-g
TGB 003246	2.62±0.03	bcd	1.89±0.07	b-e	2.26±0.17	a-d	2.13±0.12	b-f	1.98±0.12	a-d	2.05±0.08	b-f
TR 44433	1.90±0.03	fgh	1.77±0.10	b-g	1.84±0.06	g-j	2.55±0.19	a	2.24±0.03	ab	2.40±0.11	a
TGB 003232	1.64±0.06	h	1.58±0.07	e-h	1.61±0.04	j	2.15±0.07	b-f	2.03±0.03	a-d	2.09±0.04	b-f
TR 63581	2.14±0.04	e-h	1.70±0.08	b-g	1.92±0.10	f-ı	2.15±0.05	b-f	2.13±0.07	a-d	2.14±0.04	a-f
TR 37373	3.18±0.09	a	1.62±0.06	d-g	2.40±0.35	ab	2.29±0.12	a-e	1.96±0.11	a-d	2.13±0.10	a-f
TGB 003249	2.95±0.10	abc	1.68±0.12	c-g	2.32±0.29	a-d	1.92±0.04	d-g	2.20±0.18	abc	2.06±0.10	b-f
TR 63501	2.47±0.22	de	1.95±0.00	bc	2.21±0.15	b-e	2.19±0.09	a-f	2.28±0.10	ab	2.24±0.06	a-d
TR 63575	1.90±0.00	fgh	1.83±0.07	b-g	1.86±0.03	g-j	1.97±0.08	c-g	1.94±0.06	a-d	1.96±0.05	d-h
TGB 000534	2.67±0.10	bcd	1.72±0.08	b-g	2.20±0.22	b-f	2.29±0.07	a-e	2.12±0.04	a-d	2.21±0.05	a-e
TR 63497	2.38±0.01	def	1.91±0.09	bcd	2.14±0.11	b-f	2.17±0.01	a-f	2.22±0.01	ab	2.20±0.01	a-e
TGB 003248	2.48±0.21	de	1.64±0.02	c-g	2.06±0.21	d-h	2.14±0.04	b-f	2.09±1.18	a-d	2.12±0.08	a-f
TGB 000543	1.95±0.07	fgh	1.59±0.07	d-h	1.77±0.09	ıj	1.97±0.06	c-g	1.98±0.08	a-d	1.98±0.04	c-h
TGB 000526	1.82±0.05	gh	1.80±0.09	b-g	1.81±0.05	hıj	2.08±0.06	c-g	2.07±0.18	a-d	2.08±0.08	b-f
TGB 000521	3.36±0.20	a	1.65±0.08	c-g	2.51±0.39	a	2.30±0.17	a-d	2.13±0.16	a-d	2.22±0.11	a-e
Çam buğdayı	2.56±0.25	cde	1.72±0.01	b-g	2.14±0.22	b-f	2.24±0.05	a-e	2.00±0.11	a-d	2.12±0.07	a-f
Çalı basıran	2.98±0.00	abc	1.84±0.01	b-f	2.41±0.26	ab	2.49±0.21	ab	2.02±0.01	a-d	2.26±0.14	abc
Ak sunteri	2.23±0.16	d-g	2.26±0.14	a	2.24±0.09	a-d	2.18±0.06	a-f	2.11±0.07	a-d	2.14±0.04	a-f
Örmece	1.80±0.03	gh	1.61±0.01	d-h	1.71±0.04	ıj	2.01±0.01	c-g	2.10±0.00	a-d	2.06±0.02	b-f
Dimenit	2.12±0.10	e-h	2.00±0.10	ab	2.06±0.07	d-h	2.34±0.06	abc	2.20±0.15	abc	2.27±0.08	ab
Zerun	2.38±0.18	def	1.80±0.11	b-g	2.09±0.16	c-g	2.00±0.07	c-g	1.91±0.01	bcd	1.96±0.04	d-h
Ortalama Average	2.39		1.75		2.03		2.16		2.07		2.12	
Dağdaş 94	1.73±0.02	h	1.65±0.02	c-g	1.69±0.02	ıj	1.72±0.03	g	1.74±0.02	d	1.73±0.02	h
Müftbey	1.69±0.04	h	1.51±0.04	gh	1.60±0.05	j	1.93±0.07	d-g	2.21±0.17	ab	2.07±0.10	b-f
Gün 91	1.79±0.10	gh	1.57±0.05	fgh	1.68±0.07	ıj	1.81±0.05	fg	2.06±0.05	a-d	1.94±0.06	e-h
Aldane	1.71±0.08	h	1.53±0.04	fgh	1.62±0.06	j	1.80±0.02	fg	1.73±0.00	d	1.76±0.02	gh
Bezostaja 1	2.11±0.06	e-h	1.80±0.09	b-g	1.96±0.08	e-ı	1.90±0.05	efg	2.39±1.15	a	2.14±0.13	a-f
Ortalama Average	1.81		1.61		1.71		1.83		2.03		1.93	
Genel ortalama General average	2.28 a**		1.73 b		2.01		2.10		2.06		2.08	
V.K. (%) C.V. (%)	8.42		7.09		8.04		7.18		8.42		7.81	

** : Aynı harf grubuna giren ortalamalar arasında $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation.

TEŞEKKÜR

Araştırma Tokat Gaziosmanpaşa Üniversitesi Bilimsel Araştırma Projeleri tarafından desteklenmiştir. Ayrıca çalışma Tokat Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü'nde sunulan Doktora Tezinin bir kısmını kapsamakta olup, III. Uluslararası (XV. Ulusal) Tarla Bitkileri Kongresi'nde özet bildiri olarak sunulmuştur.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Physiological and Biochemical Mechanisms of Salinity Tolerance in Tall Fescue: A Comparative Analysis of Cultivars Differing in Salinity Tolerance

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ABSTRACT

Salinity stress causes an increasingly pervasive threat to agricultural productivity, including turf and forage grasses. This study aimed to reveal how salinity stress affects the physiological and biochemical levels of *Festuca arundinacea* (tall fescue) cultivars differing in salinity tolerance. The salinity stress treatments were assessed through the evaluation of growth measures, biochemical profiles, and the activity of antioxidative enzymes in four tall fescue cultivars having different salinity stress tolerance (tolerant, moderate-high, moderate-low, and sensitive). The results revealed that the tolerant cultivar named 'Titan RX' (tolerant cv.) showed increased root growth and maintained physiological status under salinity stress conditions. This was attributed to the activation of the biosynthesis for the free proline and the antioxidative status (total phenolic and flavonoid contents, DPPH activity, enzymes of CAT, and GR). The other cultivars exhibited lower values in these parameters compared to the tolerant cv., indicating their lesser capacity for salinity tolerance. The results indicated that the cv. 'Titan RX' could potentially be utilized to generate tall fescue crops that can cope with salinity stress conditions.

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Kamışsı Yumak Bitkisinde Tuzluluk Toleransının Fizyolojik ve Biyokimyasal Mekanizması: Tuz Stresine Farklı Hassasiyet Gösteren Çeşitlerin Karşılaştırmalı Analizi

ÖZET

Bu çalışma, farklı tuzluluk toleransına sahip *Festuca arundinacea* (kamışsı yumak) çeşitlerinde tuzluluk stresinin fizyolojik ve biyokimyasal düzeydeki etkilerini ortaya koymayı amaçlamıştır. Tuzluluk stresi uygulamaları, farklı tuzluluk stres toleransına (tolerant, orta-yüksek, orta-düşük ve hassas) sahip dört kamışsı yumak çeşidinde büyüme parametreleri, biyokimyasal profiller ve antioksidan enzimlerin aktivitesinin değerlendirilmesi yoluyla değerlendirilmiştir. Sonuçlar, 'Titan RX' adlı tolerant çeşidin tuzluluk stresi koşullarında kök büyümesini artırdığını ve fizyolojik durumunu koruduğunu ortaya koymuştur. Bu durum, serbest prolin biyosentezinin ve antioksidan mekanizmaların (toplam fenolik ve flavonoid içerikleri, DPPH aktivitesi, CAT ve GR enzimleri) aktivasyonu ile ilişkilendirilmiştir. Diğer çeşitler bu parametrelerde tolerant çeşide kıyasla daha düşük değerlere sahip olmuş ve sonuçlar bu çeşitlerde tuzluluk toleransı kapasitelerinin daha sınırlı olduğunu göstermiştir. Elde edilen bulgular, 'Titan RX' çeşidinin, tuzluluk stresine dayanıklı kamışsı yumak bitkileri geliştirmek için potansiyel bir kaynak olarak kullanılabileceğini göstermektedir.

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INTRODUCTION

Salinity stress has become more serious year by year, resulting in major yield and quality losses in agricultural production (El Sabagh et al., 2020). Salinity stress has been linked to physiological changes in plants, including turf and forage grasses, which may cause adverse effects, including mortality (Demirkol and Yilmaz, 2023). Therefore, an increasing challenge has been observed in recent studies for turfgrass or forage crop breeders to focus on developing salinity-tolerant cultivars (Oral et al., 2019; Bushman et al., 2020; Liu et al., 2023).

Plants have adapted and changed their metabolic processes to deal with salinity stress (Demirkol, 2020). Stimulating the antioxidant defense mechanism is a crucial strategy for minimizing the damaging effects of salinity stress (Begum et al., 2022). Studies have indicated that the expression levels of functional genes such as WRKY, bZIP, MYB, and DREB were up-regulated in salinity-tolerant plants under stress conditions (Price et al., 2022). These molecular regulatory mechanisms promote the activity of antioxidant enzymes (Kaya et al., 2020). Earlier studies have reported that plants with enhanced antioxidant defense systems are more resistant to salinity stress (Demirkol, 2021). Enhanced antioxidant activity is associated with a variety of physiological strategies, including the biosynthesis of endogenous hormones, closure of stomata, scavenging of reactive oxygen species (ROS), accumulation of protective osmolytes, cell membrane stability, and protein structure maintenance (Hussain et al., 2021; Yilmaz, 2019).

Festuca arundinacea Schreb. (tall fescue) It is a main cool-season perennial forage, turfgrass, and cover crop (Sato, 2022). The strong adaptability of tall fescue increases its usage worldwide. However, regional salinization restricts the growth of tall fescue, affecting turf persistence and forage yield (Chen et al., 2022; Kaplan et al., 2017).

The objective of this study was to assess the impact of salinity-induced stress conditions on growth parameters and the physio-biochemical status of tall fescue cultivars characterized by varying degrees of salinity tolerance.

MATERIALS and METHODS

Plant materials and preliminary study

The seeds of four tall fescue cultivars ('Grande', 'Starlet', 'Titan RX', 'Rendition') were used for the experiment, with the anticipation of observing variation in their resistance to salinity. The seeds were sterilized in a 70% (v/v) ethanol solution and shaken for 1 minute. Afterward, the seeds were washed three times with sterile distilled water.

The salinity stress tolerances of the cultivars were assessed in petri dishes in a preliminary study to confirm their different salinity tolerances. The findings classified 'Titan RX' as tolerant, 'Starlet' as moderate-high, 'Rendition' as moderate-low, and 'Grande' as sensitive to salinity stress (data not shown).

Growth and stress conditions

The experiment was performed in the greenhouse of the Agricultural Research Center in Tokat Gaziosmanpasa University. The seeds were planted in pots filled with field soil and were subjected to irrigation using Hoagland's nutrient solution at 80% of field capacity in two days. Afterward, three-week-old seedlings with two leaves were separated into five groups, each irrigated with varied salinity levels (0, 50, 100, 150, 200 mM). The samples were harvested at three weeks after the application of salinity stress treatments. During the research period, greenhouse conditions were maintained within the ecological requirements of the tall fescue cultivars to prevent the occurrence of any secondary stress.

Growth parameters and relative water content

The harvested plants' roots and shoots were measured to determine their lengths (cm) and dried (60°C for 72 h) weight (g/plant) values. The relative water content of the plants was assessed using the formula of Farrant (2000).

Free proline content

The protocol developed by Bates et al. (1973) was modified to determine the free proline content of the plants. Initially, a 0.3 g sample of fresh leaves was homogenized in 10 milliliters of 3% sulphosalicylic acid, and then it was centrifuged at $10,000 \times g$ for 10 minutes. After that, 0.1 ml of the solution was taken. The sample was then vortexed with 0.2 ml of ninhydrin reagent (1.25 g ninhydrin, 30 ml glacial acetic acid, and 20 ml 6M phosphoric acid), 0.2 ml of glacial acetic acid, and 0.1 ml of 3% sulphosalicylic acid. After 1 h of incubation at 95°C, the prepared sample was placed in an ice bath for 5 min to stop the reaction. Then, 1 ml toluene was added to the cooled sample, and centrifuged ($5,000 \times g$, 30 sec). After centrifugation, the upper phase was measured at 520 nm in a spectrophotometer.

Hydrogen peroxide

The hydrogen peroxide (H_2O_2) content was detected using the procedure outlined by Velikova et al. (2000). 5 ml of a 0.1% (w/v) trichloroacetic acid solution was used to homogenize leaf samples weighing 0.5 g in an ice bath. Following this, the homogenate was centrifuged for 15 min at $12\,000 \times g$. After centrifugation, 0.5 ml of the supernatant was added to 1 ml of a 1 M potassium iodide solution and 0.5 ml of a 10 mM potassium phosphate buffer (pH 7.0). The resulting mixture's absorbance was then measured at 390 nanometers with a spectrophotometer.

Malondialdehyde contents

For the purpose of evaluating membrane damage, the malondialdehyde (MDA) content was detected using the protocol explained by Ohkawa et al. (1979). 0.5 g of fresh leaf sample and 2 ml of 5% trichloroacetic acid were mixed together and homogenized. Centrifugation was performed on the resultant extract for 10 min at $15\,000 \times g$. Subsequently, 0.5 ml was extracted from the supernatant of the centrifuged sample and mixed with 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA. The resulting mixture was then heated to $95^\circ C$ for 30 minutes. After the incubation period, the mixture was cooled in an ice bath for 5 min, and then centrifugation at $10\,000 \times g$ was performed for 15 min to terminate the reaction. Finally, the absorbance of the supernatant that was obtained after centrifugation was measured at 532 and 600 nm.

Total chlorophyll content and electrolyte leakage

The acetonic extract was filtered and then centrifuged at $10\,000 \times g$ for 15 min. Afterward, supernatant was used to measure chlorophyll a and b contents using a spectrophotometer at 645 and 663 nm (Arnon 1949). Electrolyte leakage in the plants was found using a conductivity meter by the formula of Rai et al. (2013).

Total phenolic content and total flavonoid content

Based on the procedure developed by Slinkard and Singleton (1977), methanolic extracts were diluted and added to 0.1 mL of Folin-Ciocalteu reagent for a 5-min reaction period. Afterward, 0.3 mL of a saturated Na_2CO_3 solution was added, and the mixture was incubated for 2 h. Absorbance was measured at 760 nm after incubation. Gallic acid was used as the reference standard, and all tests were performed in triplicate. TPC values were presented as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

To determine total flavonoid concentrations in the samples, 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethanol were mixed with 0.5 mL of plant extract (dilution 1:5 in methanol). The mixture was incubated at room temperature for 40 minutes before being detected for absorbance at 415 nm with a UV-Vis spectrophotometer. Calibration was performed using quercetin solutions. All the measurements were performed in triplicate. The total flavonoid concentration was calculated as mg of quercetin equivalents per gram of dry weight (mg QE/g DW), Marcucci et al. (1998).

DPPH assay

To analyze the radical scavenging activity of the samples, DPPH was measured using the method presented in the study of Bektaş et al. (2018). A 50 μ l aliquot of methanolic extract solution was mixed with 5 mL of 0.004% (w/v) DPPH radical methanol solution. The reaction mixture was incubated in the dark at $25^\circ C$ for 30 minutes before measuring absorbance at 517 nm. The inhibition percentage and extract concentration were plotted to determine the concentration required to inhibit DPPH by 50% (IC₅₀). The DPPH levels were measured in triplicate and expressed as μ g/ml.

Antioxidative enzymes

In the study, the levels of Ascorbate Peroxidase (APX), Catalase (CAT), Glutathione Reductase (GR), and Superoxide Dismutase (SOD), key antioxidative enzymes, were analyzed to assess their roles in stress response according to the following method. The enzymatic activity of APX, CAT, SOD, and GR was detected using procedures developed by Wang et al. (1991), Chance and Maehly (1955), Beauchamp and Fridovich (1971) and Sgherri et al. (1994), respectively. Antioxidant enzyme activities were normalized to total protein concentration (per mg protein).

Statistical analyses

The variables were analyzed statistically using one-way ANOVA with the Tukey test ($p < 0.05$ and $p < 0.01$), via Minitab software (Minitab Inc., State College, PA).

RESULTS

The impact of salinity stress treatments on growth parameters

No statistically significant reduction was observed in root development in 'Titan RX' at NaCl stress doses below 200 mM NaCl, while significant decreases were observed in other cultivars (Figure 1A, 1C). In addition, 'Titan RX' showed enhanced root development under 50 mM salt stress conditions (Figure 1A, 1C).

Although significant reductions in shoot weight and length were observed in the 'Titan RX' under salinity stress, these decreases were more pronounced in the other cultivars, especially in 'Grande,' where reductions reached up to 70% under salinity stress (Figure 1B, 1D).

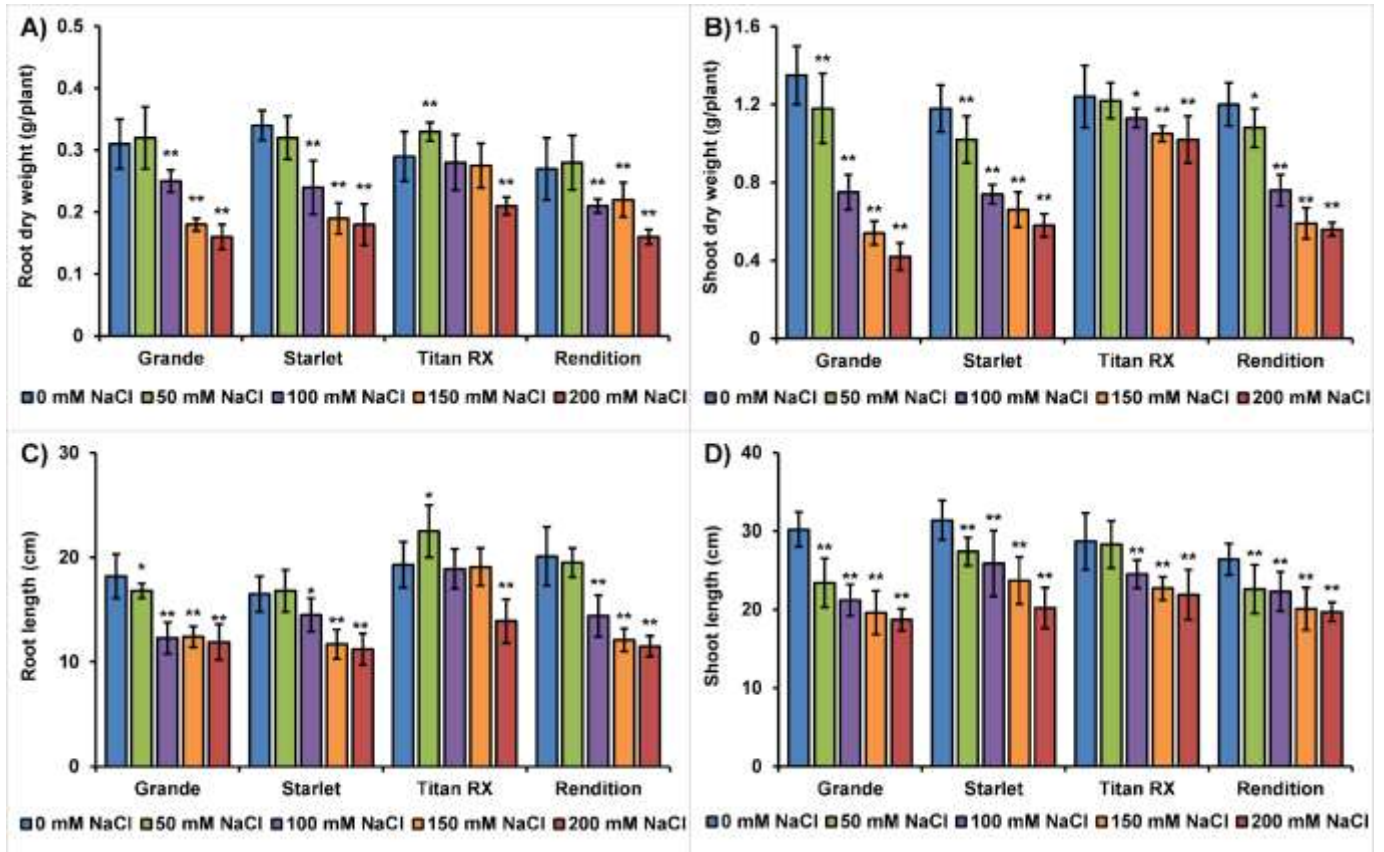


Figure 1. Growth parameters: A. Root dry weight (g/plant), B. Shoot dry weight (g/plant), C. Root length (cm), D. Shoot length (cm) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar *p < 0.05 or **p < 0.01.

Şekil 1. Farklı gruplardaki kamışsı yumak çeşitleri için büyüme özellikleri; A. Kök kuru ağırlığı (g/bitki), B. Sürgün kuru ağırlığı (g/bitki), C. Kök uzunluğu (cm), D. Sürgün uzunluğu (cm). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (*p < 0,05 veya **p < 0,01).

The impact of salinity stress on biochemical profiles

While no significant changes were observed in the relative water content of 'Titan RX' up to 150 mM NaCl, significant decreases were detected in other cultivars under salinity stress, most notably in 'Grande' (Figure 2A). Similar results were observed in the electrolyte leakage ratio in 'Titan RX', while extreme increases were detected in other cultivars under salinity stress (Figure 2B).

While the 'Titan RX' maintained its total chlorophyll content at NaCl stress doses below 200 mM NaCl, significant decreases were observed in other cultivars under all NaCl stress levels compared to their control (Figure 2C). Similar results were observed in H₂O₂ and MDA content in 'Titan RX', while extreme increases were detected in other cultivars under salinity stress (Figure 2D, 2F).

Although some increases were observed in free proline content in 'Starlet' under salinity stress, extremely higher

increases were found in 'Titan RX' (4.2 and 3.7-fold, compared to the control under 150- and 200-mM salinity, respectively) (Figure 2E). In contrast, the other cultivars either showed a decrease or no notable change in proline content compared to their control. (Figure 2E).

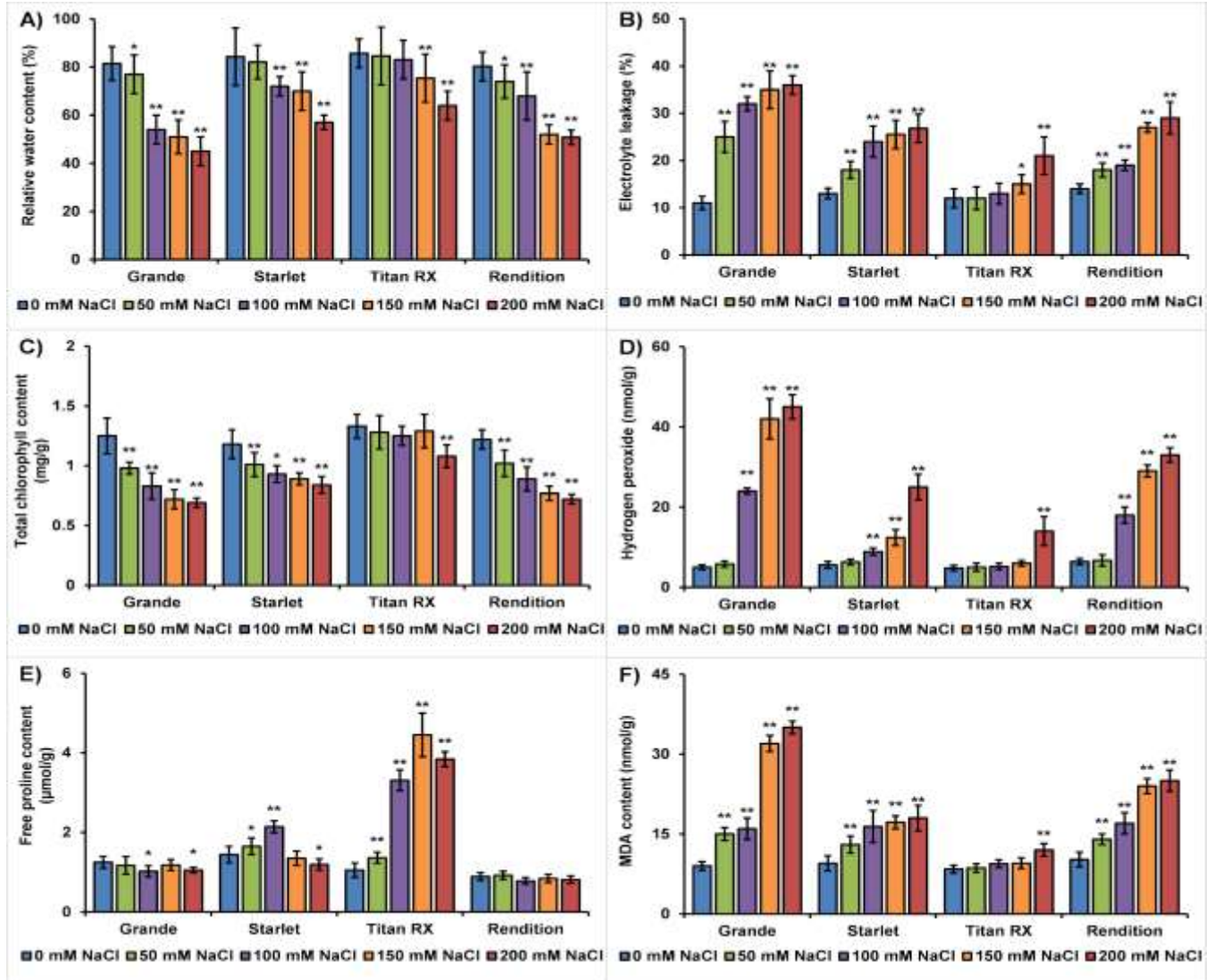


Figure 2. Biochemical status: A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content (μmol/g), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar *p < 0.05 or **p < 0.01.

Şekil 2. Farklı gruplardaki kamışsı yumak çeşitleri için büyüme özellikleri; A. Bağlı su içeriği (%), B. Elektrolit sızıntısı (%), C. Toplam klorofil içeriği (mg/g), D. Hidrojen peroksit (nmol/g). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (*p < 0,05 veya **p < 0,01).

The impact of salinity stress on total phenolics, DPPH activity, and total flavonoids

The increases were observed in total phenolics and total flavonoid contents under salinity stress, primarily in 'Titan RX', followed by 'Starlet', compared to their controls, in contrast to the others, except for 'Rendition' under 50 mM NaCl (Figure 3A, 3B). The contrary results were obtained in DPPH activity, where declines were observed under salinity stress primarily in 'Titan RX', followed by 'Starlet', compared to their controls, while no change was observed in the others (Figure 3C). Notably, a 70% decline was detected in 'Titan RX' under a 200 mM NaCl stress level (Figure 3C).

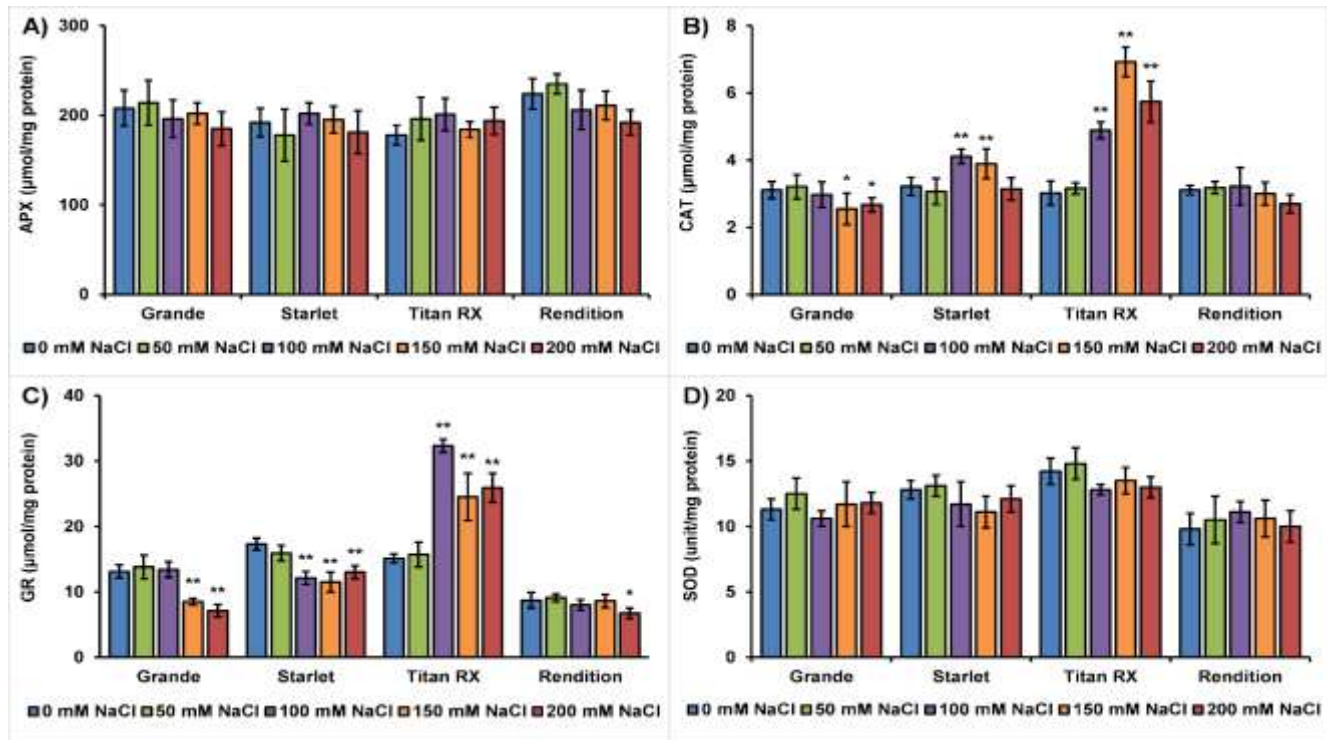


Figure 3. Biochemical status: A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content (μmol/g), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar * $p < 0.05$ or ** $p < 0.01$.

Şekil 3. Farklı gruplardaki kamışı yumak çeşitleri için büyüme özellikleri: A. Toplam fenolik miktarı (mg GAE/g), B. Toplam flavonoid miktarı (mg QE/g), C. DPPH aktivitesi (μg/ml). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (* $p < 0,05$ veya ** $p < 0,01$).

The impact of salinity stress on antioxidative enzymes

Under salinity stress conditions, CAT and GR activity levels changed significantly, with both enzymes demonstrating significant increases or declines. In contrast, no significant alterations were observed in the activity of APX and SOD (Figure 4A, 4B, 4C, 4D). Impressively, CAT and GR activities showed extreme increases in 'Titan RX', compared to the control. In contrast, these antioxidative enzymes exhibited a significant decrease in the sensitive one, 'Grande' (Figure 4B, 4C).

This study investigated the morphological, physiological, and biochemical reactions under increasing levels of salinity in four tall fescue cultivars, previously identified in preliminary tests as having varied salt stress sensitivity. The sensitivity levels of the cultivars from the preliminary study were confirmed via the evaluation of growth parameters under salinity stress conditions.

'Titan RX' showed increased or maintained root development at NaCl concentrations below 200 mM, unlike the other cultivars, which showed significant reductions (Figure 1A, 1C), indicating that in 'Titan RX', the root zone remained unaffected by osmotic and ionic stress under saline conditions. Maintaining or enhancing root development under saline conditions is a key adaptation strategy in crops to minimize the detrimental consequences of salt stress (Demirkol et al., 2019; Zou et al., 2022). In a study conducted on *Lolium multiflorum*, it was concluded that low levels of salt stress either enhanced or did not affect root development, which was suggested to be an adaptation to osmotic stress (Simsek Soysal et al., 2021).

'Titan RX' exhibited less reduction in shoot growth under salinity stress compared to other varieties (Figure 1B, 1D). Reduced plant development under salt stress could be caused by insufficient water or the harmful effects of sodium chloride, both of which can threaten physiological reactions. (Dugasa, et al. 2019). The lower reduction in root and shoot development in the 'Titan RX' under salt stress confirmed its higher tolerance to salinity compared to other cultivars.

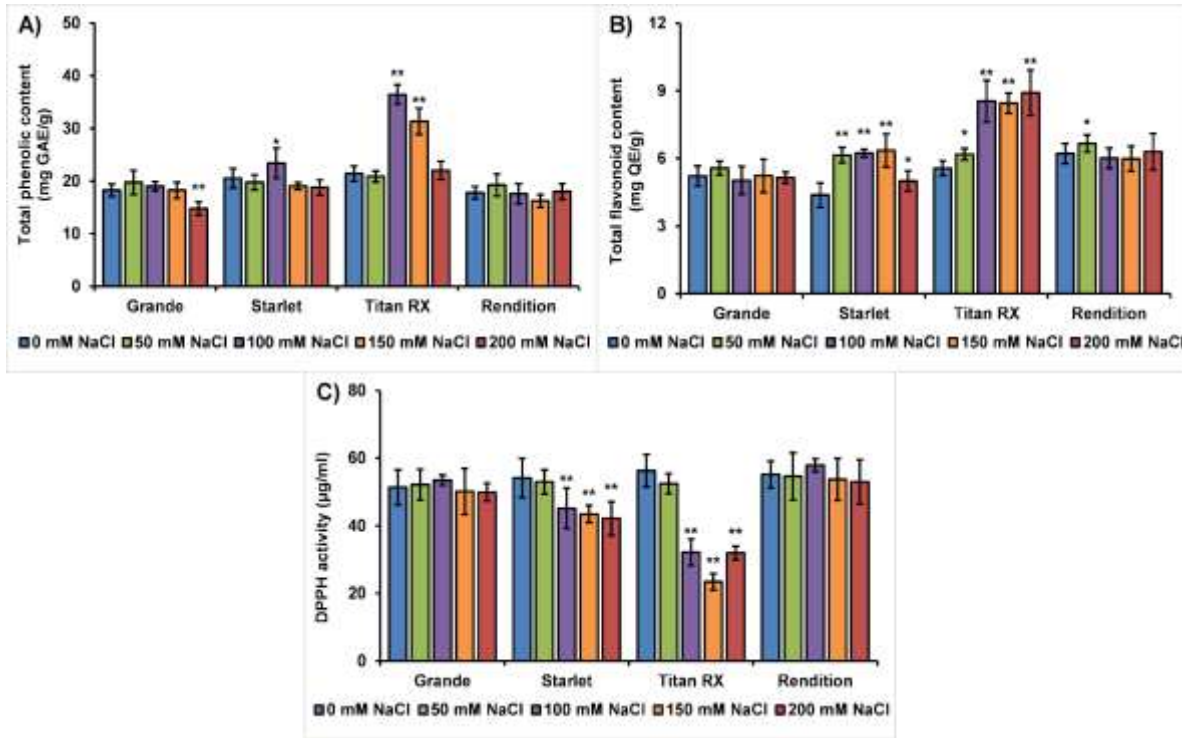


Figure 4. Biochemical status; A. Relative water content (%), B. Electrolyte leakage (%), C. Total chlorophyll content (mg/g), D. Hydrogen peroxide (nmol/g), E. Free proline content (µmol/g), F. MDA content (nmol/g) of the tall fescue cultivars in different sets. The columns represent means, and the bars represent standard errors of the four replicates. Asterisks indicate a significant difference between control (0 mM) and NaCl-stressed plants in a cultivar * $p < 0.05$ or ** $p < 0.01$.

Şekil 4. Farklı gruptaki kamışsı yumak çeşitleri için büyüme özellikleri; A. APX (µmol/mg protein), B. CAT (µmol/mg protein), C. GR (µmol/mg protein), D. SOD (ünite/mg protein). Sütunla gösterilen değerler ortalama değerleri, barlar ise dört tekrarin standart hatalarını göstermektedir. Yıldız işaretleri, bir çeşitte kontrol (0 mM) ile NaCl stresi uygulanan bitkiler arasındaki anlamlı farkı göstermektedir (* $p < 0,05$ veya ** $p < 0,01$).

'Titan RX' maintained its relative water and total chlorophyll contents up to 150 and 200 mM NaCl, respectively (Figure 2A, 2C). Other cultivars showed noteworthy declines in relative water and total chlorophyll levels in other cultivars, especially in 'Grande' and 'Rendition' (Figure 2A, 2C). Studies have revealed that reducing water in the plant leaves causes degradation chlorophyll pathway, leading to a decrease in the quantity of chlorophyll (Demirkol and Yılmaz 2023; Pour-Aboughadareh et al., 2017).

The tall fescue cultivars exhibited a higher electrolyte leakage ratio under salinity stress conditions (Figure 2B). Studies showed that salinity stress could modify the biological mechanism of cell membranes and cause increased membrane instability (Demirkol and Yılmaz 2023; Jothamani and Arulbalachandran 2020). In this study, 'Titan RX' maintained its electrolyte leakage ratio up to 150 mM NaCl, indicating that its enhanced salinity tolerance may be associated with the maintenance of cell membrane stability and the ability to rapidly repair damage. A study concluded that under salt stress, salt-tolerant plant species maintained their electrical conductivity more effectively compared to the sensitive group (Hniličková et al., 2019).

The findings showed serious up-regulations in H_2O_2 production in the tall fescue cultivars under 100, 150, and 200 mM NaCl stress, except for 'Titan RX' (Figure 2D). This indicates that the level of ROS increased in salinity-sensitive cultivars under saline conditions. Several studies have shown that excessive accumulation of ROS, particularly H_2O_2 , which causes toxic substances to cells, reduces plant chlorophyll content and damages biological membranes during salinity stress (Kim et al., 2005; Zeeshan et al., 2020).

Under salinity stress, the cultivars 'Starlet' and 'Titan RX' showed a slight and dramatic increase in free proline content, respectively. However, the other two salinity-sensitive cultivars showed declines or no significant changes when compared to their controls (Figure 2E), suggesting that the increased accumulation of free proline plays a key role in determining salinity stress resistance in tall fescue. According to studies, free proline enables plants to deal with a variety of stressors, including salinity, by maintaining turgor and redox homeostasis (Al Hinai et al., 2022; Rajasheker et al., 2019). Similar results were obtained in a study of salt-tolerant forage pea genotypes

(Demirkol, 2020).

Significant increases in MDA content were observed in the tall fescue cultivars under all NaCl stress treatments compared to their controls, except for 'Titan RX' (Figure 2F). MDA is a well-known biomarker of lipid peroxidation level and oxidative damage in plants under abiotic stress conditions (Demirci-Cekic et al., 2022). The increased MDA under salt stress has been reported in salt-susceptible crops in various studies (Jan et al., 2017; Liu et al., 2022). The detrimental impacts of NaCl stress on lipid peroxidation have been found in several plant species, and MDA has been accepted as a reliable marker of salinity tolerance (Zeeshan et al., 2020). The current data indicate that 'Titan RX' exhibited better protection against oxidative damage under salinity stress than the other cultivars.

Significant increases were detected in total phenolics and total flavonoid contents in 'Titan RX' and 'Starlet' under salinity stress treatments (Figure 3A, 3B), suggesting that these cultivars activated the antioxidative defense system to mitigate oxidative damage caused by salinity stress. In a study, moderate salinity stress was reported to increase total polyphenols in *Thymus vulgaris* (Zrig et al., 2016). These results agree with earlier studies showing that salinity stress stimulates the biosynthesis of phenolics and flavonoids as a part of a protective mechanism in plants (Bistgani et al., 2019). The enhanced biosynthesis of the compounds of total phenolics and flavonoids in 'Titan RX' and 'Starlet', highlighting their potential ability to cope with salinity stress.

Significant decreases were observed in DPPH activity in 'Titan RX' and 'Starlet' under salinity stress treatments (Figure 3C), suggesting a reduction in the radical-scavenging capacity of these cultivars under salinity stress. The previous studies have reported that increased phenolic content has led to a decrease in DPPH (IC₅₀) activity (Demirkol and Tarakci 2018; Kainama et al., 2020). The decreases in 'Titan RX' and 'Starlet' may show the protective roles by increased total phenolics, flavonoids, or antioxidative enzymes (CAT and GR) in these cultivars.

Significantly increased values were observed in CAT in 'Titan RX' and 'Starlet' under salinity stress treatments, compared to their controls (Figure 4B). Plants' antioxidant defense mechanism relies heavily on CAT, which converts H₂O₂ into water and oxygen (Riseh et al., 2024). This process is crucial in reducing the negative impacts of ROS, which accumulate under a variety of stress conditions, including salinity (Hasanuzzaman et al., 2020). The results suggest that 'Titan RX' and 'Starlet' demonstrated enhanced resistance to salinity stress compared to the other two cultivars, attributed to an upregulation of their CAT activity.

In the study, similar results were observed for GR activity, which paralleled the findings with CAT in 'Titan RX' (Figure 4C).

The recent studies have shown that GR uses NADPH to react with ROS, including hydroxyl and superoxide radicals. This process protects cells against injury caused by oxidation via maintaining redox balance (Mushtaq et al., 2020).

Although significant increases in APX and SOD activities have been identified as essential indicators of salt stress resistance in several plant species (Demirkol, 2021; Hasanuzzaman et al., 2020; Shafi et al., 2015), this impact was not detected in the tall fescue cultivars studied.

CONCLUSIONS

The results revealed that the salinity-tolerant cultivar 'Titan RX' exhibited enhanced root growth while maintaining shoot biomass, relative water content, H₂ O₂, MDA, total chlorophyll content, and electrolyte leakage rate under salinity stress. This enhanced tolerance was attributed to the activation of biosynthetic pathways for free proline, total phenolics, total flavonoid contents, and antioxidative enzymes (CAT and GR). Thus, the physiological mechanism underlying salinity stress tolerance in tall fescue has been identified, along with the critical function of antioxidative enzymes in this process. In addition, these findings suggest that 'Titan RX' has the potential to be used under saline conditions.

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Contribution Rate Statement Summary of Researchers

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Bazı Aspir Çeşitlerinde Farklı Ekim Normu Uygulamalarının Verim ve Verim Özelliklerine Etkileri

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ÖZET

Bu araştırma, Tokat-Kazova şartlarında farklı ekim normlarının bazı aspir çeşitlerinde verim ve verim özelliklerine etkilerini belirlemek amacıyla yapılmıştır. Çalışma 2017 ve 2019 yıllarında tesadüf bloklarında bölünmüş parseller deneme desenine göre üç tekerrürlü yürütülmüştür. Araştırmada sıra arası 20 cm olarak kullanılmış, ana parsellerde çeşitler (Asol, Balcı, Dinçer, Linas, Olas, Remzibey-05, Shifa), alt parsellerde ekim normları (80, 120, 160, 200 tohum m⁻²) yer almıştır. İncelenen özellikler bitki boyu, ilk dal yüksekliği, dal sayısı, tabla sayısı, tabla başına tohum sayısı, bin tohum ağırlığı, tohum verimi, yağ oranı ve yağ verimi parametreleridir. Elde edilen sonuçlara göre, her iki yılda da tüm parametreler çeşit ve ekim normu faktörlerinden önemli şekilde etkilenmişlerdir. En yüksek tohum verimleri denemenin her iki yılında da Dinçer çeşidinden sırasıyla 2686.2 ve 1541.2 kg ha⁻¹ şeklinde gerçekleşmiştir. Ekim normları bakımından her iki yılda da tohum verimleri sırasıyla 1979.0 ve 1341.1 kg ha⁻¹ ile 80 tohum m⁻² uygulamasından elde edilmiştir. Tohumdaki yağ oranları uygulanan farklı ekim normlarından etkilenmemekle birlikte, deneme yılının ilk yılında Balcı (%35.7) ve Olas (%36.3), ikinci yılında ise Linas (%31.4) çeşitlerinde daha yüksek olmuştur. Sonuç olarak, Tokat-Kazova şartlarında, Dinçer ve Shifa çeşitlerinin 80 tohum m⁻² olacak şekilde ekilmesi durumunda, daha yüksek tohum ve ham yağ verimlerinin alındığı belirlenmiştir.

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Anahtar Kelimeler

Aspir

Carthamus tinctorius L.

Ekim normu

Tohum verimi

Yağ oranı

Effects of Different Sowing Norm Applications on Yield and Yield Characteristics of Some Safflower Varieties

ABSTRACT

This research was conducted to determine the effects of different sowing norms on yield and yield characteristics of some safflower cultivars under Tokat-Kazova conditions. The study was conducted in 2017 and 2019 with three replications according to the split-plot experimental design in randomized blocks. In the research, row spacing was used as 20 cm, varieties (Asol, Balcı, Dinçer, Linas, Olas, Remzibey-05, Remzibey-05, Shifa) were placed in the main plots and sowing norms (80, 120, 160, 200 seeds m⁻²) were placed in the sub-plots. The characteristics examined are plant height, first branch plants, number of branches, head number, number of seeds per head, thousand seed weight, seed yield, oil content and oil yield. According to the results obtained, all parameters were significantly affected by variety and sowing norm factors in both years. The highest seed yields were realized as 2686.2 and 1541.2 kg ha⁻¹ from Dinçer variety in both years of the experiment, respectively. In terms of sowing norms, seed yields in both years were obtained from 80 seeds m⁻² treatment with 1979.0 and 1341.1 kg ha⁻¹, respectively. Although seed oil content was not affected by the different planting norms, it was higher in Balcı (35.7%) and Olas (36.3%) in the first year of the experiment and in Linas (31.4%) in the second year. As a result, it was determined that higher seed and crude

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oil yields were obtained when Dinçer and Shifa varieties were planted with 80 seeds m⁻² under Tokat-Kazova conditions.

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GİRİŞ

Aspir (*Carthamus tinctorius* L.) Asteraceae familyasına ait olup, birçok bölgede yetişen insanlar tarafından gıda ve endüstriyel amaçlı olmak üzere kullanılan en eski bitkilerden biridir (Weiss, 2000; Emongor, 2010; Emongor & Oagile, 2017). Aspir, geniş bir adaptasyon yeteneğinin yanında, diğer yağlı bitkilere oranla daha az su ihtiyacı olması ve kuraklığa karşı yüksek toleransı sayesinde, kuru tarım alanları için alternatif ürün haline gelerek, kıraç alanlarda rahatlıkla yetiştirilmektedir (Kaya ve ark., 2003; Gilbert, 2008; El-Lattief, 2012; Omidı ve ark., 2012; Yeilaghi ve ark., 2012). Türkiye'nin özellikle İç Anadolu, Güneydoğu Anadolu, Doğu Anadolu bölgeleri olmak üzere kurak ve yarı kurak tarım alanlarında ekilebilecek alternatif bitkiler arasındadır (Baydar & Erbaş, 2007). Karasal iklim şartlarında ve tahılların yetiştiği koşullar altında yetiştirilecek bir yağ bitkisi olarak geniş çaplı üretim potansiyeli olmasına karşın, ancak belirli bölgelerde ekim alanına sahiptir. Dünya çapında aspir ekim alanlarının sınırlı kalması, ve aspir bitkisine araştırmacılar tarafından yeterince ilgi duyulmaması (Kolsarıcı ve ark., 2003; Shahrokhnia & Sepaskhah, 2017) sonucu ihmal edilen bir bitki olmuştur (Emongor ve ark., 2015). Oysa aspir bilhassa yarı kurak bölgelerdeki nadas alanlarının azaltılması için tarımı yaygınlaştırılması gereken bitkilerin başında yer almaktadır.

Aspir yetiştiriciliğinde kültürel uygulamaların yanı sıra uygun çeşitlerin belirlenmesi ve seçimi de önemlidir. Özellikle kurak arazi şartlarına uyum sağlayabilecek çeşitlerin doğru seçimi, ekonomik olarak aspir yetiştiriciliğinde rekabetçi verim elde edilmesine katkı sağlayacaktır. Bu nedenle bir bölgeye uygun çeşitlerin belirlenebilmesi için çok sayıda genotipin kullanılması, agronomik ve kalite özelliklerinin adaptasyon denemeleri yoluyla ortaya konulması önemlidir (Erbaş ve ark., 2016).

Bitki yoğunluğu da bitki verimini belirleyen diğer önemli faktörlerden biridir (Lloveras ve ark., 2004). Aspirde ekim normu dünya genelinde büyük ölçüde çeşitlilik göstermektedir. Bitki yoğunluğu özellikle sıra aralığı olmak üzere bitkinin gelişme durumuna, büyüme ortamına ve kültürel yöntemlere bağlıdır. Birçok çalışmada bitki yoğunluğunun fazla olmasının tohum verimini arttırdığı belirtilmiştir (Hamza, 2015; Özaşık ve ark., 2019; Sefaoğlu & Özer, 2022). Ancak bazı yapılan araştırmalar sonucunda, birim alandaki bitki sayısı artışının belli bir sınıra kadar tohum veriminde artış sağladığı daha sonra tohum verimlerinin azaldığı tespit edilmiştir (Yau, 2009; Amoghein ve ark., 2012; Hamza, 2015; Sampaio ve ark., 2017). Moatshe ve ark. (2016), bitki yoğunluğu ile genotipler arasında önemli etkileşim olduğunu, bitki yoğunluğu arttıkça (62.500 bitki ha⁻¹'den 100.000 bitki ha⁻¹) tohum veriminin arttığını belirtirken, 100.000 bitki ha⁻¹'da sonra tüm genotiplerde tohum veriminin azaldığını bildirmişlerdir. Bazı araştırmacılar tarafından ise aspir bitkisinin toprakta yeterli nem olduğu müddetçe, düşük bitki popülasyonunu artan dallanma ve diğer verim komponentlerinden dolayı tolere ettiği ifade edilmiştir (Kedikanestwe, 2012; Emongor ve ark., 2013). Ayrıca Emongor ve ark. (2013), bitki yoğunluğu arttıkça tohum veriminin önemli ölçüde azaldığını belirtmiştir. Diğer taraftan Elfadl ve ark. (2009), bitki yoğunluğunun tohum verimi, yağ oranı ve verim üzerinde önemli bir etkisi olmadığını belirtmiştir. Bu çalışmada; Tokat-Kazova koşullarında yazlık olarak ekilen aspir çeşitlerinden farklı ekim normlarının bazı verim ve kalite özelliklerine etkisinin belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Araştırma, 2017 ve 2019 yılları vejetasyon dönemlerinde Tokat-Kazova şartlarında, Gaziosmanpaşa Üniversitesi, Tarımsal Uygulama ve Araştırma Merkezi deneme alanlarında yürütülmüştür. Araştırma 2018 yılında da yürütülmüş ancak bitki çıkışlarında homojenlik olmamasından dolayı 2019 yılında deneme tekrarlanmıştır. Araştırmanın yürütüldüğü Tokat ili, coğrafi olarak Karadeniz Bölgesinde olmasına rağmen Orta Anadolu ile Karadeniz iklimi arasında geçit kuşağı iklim özelliği göstermektedir. Deneme alanının uzun yıllar ve araştırma yıllarına ait bazı iklim verileri Çizelge 1'de, deneme alanları toprağının bazı fiziksel ve kimyasal özellikleri ise Çizelge 2'de verilmiştir.

Çalışmanın yürütüldüğü 2017 ve 2019 yetiştirme sezonunda ortalama sıcaklıklar ilk yıl (16.6 °C) uzun yıllarla benzer düzeyde seyrederken, ikinci deneme yılında (17.5 °C) uzun yıllardan daha yüksek olmuştur. Toplam yağışta her iki deneme yılında da uzun yıllardan fazladır. Deneme yılının ikinci yılında (244.7 mm) toplam yağış ilk yıla göre (232.2 mm) daha fazla gerçekleşmiş gibi görülse de özellikle vejetasyon süresindeki yağış miktarının önemli

olduğu Mart-Haziran ayları arasında toplam yağış ilk deneme yılında daha fazla gerçekleşmiştir. Ortalama nispi nem ise her iki deneme yılında da uzun yıllardan fazla olurken deneme yılının ilk yılında (%65.5) daha fazla ortalama nispi nem olmuştur (Çizelge 1). Deneme toprağının fiziksel ve kimyasal özellikleri her iki yılda da; killi tınlı, tuzsuz, kireçli, organik madde ve fosfor bakımından fakir iken, pH ilk yıl orta alkali ikinci yıl hafif alkali, potasyum içeriği bakımından ise ilk yıl orta, ikinci yıl fakir toprak özelliğine sahiptir (Çizelge 2).

Çizelge 1. Deneme alanına ait bazı iklim faktörleri*

Table 1. Some climatic factors of the experimental area*

İklim faktörleri <i>Climatic factors</i>	Ortalama sıcaklık (°C) <i>Average temperature (°C)</i>			Toplam yağış (mm) <i>Total precipitation (mm)</i>			Ortalama nispi nem (%) <i>Average relative humidity (%)</i>		
Aylar <i>Months</i>	2017	2019	Uzun yıllar <i>Long terms</i>	2017	2019	Uzun yıllar <i>Long terms</i>	2017	2019	Uzun yıllar <i>Long terms</i>
Mart(March)	9.2	7.2	7.3	26.2	36.8	42.2	60.1	61.8	61.6
Nisan(April)	11.8	11.5	12.4	48.2	63.5	54.6	58.2	65.2	59.2
Mayıs(May)	15.6	19.1	16.3	80.6	49.1	59.6	68.1	59.7	61.8
Haziran(June)	19.8	23.1	19.5	76.5	26.2	39.7	71.3	63.4	59.9
Temmuz(July)	18.0	21.9	22.0	0.0	16.9	11.6	72.8	59.6	57.6
Ağustos (August)	25.0	22.4	22.3	0.7	52.2	8.1	62.4	63.0	57.9
Ortalama Average	16.6	17.5	16.6	-	-	-	65.5	62.1	59.7
Toplam Total	-	-	-	232.2	244.7	215.8	-	-	-

*: Tokat Meteoroloji İstasyon Müdürlüğü (2019)

Çizelge 2. Deneme alanı toprağının bazı fiziksel ve kimyasal özellikleri*

Table 2. Some physical and chemical properties of the trial area soil*

Yıllar <i>Years</i>	Tekstür sınıfı <i>Texture class</i>	Toprak reaksiyonu <i>Soil reaction (pH)</i>	Toplam tuz <i>Total salt (%)</i>	Kireç (CaCO ₃) <i>Lime (%)</i>	Organik madde <i>Organic matter (%)</i>	Potasyum <i>Potassium (K₂O) kg da⁻¹</i>	Fosfor <i>Phosphorus (P₂O₅) kg da⁻¹</i>
2017	Killi-tınlı <i>Clay-loam</i>	8.20	0.028	20.60	1.10	157.10	5.68
2019	Killi-tınlı <i>Clay-loam</i>	7.92	0.036	9.50	1.82	89.9	5.58

*: Orta Karadeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsü Müdürlüğü.

Araştırma, Tesadüf Bloklarında Bölünmüş Parseller Deneme Desenine göre üç tekerrürlü olarak yürütülmüştür. Çalışma materyali Tohumluk Tescil ve Sertifikasyon Merkezi'nden temin edilmiş olup; Asol, Balcı, Dinçer, Linas, Olas, Remzibey-05, Shifa (Tacikistan) çeşitleri kullanılmıştır. Çalışmada kullanılan çeşitlerden Dinçer ve Shifa dikensiz çeşitler iken, Asol, Balcı, Linas, Olas ve Remzibey-05 çeşitleri dikenli çeşitlerdir. Denemede ana parsellerde 7 çeşit kullanılırken, alt parsellerde ise 80, 120, 160 ve 200 tohum m⁻² ekim normları yer almıştır. Ekim işlemi, markörle açılan sıralara el ile sıra arası 20 cm olacak şekilde yapılmıştır. Bloklar arasında 2 m, ana parseller arasında 1 m mesafe bırakılan çalışmada parseller 5 m uzunluğunda ve 4 sıralıdır. Ekimler, ekim derinliği 2-3 cm olacak şekilde her iki yılda Mart ayının ikinci yarısında yapılmıştır. Deneme sulamasız şartlarda yürütülmüştür. Gübreleme işlemi ekim öncesi 60 kg ha⁻¹ azot, 60 kg ha⁻¹ fosfor ve 60 kg ha⁻¹ potasyum kompoze gübre (15-15-15 N-P-K) şeklinde uygulanmıştır. Üst gübre olarak ise hektara 60 kg azotlu gübre (Amonyum Nitrat - %33) verilmiştir (Babaoğlu, 2010). Yabancı ot mücadelesi çapayla yapılmıştır. Hasat olgunluğuna gelen bitkiler hasat edildikten sonra sera ortamında belli bir süre kurutulmaya bırakıldıktan sonra harman edilmişlerdir. Çalışmada bitki boyu (cm), bitki başına dal sayısı (adet bitki⁻¹), bitki başına tabla sayısı (adet bitki⁻¹), bin tohum ağırlığı (g) Oruç (2014)'a, ilk dal yüksekliği (cm), tabla başına tohum sayısı (adet tabla⁻¹) Ranga Rao ve ark. (1977) ve Gencer ve ark. (1987)'na, tohum verimi (kg ha⁻¹), yağ oranı (%) ve yağ verimi (kg ha⁻¹) Köse ve Bilir (2017) ve Mariod ve ark. (2012)'na göre belirlenmiştir. Deneme yıllarından elde edilen veriler homojenite testine (Bartlett, 1950) tabi tutulmuş, yıllar ayrı ayrı denemenin kuruluş deseni olan Tesadüf Bloklarında Bölünmüş Parseller Deneme Desenine göre MSTAT-C bilgisayar programı ile varyans analizi yapılmıştır. Ortalamaları önemlilik düzeyine göre (0.05, 0.01) istatistiksel olarak karşılaştırmak için Duncan çoklu karşılaştırma testi kullanılmıştır (Yurtsever, 1984).

BULGULAR ve TARTIŞMA

Bitki Boyu (cm)

Çalışmada aspir bitkisinin bitki boyuna deneme yılının ilk yılında çeşitlerde $p < .01$ düzeyinde, ekim normlarında ise $p < .05$ düzeyinde önemli fark bulunurken, ikinci deneme yılında ise ekim normlarında $p < .01$ ve çeşit x ekim normlarının interaksyonunda $p < .05$ düzeyinde önemli farklılıklar bulunmuştur (Çizelge 3). İlk yıl çeşit ortalamalarına bakıldığında bitki boyu bakımından Shifa çeşidi (96.80 cm) en yüksek bitki boyu, Balcı çeşidi (83.78 cm) ise en düşük bitki boyu değeri almıştır. Çalışmanın ikinci yılında ise çeşitler arasındaki fark önemlilik göstermezken, Shifa (84.75 cm) çeşidi ön plana çıkmıştır. Ekim normları ortalama değerlerine bakıldığında ilk yıl en uzun bitki boyu 120 tohum m^{-2} (92.00 cm) ekim normunda, en kısa bitki boyu ise 160 tohum m^{-2} (87.54 cm) ekim normunda; ikinci yıl ise en uzun bitki boyu 80 tohum m^{-2} (85.90 cm) ekim normunda, en kısa bitki boyu ise 200 tohum m^{-2} (79.19 cm) ekim normunda tespit edilmiştir (Çizelge 3). Çalışmada her iki yılda da genel anlamda bitki sıklığı arttıkça bitki boyu değerleri azalmıştır.

Çizelge 3. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde bitki boyuna ait ortalama değerler (cm)
Table 3. Mean values of plant height in safflower cultivars grown in different planting norms (cm)

Çeşitler Cultivars	2017				
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	88.17 ±0.88	90.83±1.31	85.66±1.50	85.40±1.41	87.57±0.86 BC*
Balcı	86.27±2.06	85.17±3.74	81.70±1.19	82.00±3.07	83.78±1.29 C
Dinçer	87.20±2.11	91.13±3.33	93.33±2.02	91.87±2.78	90.88±1.31 ABC
Linaz	92.03±2.56	95.90±2.08	87.03±2.66	95.10±1.97	92.58±1.44 AB
Olas	91.00±2.40	89.83±4.57	86.60±4.85	85.70±4.87	88.28±1.95 BC
Remzibey-05	87.37±3.06	87.17±3.50	85.63±2.76	83.73±3.29	85.97±1.42 BC
Shifa	98.33±0.77	104.00±3.10	92.87±3.99	92.03±5.82	96.80±2.20 A
Ekim normu ortalaması <i>Sowing norm average</i>	90.09 ±1.11 AB**	92.00 ±1.65 A	87.54±1.28 B	87.98±1.54 AB	89.40±0.72
V.K (%)	4.05				
C.V (%)					
A.Ö.F	Çeşit:7.225	Ekim normu:3.948			
L.S.D					
Çeşitler Cultivars	2019				
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	83.97±3.74 a-e*	77.70±1.26 e-h	81.07±3.27 a-g	80.86±4.49 a-g	80.90±1.59
Balcı	85.20±2.61 a-d	77.73±3.60 e-h	76.30±1.46 fgh	75.37±1.81 gh	78.65±1.58
Dinçer	86.67±1.73 ab	85.87±1.03 abc	84.97±0.79 a-e	80.77±5.14 a-g	84.57±1.37
Linaz	86.97±3.73 ab	83.03±1.12 a-f	79.00±1.23 c-h	78.83±2.03 c-h	81.95±1.40
Olas	84.33±2.51 a-e	81.67±3.20 a-g	78.13±2.06 d-h	73.3±1.47 h	79.37±1.60
Remzibey-05	88.17±1.23 a	85.63±1.39 abc	83.13±3.38 a-f	80.27±5.50 b-h	84.30±1.68
Shifa	86.07±3.47 abc	85.20±2.87 a-d	82.87±3.98 a-f	84.87±3.82 a-e	84.75±1.56
Ekim normu ortalaması <i>Sowing norm average</i>	85.90±0.95 A**	82.40±1.03 B	80.78±1.04 B	79.19±1.40 B	82.07±0.62
V.K (%)	4.53				
C.V (%)					
A.Ö.F	Ekim normu:3.095	ÇeşitxEkim normu:6.126			
L.S.D					

* **: Aynı harf grubuna giren ortalamalar arasında sırasıyla $p < .05$ ve $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Aspir bitkisinde bitki boyu, kullanılan genotiplere (Licata ve ark., 2023), ekim sıklığı, birim alandaki bitki sayısına, çevre ve yetiştirme koşullarına göre farklılıklar göstermektedir (Süer, 2011; Yavuz, 2019). Elfadl ve ark. (2009)'da bitki yoğunluğunun bitki boyu üzerindeki etkisi çevresel koşullara göre değiştiğini belirtmişlerdir. Çalışmada da görüldüğü gibi çeşitlere ve ekim normlarına göre bitki boyu değerlerinde değişiklikler görülmüştür.

İlk yıl bitki boyu genel ortalamasının (89.40 cm), ikinci deneme yılı bitki boyu genel ortalamasına (82.07 cm) göre yüksek olması; çalışmanın ilk yılında (2017) bitki gelişiminin oldukça hızlı gerçekleştiği Mayıs ve Haziran aylarında 2019 yılına ve uzun yıllar ortalamasına göre oldukça fazla yağışın gerçekleşmesinden kaynaklanmıştır. Bunu destekler nitelikte Elfadl ve ark. (2009) 50, 100 ve 150 tohum m⁻² kullanarak yürüttükleri çalışmada birim alanda bitki yoğunluğu arttıkça bitki boyunun azaldığını belirtmişlerdir. Öte yandan bu çalışmanın aksine Köse ve Bilir (2017)'de 5 (15, 30, 45, 60 ve 75 kg ha⁻¹) ekim normunu kullanarak yürüttükleri çalışmada ekim normu bakımından en yüksek bitki boyunun 81.5 cm ile 75 kg ha⁻¹ ekim normundan elde edildiğini bildirmişlerdir.

Elde ettiğimiz değerlere bakıldığında (özellikle ikinci deneme yılında) bitki yoğunluğu azaldıkça bitkiler arasındaki ışık ve toprak nemi yönünden rekabetin azalmasıyla beraber kök sistemi gelişiminin daha iyi olması ve topraktaki besin elementlerinden daha iyi yararlanılması sonucunun bitki boyundaki artışlara yansıdığı görülmüştür. Sefaoğlu (2017)'de çalışmasında bu durumu desteklemiştir. Yavuz (2019) daha sık ekimlerde güneş ışığından yararlanmak için bitkiler arasındaki rekabetten dolayı daha uzun bitki boyu elde edildiği ifade ederek, 4 çeşit (Balcı, Dinçer, Remzibey-05 ve Yenice) ve 25, 50, 75 ve 100 tohum m⁻² kullanarak yürüttüğü çalışmasında bitki sıklığı arttıkça, bitki boyunun da arttığını, çeşitlerden bitki boyu bakımından Dinçer çeşidinin ön plana çıktığını bildirmiştir.

İlk Dal Yüksekliği (cm)

Çalışmada aspir bitkisinin ilk dal yüksekliğine deneme yılının ilk yılında çeşitler ve ekim normlarında $p < .01$ düzeyinde, çeşit x ekim normlarının interaksiyonunda ise $p < .05$ düzeyinde önemli farklılıklar bulunmuştur. İkinci deneme yılında ise çeşit x ekim normlarının interaksiyonunda $p < .05$ düzeyinde önemli bulunmuştur (Çizelge 4). Araştırmadan elde edilen sonuçlara göre ilk dal yüksekliğine ait ortalama değerler Çizelge 4'de verilmiş olup, çeşitlerin ilk dal yüksekliği ilk yıl 55.09-70.20 cm, ikinci yıl 68.39-77.29 cm arasında değiştiği saptanmıştır. Çeşitlerden ilk yıl ilk dal yükseklikleri bakımından en yüksek değer Shifa ve Linas çeşitlerinde, ikinci yıl ise Linas çeşidinde olmuştur. Ekim normu değerlerine göre ilk dal yüksekliği ilk yıl 55.93-67.47 cm; ikinci yıl 70.88-72.69 cm arasında değişmiştir. Ekim normlarına göre ilk dal yüksekliği bakımından ilk yıl 80 tohum m⁻² ekim normu ön plana çıkarken, deneme yılının ikinci yılında ekim normları arasında bir fark görülmemiştir.

Çizelge 4'te verilen genel ortalamaya göre, ilk dal yüksekliği ikinci yılda (71.88 cm) ilk yıldan (62.85 cm) fazla olmuştur. Asperde ilk dal yüksekliği, birim alanda yetişen bitki sayısı bitkiler arası rekabet ve bitki boyuna bağlı olarak değişiklik gösterebilmektedir (Oruç, 2014). Çizelge 4 ve Çizelge 5'e bakıldığında deneme yıllarında metrekaresindeki bitki sayının az olması, başka bir ifadeyle parsel içi rekabet az olduğunda, gelişmekte olan her bir bitkinin mümkün olan en alt boğumlarından itibaren yan dalların oluşmaya başladığı görülmüştür. Bu durum hem dallanmanın en alt kısımlardan başlaması, hem de bitkideki dal sayısının artmasına sebep olmuştur. Genel ortalama incelendiğinde, ikinci yıla göre ilk yılda, ilk dal yüksekliğinin düşüklüğü, diğer bir ifade ile dallanmanın aşağıdan başlaması bitkilerin gelişim dönemindeki yağışların yeterli olmasından dolayı bitkinin rekabet yaşamadığı, dolayısıyla bu durum dallanma alttan başlamasının ve dal sayısının fazla olmasına neden olmuştur.

Dal Sayısı (adet bitki⁻¹)

Çalışmadan elde edilen sonuçlara göre dal sayısı bakımından çeşitler, ekim normları ve bunların interaksiyonları arasındaki farklılıkların her iki deneme yılında da $p < .01$ düzeyinde önemli olduğu görülmüştür. Çeşitler bakımından dal sayısı ilk yıl 4.26-5.49 adet bitki⁻¹, ikinci yıl 1.82-4.43 adet bitki⁻¹ arasında değiştiği belirlenmiştir. Çeşitlerden ilk yıl dal sayısı bakımından Asol, Balcı ve Dinçer aynı grupta yer alarak en yüksek değeri, ikinci yıl ise Dinçer, Remzibey-05 ve Shifa aynı grupta yer almış ve en yüksek dallanmayı göstermiştir. Weiss (2000), aspir üretiminde ekonomik olarak 6-8 dal bulunması gerektiğini vurgulamıştır. Ancak bazı araştırmacılar asperde dal sayısının 50 adete kadar çıkabileceğini bildirmektedir (Reddy ve ark., 2004; Omid ve ark., 2009). Ekim normları değerleri bakımından dal sayısı ilk yıl 4.30-6.11 adet bitki⁻¹; ikinci yıl 3.04-4.44 adet bitki⁻¹ arasında değişmiştir. Ekim normlarına göre her iki yılda da metrekaresindeki bitki sayısının artışı ile dallanma azalmıştır. Sonuçlara göre bitki sıklığının artışının bitkilerde yaşam alanını azalttığı için bitkilerin dallanmalarını da azalttığı söylenebilir. Bu durumu destekleyecek şekilde Emongor ve ark. (2015) asperde bitki yoğunluğunun 100.000 bitki ha⁻¹'den 250.000 bitki ha⁻¹'e çıkarılmasının bitki boyunu ve dal sayısını önemli ölçüde azalttığını ifade etmişlerdir. Artan bitki yoğunluğuna bağlı olarak aspirin vejetatif büyüme ve verim bileşenlerindeki azalma, büyüme ve gelişme için gerekli olan ışık, besin maddeleri ve su için bitkiler arasındaki rekabete bağlamışlardır.

Çizelge 5'e bakıldığında, denemenin her iki yılında da farklı birkaç çeşit dallanma bakımından ön plana çıkarken, Dinçer çeşidi ilk sırada yer almıştır. Çizelge 3 ve Çizelge 5'e bakıldığında genel ortalama bitki boyu arttıkça dal sayısı artmıştır. Bu durumu destekler nitelikte Oruç (2014) bitki boyu arttıkça boğum sayısının, boğum sayısı arttıkça da dal sayısının arttığını ifade etmiştir. Ayrıca ilk dallanmanın başladığı yükseklik de, dal sayısını etkileyen bir diğer morfolojik oluşum olduğunu, ilk dallanma ne kadar toprağa yakın bir kısımdan başlıyor ise dal sayısının da o kadar fazla olduğunu belirtmiştir. Asperde dal sayısı, tabla sayısı ile doğru orantılı olduğundan dal

sayısı verimi doğrudan etkileyen özelliklerden biridir (Umrani & Bhoi, 1984). Bu durumu destekler nitelikte bitki sıklığı arttıkça, dal sayısının artmasının dekara verime yansıdığı da görülmüştür.

Çizelge 4. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde ilk dal yüksekliğine ait ortalama değerler (cm)
Table 4. Mean values of first branch height in safflower cultivars grown in different planting norms (cm)

Çeşitler <i>Cultivars</i>	2017				
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				Çeşit Ortalaması <i>Cultivar average</i>
	80	120	160	200	
Asol	51.00±1.96 kl*	63.93±4.28 b-h	58.06±2.78 f-l	61.26±5.36 d-j	58.57±2.19 BC**
Balcı	49.43±1.92 l	56.47±3.47 g-l	55.60±3.87 g-l	58.87±0.22 e-l	55.09±1.58 C
Dinçer	51.90±2.70 jkl	57.90±3.23 f-l	67.33±3.03 a-f	69.93±1.69 a-d	61.77±2.47 B
Linaz	64.10±2.66 b-h	73.37±1.91 ab	76.03±2.31 a	66.96±2.84 a-f	70.11±1.78 A
Olas	59.97±1.18 d-k	65.50±5.82 b-g	68.70±2.15 a-e	54.53±1.88 h-l	62.17±2.15 B
Remzibey-05	53.60±2.66 r-l	61.20±4.12 d-j	69.93±0.99 a-d	63.36±1.67 c-r	62.02±2.09 B
Shifa	61.50±0.98 c-j	71.30±4.65 abc	76.63±2.75 a	71.40±4.44 abc	70.20±2.23 A
Ekim normu ortalaması <i>Sowing norm average</i>	55.93±1.37 B**	64.24±1.85 A	67.47±1.88 A	63.76±1.59 A	62.85±0.95
V.K (%)	8.12				
C.V (%)					
A.Ö.F	Çeşit:5.619 Ekim normu:4.247 ÇeşitxEkim normu:8.405				
L.S.D					
Çeşitler <i>Cultivars</i>	2019				
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				Çeşit ortalaması <i>Cultivar average</i>
	80	120	160	200	
Asol	69.60±3.18 d-g*	71.67±2.10 b-f	73.77±1.40 a-e	74.87±3.41 a-d	72.47±1.28
Balcı	71.87±1.63 b-f	68.73±1.26 d-g	67.80±2.75 efg	66.20±2.95 fg	68.65±1.15
Dinçer	64.17±0.77 g	69.80±1.36 c-g	70.10±1.29 b-g	69.50±3.81 d-g	68.39±1.18
Linaz	79.03±1.93 a	78.77±3.88 a	76.57±1.71 abc	74.80±1.20 a-d	77.29±1.15
Olas	76.70±1.46 ab	75.27±3.17 a-d	72.97±4.39 a-f	68.33±1.45 d-g	73.32±1.56
Remzibey-05	66.17±1.99 fg	70.50±0.66 b-g	73.73±1.99 a-e	71.50±2.32 b-f	70.47±1.14
Shifa	68.60±1.79 d-g	74.10±2.21 a-e	73.00±4.08 a-f	74.67±5.00 a-e	72.59±1.66
Ekim normu ortalaması <i>Sowing norm average</i>	70.88±1.28	72.69±1.04	72.57±1.05	71.40±1.23	71.88±0.57
V.K (%)	4.82				
C.V (%)					
A.Ö.F	ÇeşitxEkim normu: 5.711				
L.S.D					

*, **: Aynı harf grubuna giren ortalamalar arasında sırasıyla p < .05 ve p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Tabla Sayısı (adet bitki⁻¹)

Araştırmadan elde edilen sonuçlara göre tabla sayısına çeşitler, ekim normları ve bunların interaksiyonları arasındaki farkın etkisinin her iki deneme yılında da p < .01 düzeyinde önemli olduğu görülmüştür (Çizelge 6). Çeşitler bakımından tabla sayısı ilk yıl 4.57-6.77 adet bitki⁻¹, ikinci yıl 1.92-4.23 adet bitki⁻¹ arasında değiştiği belirlenmiştir. Çeşitlerden her iki yılda tabla sayısı bakımından Dinçer çeşidi en yüksek tabla oluşumunu göstermiştir. Ekim normları değerleri bakımından tabla sayısı ilk yıl 4.76-7.60 adet bitki⁻¹; ikinci yıl 2.87-4.31 adet bitki⁻¹ arasında değişmiştir (Çizelge 6).

Çizelge 6 incelendiğinde ilk yılda oluşan tabla sayısı ikinci yıla göre daha fazla olmuştur. Nitekim bu durumun denemenin ilk yılında (2017) vejetasyon döneminde (özellikle Nisan, Mayıs ve Haziran ayları) yağış miktarının fazla olmasından kaynaklandığı düşünülmektedir. Ayrıca tabla sayısının fazlalığının ilk yıldaki dallanma sayısının fazlalığı ile doğrusal bir ilişkisi olduğu gözlenmektedir. Weiss (2000), aspride tabla sayısı 5 ile 50 adet arasında değişmekle birlikte, iyi gelişme gösteren 14 ile 16 tabla arasında ekonomik verim için yeterli olduğunu belirtmiştir. Her ne kadar bu çalışmanın özellikle ikinci yılında tabla sayısı değerleri bu aralığın altında yer alsa

da tabla sayısı yapılan diğer çalışmalarda belirtilen değerler arasındadır (Reddy ve ark., 2004; Safavi ve ark., 2011).

Çizelge 5. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde dal sayısına ait ortalama değerler (adet bitki⁻¹)
Table 5. Mean values of the number of branches in safflower cultivars grown in different planting norms (number plant⁻¹)

2017					
Çeşitler Cultivars	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				Çeşit ortalaması Cultivar average
	80	120	160	200	
Asol	6.43±0.24 b**	5.66±0.16 cde	5.10±0.10 def	4.73±0.12 fgh	5.48±0.20 A**
Balcı	7.30±0.10 a	5.06±0.12 ef	4.96±0.15 efg	4.63±0.08 fgh	5.49±0.32 A
Dinçer	6.03±0.13 bc	4.66±0.06 fgh	5.80±0.10 bcd	4.90±0.10 fgh	5.35±0.17 A
Linaz	5.00±0.17 efg	4.63±0.12 fgh	2.93±0.12 k	4.46±0.29 f ₁	4.26±0.25 D
Olas	6.20±0.10 bc	4.90±0.60 fgh	4.20±0.11 hij	3.57±0.06 jk	4.72±0.30 BC
Remzibey-05	6.20±0.15 bc	4.30±0.17 g-j	3.80±0.15 ij	3.60±0.11 jk	4.47±0.32 CD
Shifa	5.63±0.54 cde	5.83±0.03 bc	3.87±0.12 ij	4.27±0.32 g-j	4.90±0.29 B
Ekim normu ortalaması <i>Sowing norm average</i>	6.11±0.16 A**	5.00±0.12 B	4.38±0.20 C	4.30±0.12 C	4.95±0.11
V.K (%)	5.98				
C.V (%)					
A.Ö.F	Çeşit:0.378	Ekim normu:0.247	ÇeşitxEkim normu:0.653		
L.S.D					
2019					
Çeşitler Cultivars	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]				Çeşit ortalaması Cultivar average
	80	120	160	200	
Asol	4.47±0.12 b**	2.80±0.15 kl	3.70±0.11 efg	3.00±0.11 r-l	3.49±0.20 C**
Balcı	5.43±0.23 a	2.73±0.03 klm	3.70±0.10 efg	3.50±0.26 f ₁	3.84±0.30 B
Dinçer	5.43±0.12 a	3.90±0.06 c-f	3.87±0.20 def	3.40±0.11 f-j	4.15±0.24 A
Linaz	3.10±0.11 h-k	1.57±0.03 n	1.33±0.06 n	1.30±0.06 n	1.82±0.23 E
Olas	2.50±0.10 lm	2.93±1.20 jkl	2.70±0.06 klm	2.20±0.06 m	2.58±0.09 D
Remzibey-05	5.40±0.10 a	4.67±0.18 b	3.27±0.18 g-k	3.63±0.03 fgh	4.25±0.26 A
Shifa	4.77±0.17 b	4.30±0.10 bcd	4.43±0.14 bc	4.23±0.20 b-e	4.43±0.09 A
Ekim normu ortalaması <i>Sowing norm average</i>	4.44±0.25 A**	3.27±0.22 B	3.29±0.21 B	3.04±0.20 C	3.51±0.13
V.K (%)	6.54				
C.V (%)					
A.Ö.F	Çeşit:0.276	Ekim normu:0.192	ÇeşitxEkim normu:0.133		
L.S.D					

** : Aynı harf grubuna giren ortalamalar arasında p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Bitki başına tabla sayısı çeşide (Al-Juheishy, 2024), sıra aralığına ve ekim yoğunluğuna (Sefaoğlu & Özer, 2022), ekim zamanına (Koç, 2019), lokasyona (Culpan, 2023) bağlı olarak değişiklik göstermektedir. Bu durumu destekleyecek şekilde Yavuz (2019), bitki başına tabla sayıları arasındaki farkın özellikle bitkinin yetiştiği lokasyonun iklim ve toprak yapısı ile çeşit, bakım işlemleri ve bitki sıklığı bakımından farklılıklardan kaynaklandığını ifade etmiştir. Ayrıca 4 çeşit (Balcı, Dinçer, Remzibey-05 ve Yenice çeşidi) ve 25, 50, 75 ve 100 tohum m⁻² kullandığı araştırmasında bitki sıklığı arttıkça tabla sayısının azaldığını, Balcı çeşidinde bitki başına daha fazla tabla sayısı elde edildiğini bildirmiştir. Oruç ve Yılmaz (2019) 18 çeşit ve hatla yürüttükleri çalışmalarında ilk yıl 13.2-23.6 adet bitki⁻¹ ve ikinci yıl 7.5-16.2 adet bitki⁻¹ ile tabla sayısının değiştiğini belirterek, çeşitlere ve hatlara göre farklılık gösterdiğini ifade etmiştir. Bu çalışmada ise Tablo 5 ve Tablo 6'ya bakılacak olursa tabla sayısının az olması bitkideki dallanmanın az olması ile ilişkili olduğu söylenebilir. Öte yandan Patane ve ark. (2020), İtalya'da 2 yıl süre ile yürüttükleri çalışmada 25 bitki m⁻² ve 50 bitki m⁻² kullanılmış ve her iki yılda da bitki sıklığının az olduğu (25 bitki m⁻²) sıklıkta tabla sayısı daha fazla olmuştur. Bu çalışmayı da destekler nitelikte ekim sıklığı ve ekim normuna göre asperde tabla sayılarının değişkenlik gösterdiğini belirten Oad ve ark. (2002), daha seyrek ekimlerde bitki başına dal ve tabla sayısının arttığını ifade etmişlerdir.

Çizelge 6. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde tabla sayısına ait ortalama değerler(adet bitki⁻¹)
Table 6. Mean values of the number of head in safflower cultivars grown in different planting norms (number plant⁻¹)

Çeşitler Cultivars	2017						
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]						
	80	120	160	200	Çeşit ortalaması Cultivar average		
Asol	7.63±0.08 c**	5.30±0.20 f-j	6.23±0.24 de	5.90±0.20 d-g	6.27±0.27 B**		
Balcı	8.33±0.12 bc	6.10±0.11 def	5.23±0.08 g-j	4.87±0.03 hıj	6.13±0.40 BC		
Dinçer	8.60±0.05 b	6.06±0.23 def	6.43±0.08 d	5.97±0.36 d-g	6.77±0.33 A		
Linaz	5.43±0.12 eı	4.70±0.20 ıjk	2.97±0.08 m	5.20±0.11 g-j	4.57±0.29 F		
Olas	6.63±0.12 d	5.13±0.08 g-j	3.83±0.12 l	3.97±0.08 kl	4.89±0.34 E		
Remzibey-05	9.90±0.47 a	5.57±0.20 e-h	3.83±0.08 l	4.50±0.11 jkl	5.95±0.72 C		
Shifa	6.70±0.11 d	6.10±0.30 def	4.77±0.14 h-k	4.73±0.13 h-k	5.57±0.27 D		
Ekim normu ortalaması Sowing norm average	7.60±0.31 A**	5.57±0.13 B	4.76±0.27 C	5.01±0.16 C	5.73±0.17		
V.K (%)	5.81						
C.V (%)							
A.Ö.F	Çeşit:0.298	Ekim normu:0.2774	ÇeşitxEkim normu:0.734				
L.S.D							
Çeşitler Cultivars	2019						
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]						
	80	120	160	200	Çeşit ortalaması Cultivar average		
Asol	4.40±0.15 cd**	3.00±0.11 g-k	2.80±0.17 h-k	2.77±0.07 ı-l	3.25±0.21 C**		
Balcı	4.43±0.26 cd	3.80±0.10 d-g	3.20±0.20 e-j	3.67±0.37 d-g	3.77±0.17 B		
Dinçer	5.63±0.32 ab	4.03±0.03 de	3.93±0.34 def	3.33±0.13 eı	4.23±0.28 A		
Linaz	2.63±0.08 ı-l	1.77±0.03 mn	1.47±0.14 n	1.80±0.06 mn	1.92±0.14 D		
Olas	2.23±0.22 k-n	2.37±0.08 j-m	1.97±0.08 lmn	1.80±0.25 mn	2.09±0.10 D		
Remzibey-05	5.87±0.29 a	4.00±0.15 de	3.07±0.03 g-k	3.13±0.07 f-j	4.02±0.35 AB		
Shifa	5.03±0.32 bc	3.77±0.26 d-g	3.73± 0.18 d-g	3.63±0.03 d-h	4.04±0.19 AB		
Ekim normu ortalaması Sowing norm average	4.31±0.30 A**	3.24±0.19 B	2.88±0.19 C	2.87±0.17 C	3.32±0.13		
V.K (%)	10.17						
C.V (%)							
A.Ö.F	Çeşit:0.353	Ekim normu:0.282	ÇeşitxEkim normu:0.747				
L.S.D							

** : Aynı harf grubuna giren ortalamalar arasında p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Tabla Başına Tohum Sayısı (adet tabla⁻¹)

Çeşitler bakımından tabla başına tohum sayısı deneme yılının ilk yılında 17.62-24.22 adet tabla⁻¹, ikinci yılında ise 9.36-14.98 adet tabla⁻¹ arasında değer almış ve tabla başına tohum sayısı bakımından çeşitler, ekim normları ve bunların interaksiyonları arasında her iki deneme yılında da p < .01 düzeyinde önemli farklılıkların olduğu belirlenmiştir (Çizelge 7).

Tabla başına tohum sayısı bakımından çeşitlerden deneme yılının ilk yılında Dinçer çeşidi ikinci yılında ise Shifa çeşidi en yüksek tabla başına tohum sayısı görülmüştür. Ekim normları değerleri bakımından tabla başına tohum sayısı ilk yıl 17.62-24.94 adet bitki⁻¹; ikinci yıl ise 11.32-13.57 adet bitki⁻¹ arasında değişmiştir (Çizelge 7). Her iki deneme yılında da ekim normu arttıkça tabla başına tohum sayısının azaldığı söylenebilir. Bu durumu destekler nitelikte Yavuz (2019) birkaç çeşit ve 4 bitki sıklığı (25, 50, 75 ve 100 tohum m⁻²) ile yürüttüğü çalışmada metrekaredeki bitki sıklığının artması ile birlikte tabladaki tohum sayısının azaldığını ifade etmiştir. Aksine Moatshe ve ark. (2016), bitki yoğunluğu 62.500 bitki ha⁻¹'dan 100.000 bitki ha⁻¹'a çıktıkça tabla başına tohum sayısı önemli ölçüde artmış, daha sonrasında çeşit ve çevre faktörlerine göre bitki yoğunluğu arttıkça tohum sayısının azaldığını savunmuşlardır. İsmayıl-Zada (2018) yürüttüğü çalışmada 4 çeşit (Dinçer, Balcı, Olas ve Linaz) ve 4 ekim normu (30, 45, 60 ve 75 kg ha⁻¹) kullanmış ve çeşitler arasında en yüksek tabladaki tohum sayısı, bu çalışmaya paralel şekilde Dinçer çeşidinden (31.3 adet bitki⁻¹) elde edilmiştir.

Çizelge 7. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde tabla başına tohum sayısı ait ortalama değerler (adet tabla⁻¹)

Table 7. Mean values of the number of seeds per head in safflower cultivars grown in different sowing norms (number head⁻¹)

2017										
Çeşitler Cultivars	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]									
	80		120		160		200		Çeşit ortalaması Cultivar average	
Asol	27.03±0.69	bc**	20.30±0.65	e-h	19.17±	1.01 f ₁	21.33±0.79	d-g	21.95±0.97	B**
Balcı	19.70±0.37	d-g	18.87±1.15	e-h	15.03±0.60	f ₁	18.27±0.09	jkl	17.97±0.60	D
Dinçer	30.67±0.40	a	23.47±0.60	de	22.40±0.40	def	20.33±1.00	e-h	24.22±1.20	A
Linaz	23.37±0.12	de	18.87±0.49	f ₁	15.03±0.30	jkl	20.40±0.11	e-h	19.42±0.91	CD
Olas	19.80±0.53	e-h	18.03±2.30	g-j	15.53±1.88	ı-l	17.13±1.21	h-k	17.62±0.84	D
Remzibey-05	29.80±1.55	ab	17.53±0.47	g-j	19.93±0.89	e-h	12.27±0.26	l	19.88±1.96	C
Shifa	24.20±0.32	cd	18.23±0.35	g-j	20.97±0.49	d-h	13.63±0.42	kl	19.26±1.18	CD
Ekim normu ortalaması <i>Sowing norm average</i>	24.94±0.94	A**	19.33±0.54	B	18.29±0.70	BC	17.62±0.76	C	20.04±0.49	
V.K (%) C.V (%)	7.56									
A.Ö.F (L.S.D)	Çeşit:1.713		Ekim normu:1.262		Çeşitx	Ekim normu:0.875				

2019										
Çeşitler Cultivars	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]									
	80		120		160		200		Çeşit ortalaması Cultivar average	
Asol	13.87±0.71	a-e**	10.23±0.35	d-ı	12.90±1.30	a-g	11.37±1.07	c-ı	12.09±0.58	B**
Balcı	15.13±0.62	abc	13.43±1.39	a-f	11.73±0.96	c-ı	12.23±0.96	b-ı	13.13±0.59	AB
Dinçer	14.23±1.37	a-d	12.63±0.78	a-h	9.17±0.84	ghı	13.23±1.08	a-g	12.32±0.73	B
Linaz	9.93±0.57	e-ı	9.60±0.66	f ₁	8.27±0.84	ı	9.63±0.54	f ₁	9.36±0.34	C
Olas	11.37±0.78	c-ı	13.63±1.95	a-f	8.73±0.71	hı	14.93±0.44	abc	12.17±0.86	B
Remzibey-05	14.43±0.75	abc	12.10±0.89	b-ı	11.73±0.93	c-ı	11.57±0.77	c-ı	12.46±0.50	B
Shifa	16.07±1.39	ab	14.73±0.55	abc	16.70±1.06	a	12.43 ±1.24	b-h	14.98±0.68	A
Ekim normu ortalaması <i>Sowing norm average</i>	13.57±0.54	A**	12.34±0.51	AB	11.32±0.68	B	12.20±0.45	B	12.36±0.29	
V.K (%) C.V (%)	12.79									
A.Ö.F (L.S.D)	Çeşit:1.993		Ekim normu:1.316		Çeşitx	Ekim normu:3.481				

** : Aynı harf grubuna giren ortalamalar arasında p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Aspirde tabla başına tohum sayısı bir verim bileşeni olarak ifade edilerek (Polat, 2007), tabla başına tohum sayısı, çeşitlere göre değişmekle birlikte (Al-Hasnawi ve ark. 2024) tabla çapı ve tozlaşma-döllenme sürecindeki çevre koşullarının etkisi altında tohum bağlama oranıyla da ilişkilidir (Oruç, 2014). Öte yandan Uysal ve ark. (2006) yaptıkları çalışma neticesinde, genetik ve çevresel şartlara göre tabladaki tohum sayısının tabla büyüklüğü ile doğrudan bağlantılı olduğunu ifade etmişlerdir. Arslan (2007), tabla çapı, tabla sayısı ve tabladaki tohum sayısı ile tohum verimi arasında pozitif ilişki olduğunu ifade etmiştir.

Bin Tohum Ağırlığı (g)

Araştırma sonuçlarına göre bin tohum ağırlığı bakımından her iki yılda da çeşitler ve ekim normları p < .01 düzeyinde önemli olduğu, bunların interaksyonları arasındaki farkın ilk yıl p < .05 düzeyinde önemli, ikinci yıl ise önemsiz olduğu görülmüştür (Çizelge 8). Çeşitler bakımından bin tohum ağırlıkları deneme yılının ilk yılında 38.54-45.95 g, ikinci yılında ise 37.03-40.50 g arasında değişim göstermiştir. Deneme yılının ilk yılında Dinçer çeşidi başta olmak üzere Remzibey-05 ve Shifa çeşitleri ön plana çıkarken, ikinci yıl ise Dinçer, Linaz, Asol çeşitleri ön planda olmuştur. Bin tohum ağırlığı; tohumların iriliğinin ve özgül ağırlıklarının fazla olmasını ifade eden bir kalite kriteri olup, dekara verimi etkileyen faktörlerden de biridir (Oruç & Yılmaz, 2019). Bin tohum ağırlığı çeşide (Sefaoğlu & Özer, 2022; Licata ve ark., 2023), ekolojik faktörlere (Beyyavas ve ark. 2011), yazlık kışlık ekime (Oruç & Yılmaz, 2019), ekim normu ve lokasyana (Al- Juheishy, 2024), gübreleme ve gübre dozlarına (Buçak, 2019) göre değişiklik gösterdiği bildirilmiştir. Bu çalışmada da çeşitler arasındaki farkın genotipik farklılıkların yanında çevresel faktörlerden kaynaklandığı söylenebilir.

Çizelge 8. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde bin tohum ağırlıklarına ait ortalama değerler (g)
Table 8. Mean values of thousand seed weights (g) of safflower cultivars grown in different sowing norms (g)

Çeşitler Cultivars	2017 Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]								Çeşit ortalaması Cultivar average
	80		120		160		200		
Asol	42.50±0.68	c-g*	40.63±0.88	f-j	40.83±0.59	f ₁	38.73±0.78	ijk	40.67±0.51 BC**
Balcı	41.87±0.44	efg	41.67±0.64	e-h	39.57±1.23	h-k	40.47±0.54	g-j	40.89±0.43 BC
Dinçer	49.33±0.70	a	43.63±0.87	cde	44.80±1.10	bcd	46.03±0.68	b	45.95±0.74 A
Linaz	43.97±0.62	b-e	42.33±1.38	efg	41.83±0.34	efg	39.40±1.46	ijk	41.88±0.67 B
Olas	39.40±1.06	ijk	38.50±0.29	jk	38.37±2.27	jk	37.90±0.45	k	38.54±0.57 C
Remzibey-05	44.83±0.88	bc	42.73±1.09	c-g	42.97±0.78	c-f	42.83±1.32	c-f	43.34±0.51 AB
Shifa	43.80±1.30	cde	42.57±0.53	c-g	43.70±0.47	cde	42.47±0.88	d-g	43.13±0.41 AB
Ekim normu ortalaması Sowing norm average	43.67±0.69	A**	41.72±0.45	B	41.72±0.60	B	41.11±0.66	B	42.05±0.32
V.K (%)	2.86								
C.V (%)									
A.Ö.F	Çeşit: 2.727		Ekim normu: 1.003		ÇeşitxEkim normu: 1.984				
L.S.D									
Çeşitler Cultivars	2019 Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]								Çeşit ortalaması Cultivar average
	80		120		160		200		
Asol	39.83±0.64		39.10±0.55		38.00±0.66		40.13±1.03		39.27±0.40 A**
Balcı	39.50±0.53		38.40±1.04		38.17±0.84		39.20±0.95		38.82±0.40 AB
Dinçer	41.13±1.37		38.83±0.63		40.67±0.27		41.37±1.19		40.50±0.51 A
Linaz	39.50±0.47		39.63±1.07		39.43±1.34		40.50±1.14		39.77±0.47 A
Olas	36.90±0.53		37.73±0.57		38.20±0.55		36.90±0.36		37.43±0.27 B
Remzibey-05	39.43±0.47		36.40±0.76		36.30±0.06		37.80±0.44		37.48±0.44 B
Shifa	39.47±0.98		35.23±0.41		36.27±0.29		37.17±0.55		37.03±0.54 B
Ekim normu ortalaması Sowing norm average	39.39±0.36	A**	37.90±0.40	C	38.15±0.39	BC	39.00±0.45	AB	38.61±0.21
V.K (%)	3.15								
C.V (%)									
A.Ö.F	Çeşit: 1.701		Ekim normu: 1.013						
L.S.D									

*, **: Aynı harf grubuna giren ortalamalar arasında sırasıyla p < .05 ve p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Çizelge 8 incelendiğinde; bin tohum ağırlığı bakımından ekim normları arasında önemli farklılıklar saptanmış olup, ekim normları ise ilk yıl 41.11-43.67 g, ikinci yıl 37.90-39.39 g arasında değişmiştir. Denemenin ilk yılı en yüksek bin tohum ağırlığı 43.67 g ile 80 tohum m⁻² ekim normunda olurken, diğer ekim normları aynı grupta yer almıştır. İkinci yıl ise 39.39 g ile yine 80 tohum m⁻² ekim normunda görülmüştür. Ekim normları açısından bakıldığında her iki deneme yılında da seyrek ekimler bin tohum ağırlığını artırmıştır. Bu durum birim alandaki bitki sayıları azaldıkça, her bir bitkinin yaşam alanı genişlediği için bitkilerin çevre faktörlerinden daha iyi faydalanmaları neticesinde tohumlarda besin maddesi birikimi daha fazla olması ile açıklanabilir. Bazı araştırmacılar da ekim normunun artması ile bin tohum ağırlığının azaldığını bildirmiştir (Zarei ve ark., 2011; Yavuz, 2019). Bin tohum ağırlığı özellikle çiçeklenme dönemindeki (Haziran sonu ve Temmuz ayı) iklim koşullarıyla doğrudan ilişkili bir parametredir. İkinci deneme yılında bitkinin çiçeklenme döneminde ilk yıla göre sıcaklığın fazla olması bin tohum ağırlığının düşük olmasına neden olmuş olabilir.

Tohum Verimi (kg ha⁻¹)

Araştırmadan elde edilen sonuçlara göre tohum verimine ait ortalama değerler Çizelge 9'da verilmiş olup, her iki yılda da aspir çeşitleri ve ekim normları arasındaki fark p < .01 düzeyinde, bunların etkileşimleri arasındaki fark ise ilk yıl p < .05 düzeyinde önemli, ikinci yıl ise istatistiksel olarak önemsiz bulunmuştur. Aspir çeşitlerinde tohum verimi deneme yılının ilk yılında 1258.3-2686.2 kg ha⁻¹, ikinci yılında ise 849.7-1435.8 kg ha⁻¹ arasında değişim gösterirken, ekim normları ise ilk yıl 1653.6-1979.0 kg ha⁻¹, ikinci yıl 1060.7-1341.1 kg ha⁻¹ arasında

değişiklik göstermiştir. Genel olarak bakıldığında deneme yılının ilk yılında, ikinci yıla göre daha yüksek tohum verimi elde edilmiştir. Bu sonucun Çizelge 1’de görüldüğü gibi vejetasyon dönemlerindeki (özellikle Mart-Haziran arası) yıllar arasındaki yağış miktarı ile ilişkili olduğu düşünülmektedir.

Çizelge 9. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde tohum verimine ait ortalama değerler (kg ha⁻¹)
Table 9. Mean values of seed yield in safflower cultivars grown in different planting norms (kg ha⁻¹)

İl	2017				
	Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	1508.3±190.96	1420.0±72.34	1276.7±128.62	1293.3±168.08	1374.5±68.94 C**
Balcı	1681.7±154.71	1866.7±258.04	1315.0±197.88	1776.7±213.09	1660.0±109.33 BC
Dinçer	2795.0±149.08	2635.0±313.96	2770.0±342.06	2545.0±177.55	2686.2±114.82 A
Linaz	1335.0±42.52	1345.0±186.21	1233.3±64.31	1120.0±20.81	1258.3±51.18 C
Olas	1510.0±202.91	1346.7±75.29	1201.7±184.14	1170.0±143.38	1307.0±79.09 C
Remzibey-05	2545.0±230.07	2003.3±112.15	1851.7±243.00	1931.7±391.70	2082.9±139.17 AB
Shifa	2478.3±301.33	2014.8±205.12	1926.7±187.44	2226.7±335.71	2162.0±129.87 AB
Ekim normu ortalaması Sowing norm average	1979.0±139.35 A**	1804.8±115.80 AB	1653.6±136.47 B	1723.3±136.65 B	1790.2±66.35
V.K (%)	14.82				
C.V (%)					
A.Ö.F	Çeşit.660.8 Ekim normu:220.9				
L.S.D					
Çeşitler Cultivars	2019				
	Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	1370.0±65.06 c-f*	1176.7±31.79 e-h	950.0±28.86 h-l	1141.7±19.22 f-ı	1159.6±48.07 BC**
Balcı	1080.0±72.51 g-k	1030.0±109.65 g-k	943.3±120.28 h-l	1233.3±40.96 d-g	1071.7±50.28 C
Dinçer	1788.3±81.46 a	1375.0±45.82 b-f	1418.3±86.61 b-e	1583.3±41.76 abc	1541.2±56.71 A
Linaz	1148.3±76.72 fgh	848.3±79.17 kl	773.3±11.66 l	893.3±47.81 ı-l	915.8±49.67 D
Olas	938.3±61.93 h-l	876.7±92.48 jkl	848.3±49.69 kl	731.7±25.87 l	848.7±34.89 D
Remzibey-05	1445.0±73.20 bcd	1118.3±61.73 g-j	1090.0±32.14 g-k	1203.3±139.32 d-g	1214.2±55.84 B
Shifa	1618.3±134.29 ab	1181.7±63.39 e-h	1401.7±47.63 b-e	1541.7±64.95 bc	1435.8±61.67 A
Ekim normu ortalaması Sowing norm average	1341.1±68.37 A**	1086.7±44.79 BC	1060.7±57.08 C	1189.8±67.69 B	1169.6±31.96
V.K (%) C.V (%)	11.09				
A.Ö.F L.S.D	Çeşit:117.9 Ekim normu:108.0 ÇeşitxEkim normu:213.7				

*, **: Aynı harf grubuna giren ortalamalar arasında sırasıyla p < .05 ve p < .01 önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Aspirde tohum verimlerine ait değerler; genotip (Koç ve ark., 2017; Şeker, 2019), lokasyon (De Oliveira Neto ve ark., 2022), ekim zamanı (Ahadi ve ark., 2011), iklim faktörleri (Şerefoğlu, 2009), besin rekabeti, bakım işlemleri ve ekim normlarının farklılığından dolayı değişiklik gösterebilir (Koutroubas ve ark., 2004; Yavuz, 2019). Çizelge 9’a bakıldığında ilk yıl Dinçer çeşidi (2686.2 kg ha⁻¹), ikinci yıl ise Dinçer (1541.2 kg ha⁻¹) ve Shifa (1435.8 kg ha⁻¹) çeşitleri ön plana çıkmıştır. Çalışma da olduğu gibi, diğer pek çok araştırmada da aspir çeşitleri arasında tohum verimi bakımından önemli farklılıkların olduğu bilinmektedir (Öztürk ve ark., 2008; Smayıl-Zada, 2018; Arslan & Culpan, 2018; Yavuz, 2019). Ayrıca Arslan (2007), aspir bitkisinin tohum veriminin bitki başına tabla sayısı ve tabla başına tohum sayısından doğrudan etkilendiğini bildirmiştir. Bitki başına tabla sayısı ve tabla başına tohum sayısının yanında bitki boyu (Pavithra ve ark., 2016) dal sayısı (Omidi ve ark., 2009) ve bin tohum ağırlıklarının (Pattar & Patil, 2020) tohum verimi üzerine etkisi olduğu bilinmektedir. Bu çalışmada diğer çeşitlere göre tohum verimi bakımından daha ön plana çıkan Dinçer çeşidinin, dal sayısı, tabla sayısı, tabladaki tohum sayısı ve bin tohum ağırlığı bakımından genellikle diğer çeşitlerden daha iyi olduğu görülmektedir.

Ekim normu tohum verimini belirleyen önemli faktörlerden biridir (Lloveras ve ark., 2004). Çalışmada da bitki sıklıklarından dolayı önemli farklılıklar oluşarak, tohum verimi açısından ekim normu uygulamalarında tam anlamıyla homojen bir artış azalış olmasa da her iki yılda 80 tohum m⁻² en yüksek tohum verimi değerini almıştır.

Gonzalez ve ark. (1994), bitki sıklıklarının tohum verimini etkilemediğini belirtirken, çeşitlerin tohum veriminde etkisinin olduğunu ifade etmiştir. Öte yandan bazı araştırmacılar ise sık ekimlerin tohum veriminde önemli bir artış sağladığını belirtilmektedir (Şerefoğlu 2009; Akış, 2013; Vaghar ve ark., 2014; Sefaoğlu, 2017).

Yağ Oranı (%)

Araştırma sonuçlarına göre yağ oranı bakımından ilk yılda çeşitler ve çeşit x ekim normlarında, ikinci deneme yılında ise çeşitler arasında $p < .01$ düzeyinde önemli fark olduğu görülmüştür (Çizelge 10). Çeşitler bakımından yağ oranı deneme yılının ilk yılında %23.56-36.32, ikinci yılında ise %25.72-31.43 arasında değişirken, ekim normları ise ilk yıl %29.50-29.68, ikinci yıl %28.63-28.98 arasında değişiklik göstermiştir. Yağ oranı bakımından ilk yıl Balcı ve Olas çeşitleri, ikinci yıl ise Linas, Olas ve Balcı çeşitleri ön plana çıkmıştır.

Çizelge 10. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde yağ oranına ait ortalama değerler (%)

Çeşitler Cultivars	2017				
	Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	30.33±0.38 b**	30.63±0.27 b	30.40±0.65 b	28.93±0.32 b	30.07±0.27 B**
Balcı	36.10±0.35 a	35.50±0.76 a	36.03±0.82 a	35.30±0.80 a	35.73±0.32 A
Dinçer	25.13±0.92 cde	24.33±0.12 cde	23.93±0.32 cde	24.47±0.18 cde	24.47±0.25 D
Linas	30.23±0.78 b	30.97±1.11 b	31.23±0.95 b	31.03±0.73 b	30.87±0.40 B
Olas	36.10±0.40 a	36.33±0.23 a	36.27±0.24 a	36.60±0.25 a	36.32±0.13 A
Remzibey-05	26.50±0.50 c	25.97±0.58 cd	25.77±0.27 cde	26.00±0.87 cd	26.06±0.27 C
Shifa	23.40±0.70 de	23.10±1.29 e	23.53±1.12 de	24.20±0.30 cde	23.56±0.42 D
Ekim normu ortalaması Sowing norm average	29.68±1.06	29.55±1.11	29.59±1.13	29.50±1.06	29.58±0.54
V.K (%) C.V (%)	3.58				
A.Ö.F L.S.D	Çeşit: 1.576 ÇeşitxEkim normu: 2.332				
Çeşitler Cultivars	2019				
	Ekim normları (tohum m ⁻²) [Sowing norms (seed m ⁻²)]				
	80	120	160	200	Çeşit ortalaması Cultivar average
Asol	29.70±0.85	29.40±0.69	30.17±0.38	30.13±0.30	29.85±0.27 B**
Balcı	31.00±0.75	31.13±0.27	30.07±1.08	31.50±0.46	30.92±0.34 AB
Dinçer	25.53±0.72	25.73±0.42	25.00±0.29	26.63±0.47	25.72±0.28 D
Linas	32.23±0.24	30.73±0.45	31.13±0.47	31.63±0.12	31.43±0.23 A
Olas	30.30±1.23	29.33±0.29	31.20±0.52	30.20±0.29	30.26±0.36 AB
Remzibey-05	26.67±0.70	27.90±0.72	27.87±0.50	26.53±0.35	27.24±0.32 C
Shifa	26.43±0.09	26.17±0.23	27.40±0.20	25.83±0.29	26.46±0.19 CD
Ekim normu ortalaması Sowing norm average	28.84±0.59	28.63±0.46	28.98±0.50	28.92±0.53	28.84±0.26
V.K (%) C.V (%)	3.08				
A.Ö.F L.S.D	Çeşit: 1.192				

** : Aynı harf grubuna giren ortalamalar arasında $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Genel ortalamaya göre yağ oranları incelendiğinde, deneme yılının ilk yılında yağ oranları daha yüksek değer almıştır. Çizelge 1 incelendiğinde ikinci yılda yağ oranlarının daha az olmasının, ikinci yıl ortalama sıcaklık değerleri ve hissedilen kuraklıktan kaynaklandığı düşünülmektedir. Bu durumu destekler nitelikte Gonzalez ve ark. (1994) ile Öztürk (2003), yağ oranının çevre koşullarından, bilhassa da yıllar arasında iklim faktörlerinde ortaya çıkan sıcaklık değişimlerinden önemli derecede etkilendiğini; sıcak ve daha kurak geçen yıllarda yağ oranında düşüşlerin olduğunu bildirmişlerdir.

Çalışmada çeşitler arasındaki yağ oranı farklılıkları, çeşitlerin genetik yapısının farklı olmasıyla (Beyyavas ve ark., 2011; Erbaş ve ark., 2016) birlikte ekolojik şartlar ile uygulanan kültürel işlemlerden de etkilenebilmektedir (Aktaş, 2022). Katar ve ark. (2016)'da yağ oranlarının çeşitlere göre değiştiğini bildirmiştir. Çizelge 9 ve Çizelge 10 incelendiğinde tohum verimi bakımından ön plana çıkan Dinçer ve Shifa çeşitleri yağ oranı bakımından ön

planda olmamıştır. Genel bir ifade ile dikenli aspir çeşitleri dikensiz çeşitlere göre soğuğa ve kurağa toleransları daha iyi, yağ içeriklerinin daha fazla olduğu bilinmektedir (Erbaş & Baydar, 2017). Çalışmada da dikensiz çeşitler Dinçer ve Shifa çeşitleri iken, diğer çeşitler dikenli çeşitlerdir. Dinçer ve Shifa çeşitlerinin yağ oranlarının az olması asperde dikensiz çeşitlerde yağ oranının dikenli çeşitlere göre daha az olması ile ilişkilendirilir. Çizelge 10'da görüldüğü gibi her iki yılda da ekim normunun yağ oranına etkisinin olmadığı görülmüştür. Bu çalışmaya benzer Elfadl ve ark. (2009) ve Ismayıl-zada (2018) bitki yoğunluğunun yağ oranlarını etkilemediğini belirtmişlerdir. Sefaoğlu (2017) ve Yavuz (2019) ise yağ oranlarında göre dalgalı bir değişim olsa da bitki sıklıklarının yağ oranını değiştirdiğini tespit etmiştir.

Yağ Verimi (kg ha⁻¹)

Araştırma sonuçlarına göre yağ verimleri bakımından deneme yılının ilk yılında çeşitler ve çeşit x ekim normları $p < 0.05$, ekim normları bakımından $p < .01$ düzeyinde önemli olduğu, ikinci deneme yılında ise çeşit ve ekim normlarının $p < .01$ düzeyinde önemli olduğu görülmüştür (Çizelge 11). Çeşitler bakımından yağ verimi deneme yılının ilk yılında 387.2-657.2 kg ha⁻¹, ikinci yılında ise 256.7-396.4 kg ha⁻¹ arasında değişim gösterirken, ekim normları ise ilk yıl 467.4-567.0 kg ha⁻¹, ikinci yıl 302.8-381.0 kg ha⁻¹ arasında değişiklik göstermiştir. Çizelge 11'de görüldüğü gibi ilk yıl Dinçer çeşidi (657.2 kg ha⁻¹), ikinci yıl ise Dinçer (396.4 kg ha⁻¹) ve Shifa (379.6 kg ha⁻¹) çeşitleri ön plana çıkmıştır.

Çizelge 11. Farklı ekim normlarında yetiştirilen aspir çeşitlerinde yağ verimine ait ortalama değerler (kg ha⁻¹)

Table 11. Mean values of oil yield in safflower cultivars grown in different planting norms (kg ha⁻¹)

Çeşitler Cultivars	2017									
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]									
	80		120		160		200		Çeşit ortalaması Cultivar average	
Asol	457.0±55.04	f-k*	434.0±19.08	g-k	386.3±31.75	ijk	374.3±49.14	jk	412.9±20.34	CD*
Balcı	608.0±60.68	a-f	659.7±82.22	ab	474.7±76.23	d-k	629.0±84.51	a-d	592.8±38.95	AB
Dinçer	702.0±40.15	a	641.3±75.29	abc	662.3±78.59	ab	623.3±42.95	a-e	657.2±27.81	A
Linaz	403.7±6.83	h-k	412.3±45.11	h-k	385.0±22.14	ijk	348.0±14.43	k	387.2±13.48	D
Olas	547.3±80.02	a-h	489.3±29.58	c-k	436.7±68.10	g-k	427.7±49.47	g-k	475.2±29.33	BCD
Remzibey-05	677.0±75.03	ab	522.3±40.33	b-j	475.7±58.12	d-k	502.3±103.04	c-k	544.3±39.04	ABC
Shifa	574.7±55.65	a-g	471.0±73.93	e-k	451.0±34.67	f-k	538.7±81.17	b-ı	508.8±31.15	BCD
Ekim normu Ortalaması	567.0±29.06	A**	518.6±27.01	AB	467.4±26.47	B	491.9±31.57	B	511.2±14.61	
Sowing norm average										
V.K (%) C.V (%)		15.71								
A.Ö.F L.S.D		Çeşit: 134.5		Ekim normu:66.9		ÇeşitxEkim normu:132.4				

Çeşitler Cultivars	2019									
	Ekim normları (tohum m ⁻²) [<i>Sowing norms (seed m⁻²)</i>]									
	80		120		160		200		Çeşit ortalaması Cultivar average	
Asol	405.7±8.19		345.7±4.91		286.3±5.78		344.3±6.33		345.5±13.01	B**
Balcı	336.0±28.02		320.7±34.18		286.0±46.18		388.7±13.93		332.8±17.85	B
Dinçer	456.7±22.87		353.7±5.92		354.0±19.65		421.3±17.14		396.4±15.34	A
Linaz	370.0±25.77		260.7±23.16		241.7±7.33		282.3±15.91		288.7±16.96	C
Olas	284.3±21.88		257.3±27.90		264.0±11.06		221.3±9.68		256.7±10.67	C
Remzibey-05	386.7±29.38		311.7±15.71		303.7±12.73		320.0±41.01		330.5±15.26	B
Shifa	427.7±34.36		308.7±15.16		384.3±16.33		397.7±12.91		379.6±16.08	A
Ekim normu ortalaması (Sowing norm average)	381.0±14.43	A**	308.3±10.18	BC	302.8±12.35	C	339.4±15.94	B	332.8±7.41	
V.K (%) C.V (%)		12.13								
A.Ö.F L.S.D		Çeşit:32.8		Ekim normu:33.6						

*, **: Aynı harf grubuna giren ortalamalar arasında sırasıyla $p < .05$ ve $p < .01$ önem düzeyine göre fark yoktur. V.K: Varyasyon katsayısı, C.V: Coefficient of variation, A.Ö.F: Asgari Önemli Fark, L.S.D: Least Significant Difference, ± : Standart Hata.

Ham yağ verimlerine ait değerler arasındaki farklılığın kullanılan çeşit, özellikle araştırmaların yürütüldüğü lokasyonun iklim ve toprak yapısındaki farklılıklar, farklı bakım işlemleri ve ekim sıklığı bakımından farklılıklardan kaynaklanabileceği ifade edilmiştir (Yavuz, 2019). Çizelge 11'de görüldüğü gibi deneme yılının ilk yılındaki yağ verimleri ikinci yıla göre yüksek olmuştur. Bu durum aynı zamanda deneme yılının ilk yılındaki

dekara tohum verimi ve yağ oranının yüksek olması ile ilişkilendirilebilir. Zaten birçok araştırmacı tarafından da yağ veriminin önemli ölçüde hem yağ içeriği hem de tohum verimi ile ilişkili olduğu belirtilmiştir (Bidgoli ve ark., 2006; Arslan, 2007). Bu durumu destekler nitelikte Oruç (2014)'de tohum verimi yüksek olan çeşit ve hatların yağ veriminin yüksek olduğu belirterek, yağ verimi bitkinin tohum verimi ve yağ oranı ile ilişkili bir değer olduğu için tohum verimi ile benzer sonuçlar gösterdiğini ifade etmiştir. Ayrıca Omid Tabrizi (2000), yağ veriminin tohum verimi ile anlamlı ve pozitif yönde ilişkili olduğunu ve tohum verimi arttıkça yağ veriminin de artacağını bildirmiştir. Bu çalışmada da Çizelge 9,10,11 incelendiğinde genotiplerin tohum verimi ve yağ oranı bakımından farklılıklar göstermesi yağ veriminde farklılıklar göstermesine sebep olmuştur. Ekim normları bakımından yağ oranları değişmediği halde yağ verimlerinin ekim normunun az olduğu uygulamalarda olmasının sebebinin bu ekim normalarında tohum veriminin fazlalığından kaynaklandığı görülmektedir. Öte yandan başka araştırmacılar yağ verimine ekim normunun etkisinin olmadığını belirtmiştir (Moghaddasi & Omid, 2015).

SONUÇ ve ÖNERİLER

Çalışmada incelenen tüm parametreler bakımından incelenen aspir çeşitleri arasında önemli farklılıklar meydana gelmiştir. Araştırmadan elde edilen verilerle yapılan varyans analizleri sonucunda, ekim normu uygulamalarının aspir çeşitlerinde her iki deneme yılında da bitki boyu, dal sayısı, tabla sayısı, tabladaki tohum sayısı, bin tohum ağırlığı, tohum verimi, yağ oranı ve yağ verimine önemli etkilerinin olduğu saptanmıştır. Araştırma sonuçlarına göre, belli noktaya kadar (160 tohum m⁻²) bitki yoğunluğu arttıkça tohum verimi düşmüştür. En yüksek tohum verimi ise deneme yılının ilk ve ikinci yılında da 80 tohum m⁻² ile Dinçer çeşidinden (2686.2 kg ha⁻¹), ikinci yıl ise Dinçer (1541.2 kg ha⁻¹) ve Shifa (1435.8 kg ha⁻¹) çeşitlerinden elde edilmiştir. Yağ oranı bakımından ekim normları arasında her iki yılda istatistiksel bir fark bulunmamasına karşın en yüksek yağ oranı ilk yıl Balcı (%35.7) ve Olas (%36.6), ikinci yıl ise Linas (%31.4) çeşidinden elde edilmiştir. Çalışmanın sonucu bütün olarak ele alındığında; Tokat-Kazova koşullarında yazlık aspir ekiminde kullanılacak uygun bitki sıklığının 80 tohum m⁻² olduğu, Dinçer ve Shifa çeşitlerinin diğer çeşitlere göre daha iyi sonuçlar verdiği tespit edilmiştir.

TEŞEKKÜR

Bu çalışma III. Uluslararası (XV. Ulusal) Tarla Bitkileri Kongresi'nde özet bildiri olarak sunulmuştur.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Influences of Light-Emitting Diodes (LEDs) and Culture Media on Micropropagation of Native Hemp (*Cannabis sativa*) Population from Türkiye

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ABSTRACT

Light-emitting diodes (LEDs) have historically been the standard for supplemental lighting in industrial hemp (*Cannabis sativa*) cultivation in a greenhouse, but there is a lack of scientific understanding in *C. sativa* regarding different media under various LEDs in vitro conditions. The aim of this study was to determine the top-performing LEDs and nutrient media for micropropagation of *C. sativa*. In this study, two different spectra (white and red LEDs) and different media [MS, B5, MS+B5, ½ MS, ½ B5, ½ MS + ½ B5, and control (without medium)] were used, maintaining constant light photoperiod. The diversity of light spectra and nutrient media showed statistically significant differences for the growth parameters of *C. sativa*. Red LED provided an extraordinarily high shoot length of 7.37 cm, whereas white LED exhibited the highest root length with 6.57 cm. Also, nutrient media were found to be effective on shoot growth in full-strength media, while on root growth in half-strength media. Among the plant growth parameters, the number of shoots and nodes per plant is of great importance for clonal propagation. B5 medium under red LED provided an extraordinarily high shoot number with 4.64, whereas MS medium under white LED exhibited the highest node number with 5.87. Each LED source required the respective medium adjustment for the best results of the desired parameters. In this respect, the study may be advantageous for the selection of the appropriate nutrient medium and led spectrum in micropropagation of *C. sativa*.

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Keywords

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Türkiye'deki Yerel Kenevir (*Cannabis sativa*) Popülasyonunun Mikroçoğaltımı Üzerinde Işık Yayan Diyotların (LED'ler) ve Kültür Ortamlarının Etkileri

ÖZET

Işık yayan diyotlar (LED'ler), tarihsel olarak serada endüstriyel kenevir (*Cannabis sativa*) yetiştiriciliğinde ek aydınlatma için standart olmuştur, ancak *C. sativa*'da in vitro koşullarında çeşitli LED'ler altında farklı ortamlar konusunda bilimsel anlayış eksikliği vardır. Bu çalışmanın amacı, *C. sativa*'nın mikro çoğaltılması için en iyi performans gösteren LED'leri ve besin ortamlarını belirlemektir. Bu çalışmada, sabit ışık fotoperiyodu korunarak iki farklı spektrum (beyaz ve kırmızı LED'ler) ve farklı ortamlar [MS, B5, MS + B5, ½ MS, ½ B5, ½ MS + ½ B5 ve kontrol (ortamsız)] kullanıldı. Işık spektrumlarının ve besin ortamlarının çeşitliliği, *C. sativa*'nın büyüme paarametreleri için istatistiksel olarak anlamlı farklılıklar gösterdi. Kırmızı LED, 7,37 cm ile olağanüstü yüksek bir sürgün uzunluğu sağlarken, beyaz LED 6,57 cm ile en yüksek kök uzunluğunu sergiledi. Ayrıca, besin ortamlarının tam güçteki ortamlarda sürgün büyümesinde, yarı güçteki ortamlarda ise kök büyümesinde etkili olduğu bulunmuştur. Bitki büyüme parametreleri arasında, bitki başına sürgün ve nod sayıları klonal çoğaltım için büyük önem taşımaktadır. Kırmızı LED altında B5 ortamı 4.64 ile olağanüstü yüksek bir sürgün sayısı sağlarken, beyaz LED altında

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Endüstriyel kenevir
Cannabis sativa
Kırmızı LED
Beyaz LED
Kültür ortamı
Mikro çoğaltma

MS ortamı 5.87 ile en yüksek nod sayısını sergilemiştir. İstenilen parametrelerin en iyi sonuçları için her bir LED kaynağı ilgili ortam ayarlamasını gerektirmiştir. Bu açıdan, çalışma *C. sativa*'nın mikroçoğaltımında uygun besin ortamının ve LED spektrumunun seçilmesi için avantajlı olabilir.

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INTRODUCTION

Cannabis sativa L. which is commonly known as cannabis, hemp and marijuana, an annual dioecious species belonging to Cannabaceae family is an important multipurpose crop cultivated for industrial, medicinal and nutritional uses since ancient times (Bonini et al., 2018, Akıncı & Karaman, 2024): its stem has fiber serving as feedstock for textile, paper, and mechanical industries and in the construction sectors (Ceyhan et al., 2022); its seeds are rich in nutrients as a source of nutritious oil and protein; Its flowers, leaves and roots contain commercially serious bioactive compounds that have a wide spectrum of biological activity (Esposito et al., 2021; Liu et al., 2022). However, in the 20th century, its cultivation was legally restricted in many countries due to the psychoactive effect of one of its compounds, Δ^9 - Δ^9 -tetrahydrocannabinol (THC) (Liu et al., 2022). In the commercial production of industrial hemp, the threshold THC amounts in its flowering plant parts range from 0.1% (several Australian states, Columbia, Mexico, Switzerland and Uruguay), to 0.2% (Europe), to 0.3% (Brazil, Canada, China and USA) of dry weight according to the legal permission of each country's governments (Anonym, 2016; Adhikary et al., 2021). Due to these legal restrictions, research and development studies on *C. sativa* have been slow.

The cultivation of this economically valuable plant is increasing day by day. Traditionally, the fiber type (commonly called hemp, with Δ^9 -THC < 0.3%) of *C. sativa* is predominantly monoecious (hermaphrodite), mostly cultivated by seeds, as this characteristic produces more uniform material, and its cultivation has been highly mechanized (Chandra et al., 2017). In contrast, drug type (commonly called cannabis, with Δ^9 -THC > 0.3%) of *C. sativa* is often propagated using clonal methods (Caplan et al., 2018, Campbell et al., 2019) to reduce the high levels of phenotypic diversity exhibited in seedling populations and to produce high-quality, genetically and phenotypically uniform crops that meet consumer preferences and comply with government regulations (Lata et al., 2010). Although more expensive than seed, this approach is efficiently used by some *C. sativa* producers to commercially produce a more uniform crop containing CBD (Cannabidiol) and other non-psychoactive cannabinoids (Fike et al., 2020; Mark et al., 2020). Also, many scientists are trying to produce new varieties that can serve the purpose due to the economic importance of *C. sativa*. The predominantly annual dioecious nature of *C. sativa* (Clarke et al., 2016) is an important obstacle to slowing down traditional breeding efforts. Therefore, clonal multiple propagation practices offer great service for the production of uniform crops of male and female individuals to be used for the breeding program of *C. sativa*.

Micropropagation by tissue culture is an effective method for mass propagation of disease-free plants in a highly controlled environment using aseptic techniques and has been actively used for many commercial plants for a long time (Abdalla et al., 2022; Xue et al., 2023; Ioannidis et al., 2023; Miladinova-Georgieva et al., 2022, Özyalın & Yaman, 2023). Successful in vitro propagation of plants depends on explant source (He et al., 2023; Papafotiou et al., 2023), basal medium type (Suraya et al., 2021; Meyad et al., 2023), and climatic conditions such as light, temperature, humidity, and others (Yadav et al., 2020; Wojtania et al., 2023). Moreover, the type and concentration of basal media have a significant effect, both on shoot multiplication and rooting, as well as their promotion of the formation of strong plants with well-developed leaves (Garcia et al., 2011; Page et al., 2020).

Light, which is a physical factor, plays an important key role in processes of morphogenesis, photosynthesis, and primary and secondary metabolism of plants, which ensures the survival of the in vitro plant cultures (Azmi et al., 2016; Khurshid et al., 2020). The use of light-emitting diodes (LEDs) among the light sources is widely used in clonal reproduction techniques from *C. sativa*, especially in greenhouse conditions (Namdar et al., 2019), due to the many advantages, such as lower heat emission and more efficient energy utilization, as well as significantly longer lifetimes. However, the effects of LED spectra on *C. sativa* have not yet been previously elucidated in vitro culture. Therefore, the aim of the present study was to determine the effects of different media under various LED spectra on in vitro shoot proliferation and rooting in order to improve the in vitro *C. sativa* micropropagation system.

MATERIAL and METHOD

Material

The seeds of *Cannabis sativa* L., dioecious genotype Narlısaray, a native population of Türkiye, were obtained from the Hemp Research Institute of Yozgat Bozok University.

Surface Disinfection and Germination Condition

The seeds were washed with running tap water. After initial washing, the seeds were treated with 70% ethanol for 1 min. Lastly, the surface of seeds was disinfected by shaking in a solution containing 0.5% NaOCl (15% v v⁻¹ commercial bleach) and 0.1% Tween 20 (v v⁻¹) for 15 min. Finally, the seeds were thoroughly rinsed 4-5 times with de-ionized sterile water and cultured in ½ × Murashige & Skoog basal salts (MS, Murashige and Skoog, 1962) supplemented with 1.5 % sucrose. The pH of the medium was adjusted to 5.7±0.1 with 0.1 N NaOH or 0.1 N HCl before the addition of 0.6% (w v⁻¹) plant agar (Duchefa), and then autoclaved at 121 °C for 20 min. The seeds were maintained in a growth chamber at room temperature (25 ± 1 °C) under 16/8 h light/dark photoperiod, 60% relative humidity, and cool white, fluorescent light (36 W/830 G13 1214 mm) intensity of about 40 µmol m⁻²s⁻¹ PPFD for 15 days. After two weeks, the apical shoots with a single node excised from germinated seedlings (healthy zygotic embryos) were used as an explant source.

Culture Media

The apical shoots were cultured onto seven different media prepared separately in a 100 ml culture flask. Two different basal nutrient media (MS basal salts+vitamins and Gamborg B5 vitamins (B5, Gamborg et al., 1968)) and their different concentrations (full and half-strength) and their combinations were used as the medium for the culture: MS, B5, MS+B5, ½ MS, ½ B5, ½ MS + ½ B5, and control (without medium). All media were supplemented 3% (w/v⁻¹) sucrose and 0.64% (w/v⁻¹) plant agar.

All cultures were incubated in growth rooms for 5 weeks at 24 ± 2 °C temperature under two different spectra (red and white) with 16/8 h light/dark photoperiod by light-emitting diodes (LEDs) fluorescent tubes (Lumilux, L 36W/865, cool daylight, Osram, Germany): white LED and red LED (λ = 650 nm), relative humidity 60 %. All media were separately tested at white and red LEDs to evaluate their effects on shoot proliferation (Figure 1).

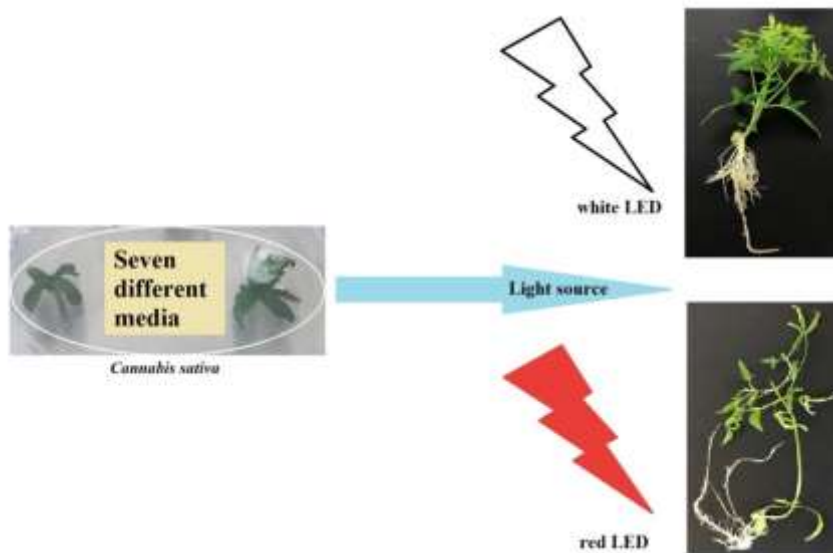


Figure 1. In vitro culture of *Cannabis sativa* on seven different nutrient media under two different LEDs
Şekil 1. İki farklı LED altında yedi farklı besin ortamında *Cannabis sativa*'nın in vitro kültürü

Analysis of Growth Parameters

Three weeks after the beginning of the culture, the apical segments of the in vitro-grown shoots were cut to form new shoots from the nodal parts. After this treatment, the in vitro shoots were continued to culture for another two weeks. In total, explants were cultured for 5 weeks. Then, the observations were recorded as shoot vitality, root formation, number of shoots per plant, number of roots per plant, number of nodes per plant, number of leaves per plant, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight.

Experimental Design and Statistical Analysis

The experimental design was completely randomized with four replicates using five explants in each culture flask. In the experiment, seven different culture media, including a control under two different (white and red) light sources, a total of 14 treatments, were used. These data were analyzed by One-Way analysis of variance (ANOVA) using the SPSS statistical program. The mean values were given with standard error ($\pm$ SE) and compared with Duncan's multiple range test at $P < 0.05$ for significant differences. The mean values of the applications were compared using the Duncan test. Percentages were subjected to arcsine transformation before statistical analysis (Snedecor and Cochran 1967). Hierarchical cluster analysis (HCA) was used to evaluate all parameters and to select the best combination of LED spectra and different culture media for *in vitro* culture plantlets of *C. sativa*. HCA findings based on between-groups linkage and squared Euclidean distance are presented as a dendrogram.

RESULTS

Effect of different LEDs and basal media on the growth parameters of *C. sativa*

In this study, the plant growth of *C. sativa* was very critically affected by light and nutrient media, which are environmental factors. The red LEDs produced the highest mean shoot vitality, shoot length, number of shoots per plant and number of roots per plant whereas the white LEDs promoted higher root formation, root length, number of nodes per plant, number of leaves per plant, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight (Table 1).

Table 1. Effect of different LEDs on the growth parameters of *C. sativa* in vitro condition.

Çizelge 1. Farklı LED'lerin in vitro koşullarda C. sativa'nın büyüme parametreleri üzerine etkisi.

Growth parameters	Red LED	White LED
Shoot vitality (%)	88.5	78.6
Root formation (%)	61.5	67.5
Shoot length (cm)	7.37	4.85
Root length (cm)	4.45	6.57
Number of shoots per plant	3.51	2.62
Number of roots per plant	2.70	2.33
Number of nodes per plant	2.24	3.61
Number of leaves per plant	11.06	12.73
Shoot fresh weight (g)	1.41	1.75
Shoot dry weight (g)	0.23	0.26
Root fresh weight (g)	0.24	0.82
Root dry weight (g)	0.03	0.08

The explants were separately cultured on various media (MS, B5, MS+B5, $\frac{1}{2}$ MS, $\frac{1}{2}$ B5, $\frac{1}{2}$ MS + $\frac{1}{2}$ B5, and control) supplemented with no plant growth regulators. The effect of these media on the growth parameters of *C. sativa* is given in Table 2. MS medium displayed a stronger effect on parameters related to shoot growth. However, $\frac{1}{2}$ B5 and $\frac{1}{2}$ MS + $\frac{1}{2}$ B5 media showed a higher benefit on root growth characteristics.

Table 2. Effect of different nutrient media on the growth parameters of *C. sativa* in vitro condition.

Çizelge 2. Farklı besin ortamlarının C. sativa'nın in vitro büyüme parametreleri üzerine etkisi.

Growth parameters	Control	MS	B5	MS+B5	1/2 MS	1/2 B5	1/2MS+1/2 B5
Shoot vitality (%)	16.7 $\pm$ 7.5 c	100.0 a	100.0 a	100.0 a	70.8 $\pm$ 9.6 b	97.2 $\pm$ 2.8 a	100.0 a
Root formation (%)	27.8 $\pm$ 14.1 b	83.3 $\pm$ 6.1 a	63.9 $\pm$ 13.9 a	55.6 $\pm$ 11.1 ab	54.2 $\pm$ 9.6 ab	83.3 $\pm$ 4.2 a	83.4 $\pm$ 7.5 a
Shoot length (cm)	1.00 $\pm$ 0.5 c	7.76 $\pm$ 1.1 a	7.38 $\pm$ 0.9 ab	7.04 $\pm$ 0.7 ab	5.42 $\pm$ 0.2 b	7.50 $\pm$ 0.3 ab	6.65 $\pm$ 0.8 ab
Root length (cm)	0.73 $\pm$ 0.4 d	6.43 $\pm$ 0.7 abc	5.31 $\pm$ 0.6 bc	5.05 $\pm$ 0.3 c	7.67 $\pm$ 0.4 a	6.04 $\pm$ 1.3 abc	7.34 $\pm$ 0.5 ab
Number of shoots per plant	0.88 $\pm$ 0.4 c	3.68 $\pm$ 0.2 ab	4.10 $\pm$ 0.2 a	3.53 $\pm$ 0.3 ab	2.74 $\pm$ 0.4 b	3.39 $\pm$ 0.3 ab	3.17 $\pm$ 0.4 ab
Number of roots per plant	0.96 $\pm$ 0.4 b	2.64 $\pm$ 0.2 a	2.70 $\pm$ 0.3 a	3.11 $\pm$ 0.4 a	2.42 $\pm$ 0.3 a	3.09 $\pm$ 0.2 a	2.67 $\pm$ 0.2 a
Number of nodes per plant	0.75 $\pm$ 0.3 c	4.27 $\pm$ 0.7 a	3.70 $\pm$ 0.4 ab	2.57 $\pm$ 0.1 b	3.05 $\pm$ 0.5 ab	2.45 $\pm$ 0.3 b	3.71 $\pm$ 0.5 ab
Number of leaves per plant	1.00 $\pm$ 0.5 d	15.47 $\pm$ 0.3 a	14.94 $\pm$ 0.5 ab	12.21 $\pm$ 0.5 c	12.64 $\pm$ 0.7 bc	13.09 $\pm$ 1.7 abc	13.92 $\pm$ 0.5 abc
Shoot fresh weight (g)	$\leq$ 0.01	2.06	2.53	1.84	1.08	1.69	1.87
Shoot dry weight (g)	$\leq$ 0.01	0.38	0.33	0.24	0.19	0.28	0.30
Root fresh weight (g)	$\leq$ 0.01	0.67	0.48	0.16	1.12	0.66	0.63
Root dry weight (g)	$\leq$ 0.01	0.07	0.05	0.02	0.10	0.06	0.06

Data expressed as the mean value $\pm$ SE (n = 6). Different lowercase (a–d) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$).

Effect of basal media under different LEDs on the growth parameters of *C. sativa*

The treatments with the different basal media under different LEDs such as red LED or white LED promoted significant responses on shoot and root growth of *C. sativa* (Table 3).

Shoot vitality was affected by a different basal media and LEDs (Table 3). High shoot vitality (33.3-100%) occurred under both LEDS in all media except control media with white LEDs

Rooting was seen from the cut ends of the shoots all media tested under red and white LED. Root formation ratio was fluctuated between 0 % and % 100% (Table 3). The highest frequency of root proliferation (100%) was achieved on ½ MS+½ B5 medium (100%) under the white LED. Whereas B5 medium with white LED (94.4%) followed this combination. No root formation occurred in control medium with white LED.

Table 3. Shoot vitality, root formation, shoot length, root length in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 3. Farklı renkteki LED'ler altında farklı besin ortamlarında yetiştirilen *C. sativa* fidelerinde sürgün canlılığı, kök oluşumu, sürgün uzunluğu, kök uzunluğu.

Color LEDs*, media	Shoot vitality (%)	Root formation (%)	Shoot length (cm)	Root length (cm)
Red LED, control	33.3±0.0 d	55.5±9.0 cd	2.00±0.1 g	1.47±0.4 g
Red LED, MS	100.0±0.0 a	83.3±10.4 abc	10.10±0.4 a	4.88±0.2 d
Red LED, B5	100.0±0.0 a	33.3±0.0 d	9.33±0.0 b	4.07±0.1 e
Red LED, MS+B5	100.0±0.0 a	33.3±0.0 d	8.47±0.1 c	4.42±0.1 de
Red LED, ½ MS	94.4±5.6 ab	75.0±3.2 bc	5.56±0.4 f	6.81±0.3 b
Red LED, ½ B5	91.7±4.8 b	83.3±10.4 abc	7.69±0.4 de	3.23±0.4 f
Red LED, ½ MS+½ B5	100.0±0.0 a	66.7±0.0 cd	8.41±0.2 cd	6.29±0.2 bc
White LED, control	0.0±0.0 e	0.0±0.0 e	0.0±0.0 h	0.0±0.0 h
White LED, MS	100.0±0.0 a	83.3±9.6 abc	5.43±0.0 f	7.98±0.2 a
White LED, B5	100.0±0.0 a	94.4±5.6 ab	5.43±0.2 f	6.55±0.0 b
White LED, MS+B5	100.0±0.0 a	77.8±11.1 bc	5.61±0.4 f	5.68±0.2 c
White LED, ½ MS	50.0±0.0 c	33.3±0.0 d	5.28±0.0 f	8.53±0.1 a
White LED, ½ B5	100.0±0.0 a	83.3±0.0 abc	7.30±0.3 e	8.84±0.2 a
White LED, ½ MS+½ B5	100.0±0.0 a	100.0±0.0 a	4.89±0.1 f	8.40±0.1 a

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–h) within column for each parameter indicate significant differences between means (Duncan test; p ≤ 0.05). *red LED (λ = 650 nm); White LED.

Shoot length was also influenced by different basal media under the different LEDs. The maximum shoot length (10.10±0.4 cm) was obtained on MS medium under red LEDs. B5 medium x red LED combination also showed high shoot length (9.33±0.0). Whereas the shoot length was drastically decreased on the control (0.0 %) under white LED. Different media and LED types also affected root length, and the highest root length was observed in ½ B5 medium (8.84±0.2 cm) and ½ MS (8.53±0.1 cm) and ½ MS+½ B5 (8.40±0.1 cm), and MS medium (7.98±0.2 cm) under the white LED. Because there is no root formation as indicated in root formation, the lowest root length was observed in the control medium under both red and white LEDs (1.47±0.4 cm and 0.0±0.0 cm, respectively).

A significant effect of all basal media under different color LEDs was observed on the number of shoots per plant, number of roots per plant, number of nodes per plant, and number of leaves per plant (Table 4).

The number of shoots per plant was varied in response to the different media and light sources (Table 4). The red LED exhibited the highest number of shoots per plant in B5 medium (4.64±0.1) and MS medium (4.11±0.2) and in ½ MS+½ B5 (4.00±0.3), and MS+B5 (3.83±0.5). Under white LED, only ½ B5 medium (4.07±0.2) was statistically in the same group as these media under red LED.

The number of roots per plant a significantly affected by different LEDs and basal media (Table 4). The greatest number of roots per plant was observed in MS+B5 (3.56±0.6) medium under the white LED.

All treatments showed a significant effect on the number of nodes per plant (Table 4). The highest number of nodes per plant was observed in MS medium under the white LED (5.87±0.1).

LED colors had a serious effect on the number of leaves per plant (Table 4). Statistically, the highest values were recorded under the white LED in ½ B5 medium (16.84±0.4) and MS medium (16.06±0.2), and in B5 medium (15.94±0.3).

The shoot and root activities of *C. sativa* seedlings grown under the red and white LEDs varied in response to the different media (Table 5).

Shoot fresh weight both treatments showed a significant effect (Table 5). A high level of shoot fresh weight was

observed in B5 medium under the white LED (3.37 ± 1.1 g) which was statistically significantly different from other applications.

Table 4. Number of shoots per plant, number of roots per plant, number of nodes per plant, number of leaves per plant in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 4. Farklı renklerdeki LED'ler altında farklı besin ortamlarında yetiştirilen *C. sativa* fidelerinde bitki başına sürgün sayısı, bitki başına kök sayısı, bitki başına düğüm sayısı, bitki başına yaprak sayısı.

Color LEDs*, media	Number of shoots per plant		Number of roots per plant		Number of nodes per plant		Number of leaves per plant	
Red LED, control	1.75±0.1	e	1.92±0.3	c	1.50±0.0	f	2.00±0.0	f
Red LED, MS	4.11±0.2	ab	2.53±0.3	abc	2.67±0.3	c	14.89±0.4	b
Red LED, B5	4.64±0.1	a	3.23±0.1	ab	2.79±0.0	c	13.93±0.3	bc
Red LED, MS+B5	3.83±0.5	ab	2.67±0.3	abc	2.36±0.2	cde	13.30±0.2	c
Red LED, ½ MS	3.56±0.2	bc	2.51±0.3	abc	1.84±0.1	ef	10.98±0.0	d
Red LED, ½ B5	2.70±0.2	cd	3.17±0.3	ab	1.98±0.3	def	9.34±0.3	e
Red LED, ½ MS+½ B5	4.00±0.3	ab	2.83±0.4	abc	2.55±0.2	cd	13.00±0.8	c
White LED, control	0.0±0.0	f	0.0±0.0	d	0.0±0.0	g	0.0±0.0	g
White LED, MS	3.25±0.3	bc	2.75±0.1	abc	5.87±0.1	a	16.06±0.2	a
White LED, B5	3.56±0.1	bc	2.17±0.3	bc	4.61±0.3	b	15.94±0.3	a
White LED, MS+B5	3.22±0.2	bc	3.56±0.6	a	2.78±0.1	c	11.11±0.4	d
White LED, ½ MS	1.92±0.5	de	2.33±0.6	bc	4.25±0.1	b	14.29±0.2	b
White LED, ½ B5	4.07±0.2	ab	3.00±0.1	abc	2.92±0.3	c	16.84±0.4	a
White LED, ½ MS+½ B5	2.33±0.2	de	2.50±0.1	abc	4.86±0.1	b	14.83±0.2	b

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–g) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$). *red LED ($\lambda = 650$ nm); White LED.

Shoot dry weight varied in response to the different media and LED colors (Table 5). The highest shoot dry weight was obtained in MS medium (0.38 ± 0.0 g) under both red LED and white LED, followed by B5 medium (0.37 ± 0.0 g), ½ B5 medium (0.36 ± 0.0 g), and ½ MS+½ B5 (0.35 ± 0.0 g) under the red LED.

Root fresh weight LED colors had a significant effect on root fresh weight (Table 5). The higher level of root fresh weight was observed under the white LED in ½ MS medium (1.48 ± 0.8 g), followed by ½ MS+½ B5 medium (1.08 ± 0.1 g), ½ B5 medium (1.07 ± 0.1 g), MS medium (1.05 ± 0.1 g), B5 medium (0.83 ± 0.0 g), and also those did not differ statistically from the red LED in ½ MS medium (0.76 ± 0.2 g).

Table 5. Shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 5. Farklı renkteki LED'ler altında farklı besin ortamlarında yetiştirilen *C. sativa* fidelerinde sürgün taze ağırlığı, sürgün kuru ağırlığı, kök taze ağırlığı, kök kuru ağırlığı.

Color LEDs*, media	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
Red LED, control	0.0±0.0	d	0.0±0.0	d	0.00±0.0	c	0.0±0.0	d
Red LED, MS	2.18±0.1	b	0.38±0.0	a	0.29±0.1	bc	0.04±0.0	bcd
Red LED, B5	1.69±0.2	bc	0.29±0.0	abc	0.13±0.1	c	0.02±0.0	cd
Red LED, MS+B5	1.91±0.1	bc	0.26±0.0	abc	0.08±0.0	c	0.01±0.0	d
Red LED, ½ MS	1.31±0.1	bc	0.22±0.0	bc	0.76±0.2	abc	0.08±0.0	abcd
Red LED, ½ B5	1.30±0.1	bc	0.2±0.0	c	0.25±0.1	bc	0.03±0.0	bcd
Red LED, ½ MS+½ B5	1.48±0.0	bc	0.24±0.0	abc	0.19±0.1	bc	0.02±0.0	bcd
White LED, control	0.0±0.0	d	0.0±0.0	d	0.0±0.0	c	0.0±0.0	d
White LED, MS	1.94±0.5	bc	0.38±0.1	a	1.05±0.4	ab	0.10±0.0	ab
White LED, B5	3.37±1.1	a	0.37±0.0	a	0.83±0.0	abc	0.09±0.0	abc
White LED, MS+B5	1.76±0.2	bc	0.22±0.1	bc	0.25±0.0	bc	0.03±0.0	bcd
White LED, ½ MS	0.86±0.2	cd	0.16±0.0	c	1.48±0.8	a	0.12±0.1	a
White LED, ½ B5	2.07±0.3	b	0.36±0.0	ab	1.07±0.4	ab	0.10±0.0	ab
White LED, ½ MS+½ B5	2.27±0.1	b	0.35±0.0	ab	1.08±0.1	ab	0.10±0.0	ab

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–d) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$). *red LED ($\lambda = 650$ nm); White LED.

Root dry weight LED colors had a significant effect on root dry weight. similar to root fresh weight, the greater root dry weight occurred under the white LED in $\frac{1}{2}$ MS medium (0.12 ± 0.1 g), followed by $\frac{1}{2}$ MS+ $\frac{1}{2}$ B5 medium (0.10 ± 0.0 g), $\frac{1}{2}$ B5 medium (0.10 ± 0.0 g), MS medium (0.10 ± 0.0 g), B5 medium (0.09 ± 0.0 g), and also those did not differ statistically from the red LED in $\frac{1}{2}$ MS medium (0.08 ± 0.0 g).

Hierarchical cluster analysis

A hierarchical cluster analysis (HCA) was also used to identify the possible nearest and similarity of all growth parameters analyzed of *C. sativa* in vitro culture (Figure 2). According to the dendrogram results of HCA, four major clusters were observed. These clusters contain: Cluster I: White LED, control. Cluster II: White LED, $\frac{1}{2}$ MS; Red LED, control. Cluster III: White LED, B5; White LED, $\frac{1}{2}$ MS+ $\frac{1}{2}$ B5; White LED, MS; White LED, MS+B5; White LED, $\frac{1}{2}$ B5. Cluster IV: Red LED, B5; Red LED, $\frac{1}{2}$ MS+ $\frac{1}{2}$ B5; Red LED, MS; Red LED, $\frac{1}{2}$ B5; Red LED, $\frac{1}{2}$ MS; Red LED, MS+B5.

Regarding the clustering seen in HCA, we can speculate about the responses of in vitro growth similarities of different media used in this study under LED colors. Among the treatments, the white LED inhibited the growth of *C. sativa* in a medium that did not contain any basal medium. So, the White LED control clustered distinctly from the other treatments.

White LED, $\frac{1}{2}$ MS, and Red LED, control clustered together, showed similar low impact on shoot vitality, root formation, number of shoots per plant, number of roots per plant, shoot fresh weight, and shoot dry weight in terms of analyzed parameters.

As cluster III strikingly indicates, the media under white LED, cluster IV covers the media under red LED. This shows that the white LED and red LED have significantly different effects on the in vitro growth of *C. sativa*.

For micropropagation from the nodal segment as an explant, the number of nodes on in vitro growing shoots is as important as shoot vitality. When examined from this point of view, the MS medium under the white LED was the most effective treatment. According to the HCA results, we can speculate that treatments in cluster III were higher than those in cluster IV.

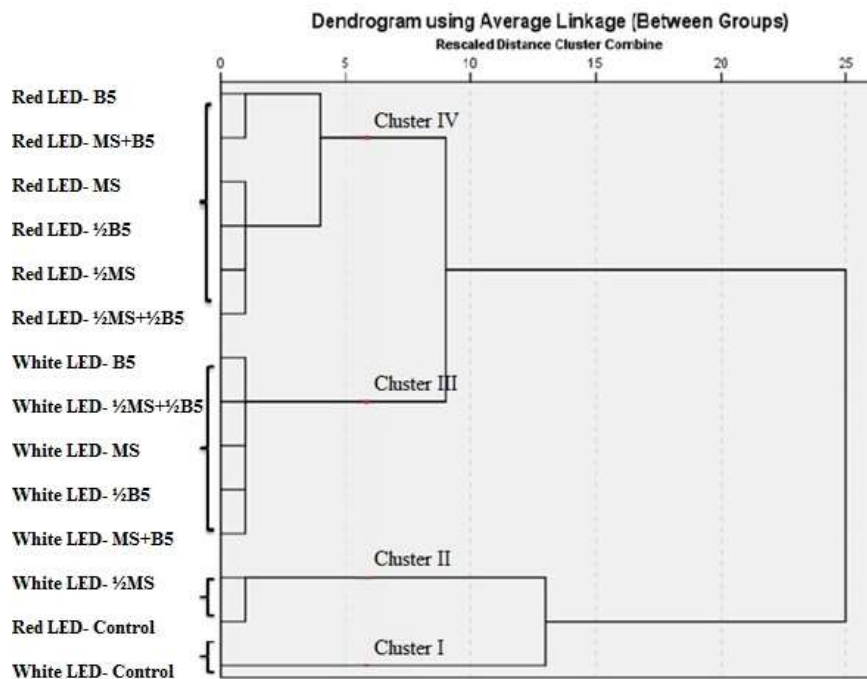


Figure 2. Dendrogram of the nutrient media under the red LED and the white LED for the twelve growth parameters of *C. sativa*.

Şekil 2. *C. sativa*'nın on iki büyüme parametresi için kırmızı LED ve beyaz LED altında besin ortamlarının dendrogramı.

DISCUSSION

In this study, the effects of light and culture medium were investigated in order to produce mass micro production from *C. sativa* under in vitro conditions. Miler et al. (2019) indicated that the success of an in vitro culture system

is related to the light source and basal medium type; it is important to identify the best spectra and suitable culture medium that provide the production of plants with desired characteristics. The most desirable growth parameters for clonal micropropagation are a great number of nodes and shoots on the plant, which is effective in the rapid reproduction of the plant.

Light source has a serious impact on the regulation of plant metabolism and morphology, as well as many other factors necessary for healthy plant growth (Davis & Burns, 2016; Yuanchun et al., 2021). Different light sources with various colors have actively been used in greenhouse and in vitro plant cultivation (Hung et al., 2016; Bantis et al., 2018). Spectral quality can have a profound effect on the growth, development, and physiology of plants. Especially, the spectra during in vitro culture are of serious importance not only for healthy morphological characteristics but also for the efficiency of adventitious root formation and shoot vitality (Iacona & Muleo, 2010). Among various light sources, red LEDs are mostly used for plant growth because wavelengths around 660 nm are highly effective for chlorophyll absorption, which in turn is effective for leaf growth, carbohydrate accumulation, and anatomical development through optimum photosynthetic efficiency (Mengxi et al., 2011; Nam et al., 2022).

There is not enough detailed information about the effects of light sources on the plant regeneration of *C. sativa* in vitro culture conditions. Recently, many studies have been carried out to determine the effect of light on *C. sativa*, especially increasing its phytochemical content and clonal propagation for field cultures and usually greenhouses by cutting from seed or from the mother plant (Hawley et al., 2018; Livadariu et al., 2019; Namdar et al., 2019; Jenkins & Livesay, 2021; Islam et al., 2021a; Islam et al., 2021b; Wei et al., 2021; Morello et al., 2022; Schilling et al., 2023). However, this study is almost the first report on the effect of (white and red) LEDs for *C. sativa* multiplication in vitro. *C. sativa* growth in vitro under different LED colors (red and white) resulted in higher shoot vitality, shoot length, number of shoots, and number of roots when the explants were subjected to red LED. Our findings indicated that red LED had a positive effect on shoot activity. Many studies reported that red color light stimulated shoot elongation in plants (Casal, 2013; dos Reis Oliveira et al., 2020). Similarly, Shulgina et al. (2021) recorded that red LED had the tallest plants and the greatest microshoots of *Stevia rebaudiana* Bertoni grown in vitro compared to all other LED treatments. Gnasekaran et al. (2021) indicated that the red LED exhibited the greatest number of roots, number of shoots, and shoot length from *Zingiber officinale* var. *rubrum* Theilade compared to the white LED.

On the other hand, early works showed that the white light compared to red light increased the chlorophyll a and b content, and accordingly, it influenced the number of leaves, number of nodes and fresh/dry weight of the plant (Matysiak & Kowalski, 2019; Gnasekaran et al., 2021; Nacheva et al., 2021). In this study, the white LED had the maximum node production per plant, which is important for clonal micropropagation. Similar to this study, Nacheva et al. (2021) reported that red light limited node formation. The high root length may have occurred as Silva-Navas et al. (2016) reported that white light, together with cytokinin, induces cell elongation by stimulating the accumulation of some bioactive agent on the light side of the root.

Under in vitro conditions, the types of nutrient medium have a serious effect due to their macro and micro elements. Previous literature reported that each nutrient medium had different effects on the vegetative growth of many plant species (Joo et al., 2019; Page et al., 2021).

In comparison to B5 vitamins, MS basal salts are widely used not only in monocots but also in dicotyledons. Phillips and Garda (2019) explained the composition of these media in detail, but the major difference between these two media includes the amount and relative proportions of the various salts, and more particularly, the amount and type of nitrogen. MS has higher total nitrogen levels in both nitrate and ammonium forms. This may contribute to good plant regeneration. However, sometimes low levels of ammonium ions can be the best medium for optimal growth of plant.

In the current study, these two different basal salts and their combinations were scanned: MS, B5, MS+B5, $\frac{1}{2}$ MS, $\frac{1}{2}$ B5, $\frac{1}{2}$ MS+ $\frac{1}{2}$ B5. Of these media, MS medium gave a high response on shoot vitality, shoot length, number of nodes per plant, and number of shoots, which are important for clonal micropropagation. Many previous studies have also mentioned that the MS medium is suitable for micropropagation of *C. sativa* (Lata et al., 2009; Wang et al., 2009; Chaohua et al., 2016; Lata et al., 2016). In a recent study, Page et al. (2021) reported that multiple commercial cultivars of *C. sativa* produced the best results in MS and DKW (Driver & Kuniyuki, 1984) media, while resulting in very poor growth in WPM (Lloyd & McCown, 1980), B5, and BABI (Greenway et al., 2012) media, and DKM medium offered more stable plant growth than MS for multiple subcultures of plants.

In addition, these findings showed that the root formation and root elongation were the best in half-strength nutrient media, but the maximum root number was obtained from full-strength nutrient media. This was supported by many scientists that half-strength media gave the best rooting response (Chaohua et al., 2016; Smýkalová et al., 2019; Wróbel et al., 2022). The results showed that $\frac{1}{2}$ B5 and $\frac{1}{2}$ MS+ $\frac{1}{2}$ B5 media can be preferable for rooting of *C. sativa*.

Plant growth response to varying light quality across white and red colors behaved in a manner very similar to that shown by many scientists (Matysiak & Kowalski, 2019; Shulgina et al., 2021; Gnasekaran et al., 2021;

Nacheva et al., 2021). In vitro conditions, apart from the vigor of plants attained by LED treatments, the micropropagation quantity is always considered necessary for any breeding studies, particularly for micro-shoot production of commercial plants. In the current study, culture media under LED treatments had obvious effects on the coefficients of shoot multiplication from *C. sativa*. The control treatment without basal medium inhibited the plant growth from apical meristem explants for *C. sativa* under white LEDs, but it stimulated a slight plant development under red LEDs.

However, as mentioned in the results section, MS medium was found to be effective in parameters related to shoot growth in general. Again, the MS medium showed a strong effect with slight differences under different LEDs. Under red LED, MS and B5 media showed the highest shoot vitality, shoot length, and shoot number of *C. sativa*, while the highest node and leaf numbers were recorded in MS medium under white LED. For shoot weight, both fresh and dry, white LED was found to be effective in both B5 and MS media.

In the parameters related to rooting, the highest values were generally detected under white LED. But the effect of nutrient media changed under different LEDs according to the investigated parameters. The highest values were obtained from ½ MS+½ B5 for root formation, ½ B5 for root length, MS+B5 for root number per plant, and ½ MS for root weight, both fresh and dry. Considering the in vitro propagation of *C. sativa*, ½ B5 was the greatest medium for rooting, which is statistically in the same group for these parameters.

CONCLUSION

This study is the first report about the effects of various media under different spectra of light-emitting diode (LED) light quality on the shoot growth and rhizogenesis of the *C. sativa* tissue culture seedlings. All treatments significantly but differentially influenced the vegetative growth of in vitro *C. sativa* plantlets. Because it is important to produce genetically identical and disease-free plants in breeding studies, shoot and node numbers per plant are equally valuable. From this study, we can draw the following conclusions: Firstly, LED spectra and basal medium type/concentration significantly affect both shoot multiplication and rooting, as well as their promotion of the formation of strong plants with well-developed leaves of *C. sativa* in vitro conditions. Secondly, red LED combined with MS medium greatly promotes the micro shoot through increasing shoot number and elongation, but white LED combined with MS medium improves healthier shoot growth by increasing both node number and leaf number. Thirdly, white LED considerably promotes the rooting capability of *C. sativa* in vitro culture. The ½ B5 and ½ MS+½ B5 media significantly stimulate root formation and elongation, as well as they provide the highest root number in addition to the MS+B5 medium. This study may give a general background on light spectral quality affecting hemp morphogenesis. More detailed studies are required to standardize the effects of LEDs on the growth and development of hemp cultivars and genotypes.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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Efficacy of Exogenous Absciscic Acid on Cholinesterase Enzyme Activity and Phenolic Compound Variability in *Hypericum perforatum* calli

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ABSTRACT

The aim of this study was to investigate the effect of abscisic acid (ABA) on cholinesterase enzyme activities and bioactive components of *in vitro* calli of *Hypericum perforatum*. Calli were obtained from Linsmaier Skoog (LS)/B5 medium containing 0.5 mg L⁻¹ thidiazuron (TDZ) and 0.5 mg L⁻¹ indole butyric acid (IBA). Different abscisic acid (0.01 mg L⁻¹, 0.05 mg L⁻¹, and 0.1 mg L⁻¹) applications were performed on the calli obtained in tissue culture and extracted in 80% methanol. Hispidulin, fumaric acid, acacetin, epicatechin, and naringenin compounds were analyzed in Liquid chromatography–high–resolution mass spectrometry (LC-HRMS). While epicatechin (1.09 mg L⁻¹) was found at the highest 0.05 mg L⁻¹ ABA in callus samples, fumaric acid (2.30 mg L⁻¹) and hispidulin (0.78 mg L⁻¹) compounds were detected in the highest containing of 0.1 mg L⁻¹ ABA. Acacetin (0.10 mg L⁻¹) was produced in only 0.01 mg L⁻¹ ABA, whereas naringenin (0.01 mg L⁻¹) was produced in medium containing 0.05 mg L⁻¹ ABA. The strongest Acetylcholinesterase (AChE) (0.207±0.012) and Butyrylcholinesterase (BChE) (0.243±0.019) inhibition activity was shown the best with 0.1 mg L⁻¹ ABA application. This study has shown that ABA can be used as an elicitor in callus culture of *H. perforatum*, and the amounts of related metabolites can be changed via elicitors.

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Keywords

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Hypericum perforatum L. Kalluslarında Ekzojen Absisik Asidin Kolinesteraz Enzim Aktivitesi ve Fenolik Bileşik Değişkenliği Üzerine Etkinliği

ÖZET

Bu çalışmanın amacı, absisik asidin (ABA) *Hypericum perforatum*'un *in vitro* kalluslarının kolinesteraz enzim aktiviteleri ve biyoaktif bileşenleri üzerine etkisini araştırmaktır. Kalluslar, 0.5 mg L⁻¹ thidiazuron (TDZ) ve 0.5 mg L⁻¹ indol bütirik asit (IBA) içeren Linsmaier Skoog (LS)/B5 besiyerinden elde edildi. Doku kültüründe elde edilen kalluslara farklı absisik asit (0,01 mg L⁻¹, 0,05 mg L⁻¹ ve 0,1 mg L⁻¹) uygulamaları yapıldı ve %80'lik metanolde ekstrakte edildi. Hispidulin, fumarik asit, akasetin, epikateşin, naringenin bileşikler Sıvı Kromatografisi-Yüksek Çözünürlüklü Kütle Spektrometrisi (LC-HRMS) ile analiz edildi. Kallus örneklerinde en yüksek epikateşin (1.09 mg L⁻¹) 0.05 mg L⁻¹ ABA'da bulunurken, fumarik asit (2.30 mg L⁻¹) ve hispidulin (0.78 mg L⁻¹) bileşikler en yüksek 0.1 mg L⁻¹ ABA içerenlerde tespit edildi. Sadece 0.01 mg L⁻¹ ABA içeren ortamda akasetin (0.10 mg L⁻¹) üretilirken, 0.05 mg L⁻¹ ABA içeren besin ortamında naringenin (0.01 mg L⁻¹) üretildi. En güçlü Asetilkolinesteraz (AChE) (0.207±0.012) ve Bütirilkolinesteraz BChE (0.243±0.019) inhibisyon aktivitesinin 0.1 mg L⁻¹ ABA uygulamasıyla olduğu ortaya çıktı. Bu çalışma, ABA'nın *H.perforatum*'un kallus kültüründe uyarıcı olarak kullanılabileceğini ve ilgili metabolitlerin miktarlarının elisitör aracılığıyla değiştirilebileceğini göstermiştir.

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Anahtar Kelimeler

Absisik Asit
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LC-HRMS
Kolinesteraz inhibisyonu

Atıf İçin : *Hypericum perforatum* kalluslarında ekzojen absisik asidin kolinesteraz enzim aktivitesi ve fenolik bileşik değişkenliği üzerine etkinliği. *KSÜ Tarım ve Doğa Derg* 28 (4), 1083-1094. DOI: 10.18016/ksutarimdoga.vi.1614168.

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INTRODUCTION

A genus of *Hypericum* comprises nearly 500 species, and *Hypericum perforatum* is the most well-known and investigated species (Al-Khayri et al., 2024; Altıntaş, 2025; Bal et al., 2022; Crockett & Robson, 2011). *H. perforatum* has been traditionally used in the treatment of inflammation, bacterial-viral infections, burns, stomach ulcers, mild depression, diarrhea, pain, fevers, wounds and burns (Kakouri et al., 2023; Sut et al., 2025). Aerial parts of *H. perforatum* have been widely studied because of contains phenolic compounds such naphthodianthrone (hypericin, pseudohypericin), phloroglucinol derivatives (hyperforin, adhyhyperforin) and flavonoids (quercetin, kaempferol, rutin, quercitrin, and hyperoside) (Barnes et al., 2001; Bombardelli & Morazzoni, 1995; Bruneton, 1999). Although naphthodianthrone and phloroglucinols are prestigious in *Hypericum*, flavonoids, xanthenes, and tannins are as important as these compounds, and also have synergistic effects (Song et al., 2023; Xiao et al., 2020; Zhang et al., 2020).

H. perforatum extracts have been reported to have anti-inflammatory, anti-angiogenic, anti-fibroblastic, antioxidant, and anti-depressant properties. In recent times, depression is associated with Alzheimer's, and it is a neurodegenerative disease caused by the deficiency of acetylcholine and butyrylcholine, which are hydrolyzed by acetylcholinesterase and butyrylcholinesterase (Altun et al., 2013). It has been reported that cholinesterase inhibition occurred with the methanol extract of *H. capitatum* var. *capitatum*, and the ethyl acetate extract of *H. perforatum* against the butyrylcholinesterase enzyme (Boga et al., 2016). Physiologically, amyloid-beta peptide and tau proteins are produced and expressed in the normal human body. Alzheimer's and Parkinson's diseases occur with the abnormal behavior of these proteins (Abyadeh et al., 2024; Suryawanshi et al., 2024). It has been reported that compounds such as hypericin, hyperforin, and kaempferol have neuroprotective properties in *H. perforatum* (Suryawanshi et al., 2024). Due to its medicinal effects, the production of *H. perforatum* is supported by tissue culture techniques.

Commercial production of bioactive compounds through plant cell, organ, and tissue culture is a very promising technology when natural resources are limited (Karuppusamy, 2009). Thousands of methods can be used to increase efficiency, such as genetic transformation, precursor feeding, media optimization, and elicitation in plant tissue culture (Murthy et al., 2014). Elicitation is stimulating bioactive compounds by adding trace amounts of elicitor to the plant *in vitro* culture systems. Elicitors, such as abscisic acid and salicylic acid, are administered externally through *in vitro* applications and enable the activation of the signal transduction cascade, gene expression, and secondary metabolism (Zhao et al., 2005).

Abscisic acid (ABA) is a growth regulator that governs many physiological steps and developmental times in plants. It makes many regulations such as seed dormancy, germination, lipid synthesis, protein storage, stomatal opening, and closing (Vaičiukynė et al., 2019). It has been reported that the production of abscisic acid in this way activates the antioxidant system. Another investigation (Ellman et al., 1961) the effect of abscisic acid on somatic embryogenesis in cactus. Accordingly, it has been revealed that low ABA concentrations stimulate the elongation of embryos, while high ABA concentrations inhibit growth. (Lema-Rumińska et al., 2013).

According to the literature, this study is the first investigation on the effect of abscisic acid on *H. perforatum* calli in *in vitro*. The aim of the present study was to determine; (i) callus formation in LS/B5 media, (ii) different ABA (0.01 mg L⁻¹, 0.05 mg L⁻¹ and 0.1 mg L⁻¹) concentration effect on phenolic compounds amounts of calli, and (iii) anticholinesterase activity of elicited callus of *H. perforatum*, (iv) phenolic compounds were analyzed by Liquid chromatography–high resolution mass spectrometry (LC-HRMS).

MATERIAL and METHOD

Plant Material and Seeds Surface Sterilization

Hypericum perforatum L. seeds were collected from Samsun-Balıca (41°27'97"01' and 36°33'60"67'), Turkey. Seeds were washed for 15 minutes under tap water, surface sterilized with 70 % ethanol for 1 min, then NaOCl 15 % for 5 min, rinsed 3 times in sterile deionized water. Seeds were cultured LS/B5 medium supplemented with 3 % sucrose and 0.7 % agar without plant growth regulators.

Callus Formation and Absciscic Acid Elicitation

Callus cultures were started with axillary buds of sterile seedlings. The media contained LS (Linsmaier and Skoog)/B5, including 3 % sucrose, 0.7 % agar. Five different TDZ (0.1, 0.25, 0.5, 0.75, 1 mg L⁻¹) and IBA (1, 0.75,

0.5, 0.25, 0.1 mg L⁻¹) concentrations were used for callus culture. Calli were obtained from 0.5 mg L⁻¹ TDZ and 0.5 mg L⁻¹ IBA. After 2 months of callus induction, the friable and green callus as equal fragments were transferred to new LS/B5 media containing abscisic acid (0.01 mg L⁻¹, 0.05 mg L⁻¹ and 0.1 mg L⁻¹) elicitor for 15 days. Absciscic acid (Sigma-Aldrich, USA) was dissolved in pure ethanol and filtered using a micro filter of 0.22 µ pore size. All treatments were incubated in the 16 h light/8 h dark photoperiod at 24 ± 2°C and photon flux of 90 µmol m⁻² s⁻¹ in the culture chamber.

Extraction of Elicited Callus

The calli, which were treated with ABA, were freeze-dried, lyophilized, and powdered. The samples obtained from *in vitro* were weighed (20 mg) and homogenized. After, it was added to these samples 2 ml of 80% methanol and vortexed. Then, the samples were kept in an ultrasonic bath for 30 min at 4°C and centrifuged at 4000 rpm for 15 minutes. The supernatant was used as an extract in the analyses (Gadzovska et al., 2007; Gadzovska et al., 2013).

Statistical analyses

All the experiments were conducted using three biological replicates, and all measurements were analyzed in triplicate. The compliance of the data with normal distribution was evaluated with the Shapiro-Wilk test. In the display of descriptive statistics of variables determined to be normally distributed, mean and standard deviation (SD) values were given; in the display of variables determined to be not normally distributed, median and interquartile range (IQR) values were given. Significant differences in the number of compounds between different plant extracts were evaluated with the ANOVA and Kruskal-Wallis test. IBM SPSS Statistics 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) and OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA) were used for statistical analyses and calculations. Statistical significance level was accepted as $p < 0.05$.

Anticholinesterase inhibition

Acetylcholinesterase from *Electrophorus electricus* (Electric eel) and butyrylcholinesterase from equine serum were obtained from Sigma-Aldrich (St. Louise, MO). The inhibitory effects of extracts against AChE and BChE were measured using a slight modification of Ellman's spectrophotometric method (Ellman et al., 1961) and using commercially available galantamine as the reference compound. Acetylthiocholine iodide (AChI), butyrylcholine iodide (BChI) used as the substrate in the reaction, 5'dithiobis - 2 - nitrobenzoic acid (DTNB) used as Ellman's reagent, were purchased from Sigma - Aldrich (St. Louis, MO).

The absorbance of the reaction mixture was measured at 412 nm three times within 5 min of the start of the reaction on a Thermo Fisher Scientific Multiskan Go, and the results are reported as mean ± standard deviation. Activity (%) was plotted to determine the inhibitory effects of extracts on AChE and BChE. IC₅₀ values were obtained by activity (%) versus compound plots.

LC-HRMS device and chromatographic conditions

The analyzes of the extracts were made with LC-HRMS. Epicatechin, acacetin, fumaric acid, hispidulin, naringenin, naringin, chicoric acid, hyperoside, rhamnocitrine, chlorogenic acid, chrysin, isosacuranetin, apigenin 7-glucoside compounds were determined. Hypericin, pseudohypericin and hyperforin compounds were introduced to the device.

LC-HRMS measurements were carried out on a Thermo ORBITRAP Q-EXACTIVE (Bremen, Germany) mass spectrometry-equipped ESI ion source and with Dionex LC system. Scan range was set to m/z⁻¹ 85-1500 amu, and other mass parameters are used as following: gas flow rate: 45, aux gas flow rate: 10, spray voltage: 3.80 kV, capillary temperature: 320 °C, aux gas heater temperature: 320 °C, and Slens RF is 50. A Fortis C18 column (150 x 3 mm i.d., 3 µm particle size, Istanbul, Turkey) was used for the separation of compounds. The mobile phases A and B were composed of 1% formic acid-water and 1% formic acid-methanol, respectively. The gradient program was 0-1.00 min 50% A and 50% B, 1.01-6.00 min 100% B, and finally 6.01-10 min 50% A and 50% B. The flow rate of the mobile phase was 0.35 mL min⁻¹, and the column temperature was set to 22°C. Environmental conditions were set as temperature 22.0 ± 5.0 °C and relative humidity (50 ± 15) % rh.

Identification of compounds was performed by comparison of the retention time of standard compounds (in the range of purity 95-99%, see section chemicals) and HRMS data of Bezmialem Vakıf University, Drug Application and Research Center Library-ILMER. Dihydrocapsaicin (purity 95%) was used as an internal standard for LC-HRMS measurements in order to reduce the repeatability problem caused by external effects, such as ionization repeatability, in mass spectrometry measurements (Inal et al., 2022).

RESULTS and DISCUSSION

Abscisic acid Induced Phenolic Compounds of Calli

In this study, elicited calli with ABA of *H. perforatum* were used and these calli were extracted with 80% methanol. The phytochemical profile of the extract was determined by LCHR-MS and anticholinesterase inhibition assay was investigated. Epicatechin, acacetin, fumaric acid, hispidulin, naringenin, naringin, chicoric, hyperoside, rhamnocitrin, chlorogenic acid, chrysin, isosakuranetin, and apigenin 7-glucoside compounds were determined with the LCHR-MS (Figure 1,2,3). Hypericin, pseudohypericin and hyperforin compounds were introduced to the device, but these compounds could not be detected in callus.

Initially, as shown in Table 1, epicatechin (1.09 mg L^{-1}) and naringenin (0.01 mg L^{-1}) amounts were found to be the highest at 0.05 mg L^{-1} ABA in callus of *H. perforatum*. As well as naringenin was just found at 0.05 mg L^{-1} ABA. Likewise, acacetin (0.10 mg L^{-1}) was produced only in the medium containing 0.01 mg L^{-1} ABA. On the other side, fumaric acid was produced at 0.1 mg L^{-1} ABA (2.30 mg L^{-1}) and 0.05 mg L^{-1} ABA (1.09 mg L^{-1}). Hispidulin compound had close values in 0.1 mg L^{-1} ABA and 0.05 mg L^{-1} ABA applications. Finally, naringin, chicoric, hyperoside, rhamnocitrin, chlorogenic acid, chrysin, isosakuranetin, and apigenin-7-Glucoside compounds did not detect in elicited calli (Table 1).

Table 1. Amounts of phytochemical compounds analysed by LC-HRMS in calli of *H. perforatum* L.

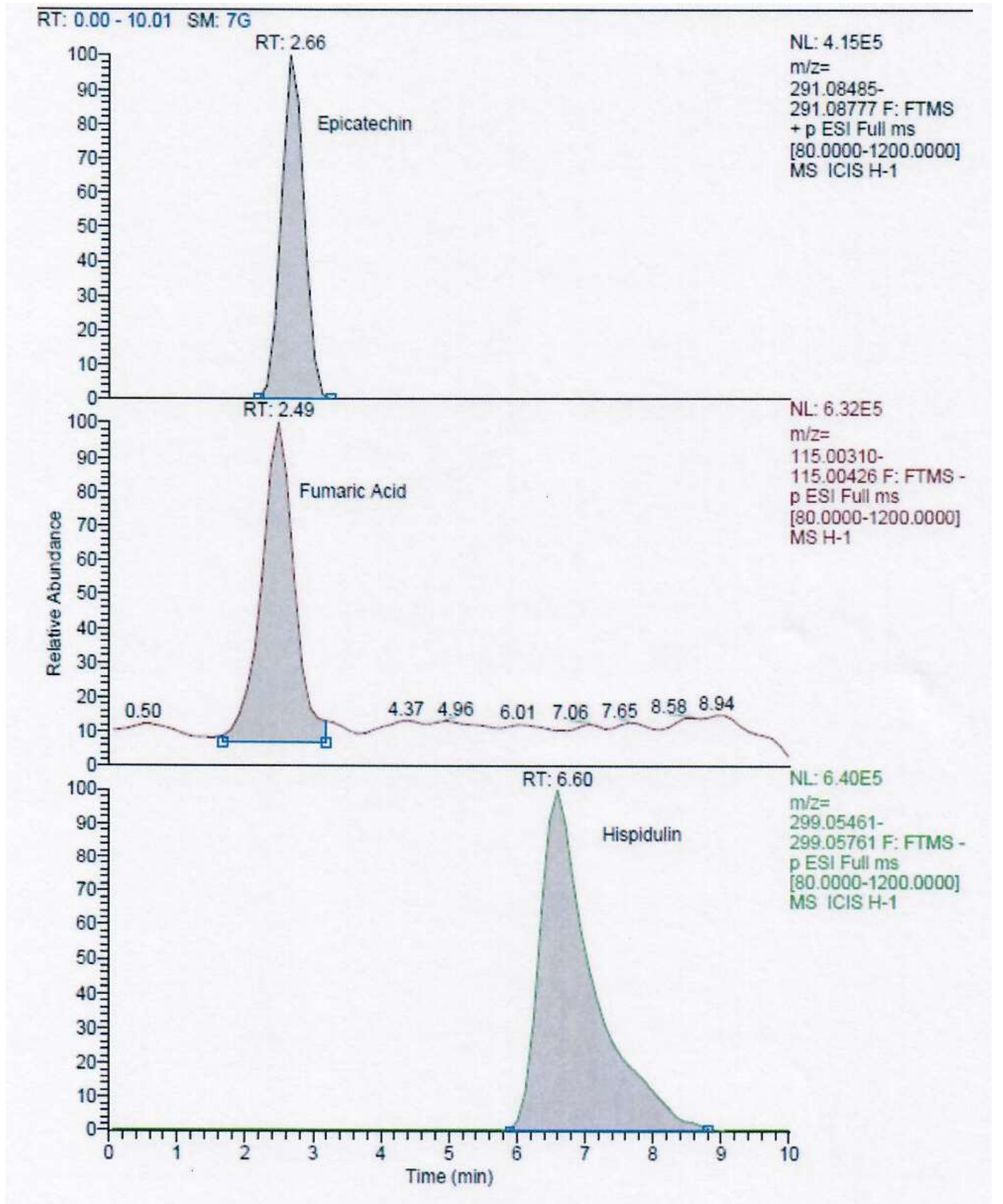
Çizelge 1. H. perforatum L. kalluslarında LC-HRMS ile analiz edilen fitokimyasal bileşik miktarları

No	Compounds	R.T.	Quantification ($\text{mg L}^{-1} \pm \text{SD}$)			Mother	Ion mode	LOD/LOQ
			0.1 mg L^{-1}	0.01 mg L^{-1}	0.05 mg L^{-1}			
			ABA	ABA	ABA	Ion (m/z) ¹⁾		(mg L^{-1})
1	Fumaric acid	2.49	2.30 ± 3.15	1.45 ± 3.15	1.09 ± 3.15	115.0037	Negative	0.26/0.88
2	Epicatechin	2.66	0.46 ± 3.62	*	1.09 ± 3.62	291.0863	Negative	0.23/0.76
3	Naringenin	5.89	*	*	0.01 ± 4.15	271.0612	Negative	0.2/0.67
4	Hispidulin	6.60	0.78 ± 1.73	0.57 ± 1.73	0.76 ± 1.73	299.0561	Negative	0.14/0.46
5	Acacetin	7.88	*	0.10 ± 1.5	*	283.0612	Negative	0.13/0.42

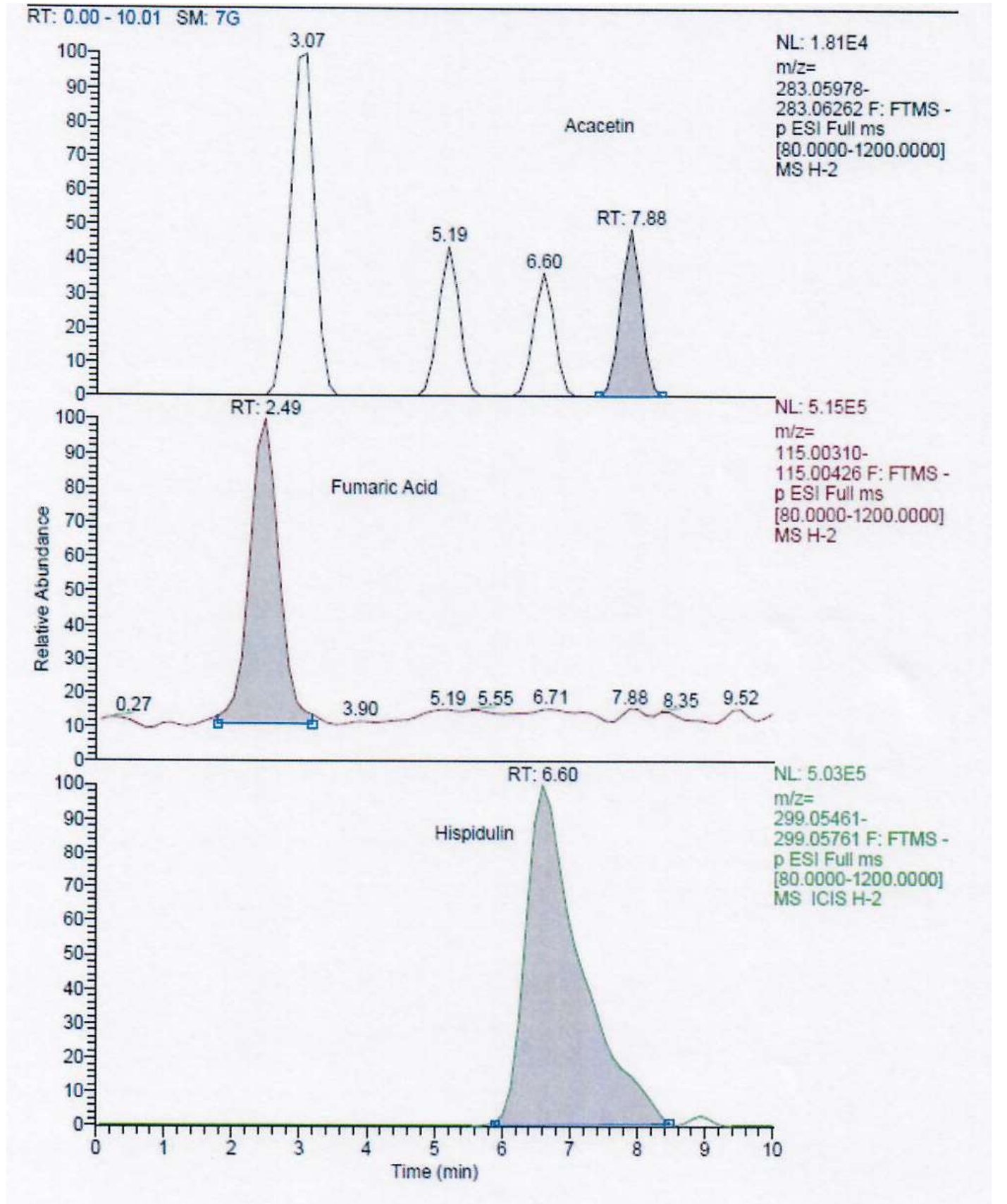
*Not Detected, SD: Standard deviation, R.T: Retention time, LOD: Limit of detection, LOQ: Limit of quantification

According to the LCHR-MS results of jasmonic acid (JA) application of *H. perforatum in vitro* samples, chlorogenic acid was at the highest value in the *in vitro* plantlet control group, while it was not produced in calli (Önlü, 2023). Accordingly, it is observed that there is no production of chlorogenic acid in *H. perforatum* calli in both JA and in our ABA applications. While epicatechin could not be detected in 0.01 mg L^{-1} ABA, acacetin, fumaric acid and hispidulin were produced. Similarly, there was no epicatechin production in 0.01 mg L^{-1} JA calli, and acacetin, fumaric acid, and hispidulin compounds were determined by Önlü (2023). As a result, it is observed that the most effective rate for epicatechin production is 0.05 mg L^{-1} ABA, and no production occurred at lower ABA rates. Epicatechin is a derivative of catechin, and studies have reported that it does not exhibit hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity effects (Pathania & Singh, 2021). Moreover, it has been reported that epicatechin inhibited the replication of Mayaro and hepatitis C diseases (Ferreira et al., 2018; Lin et al., 2013).

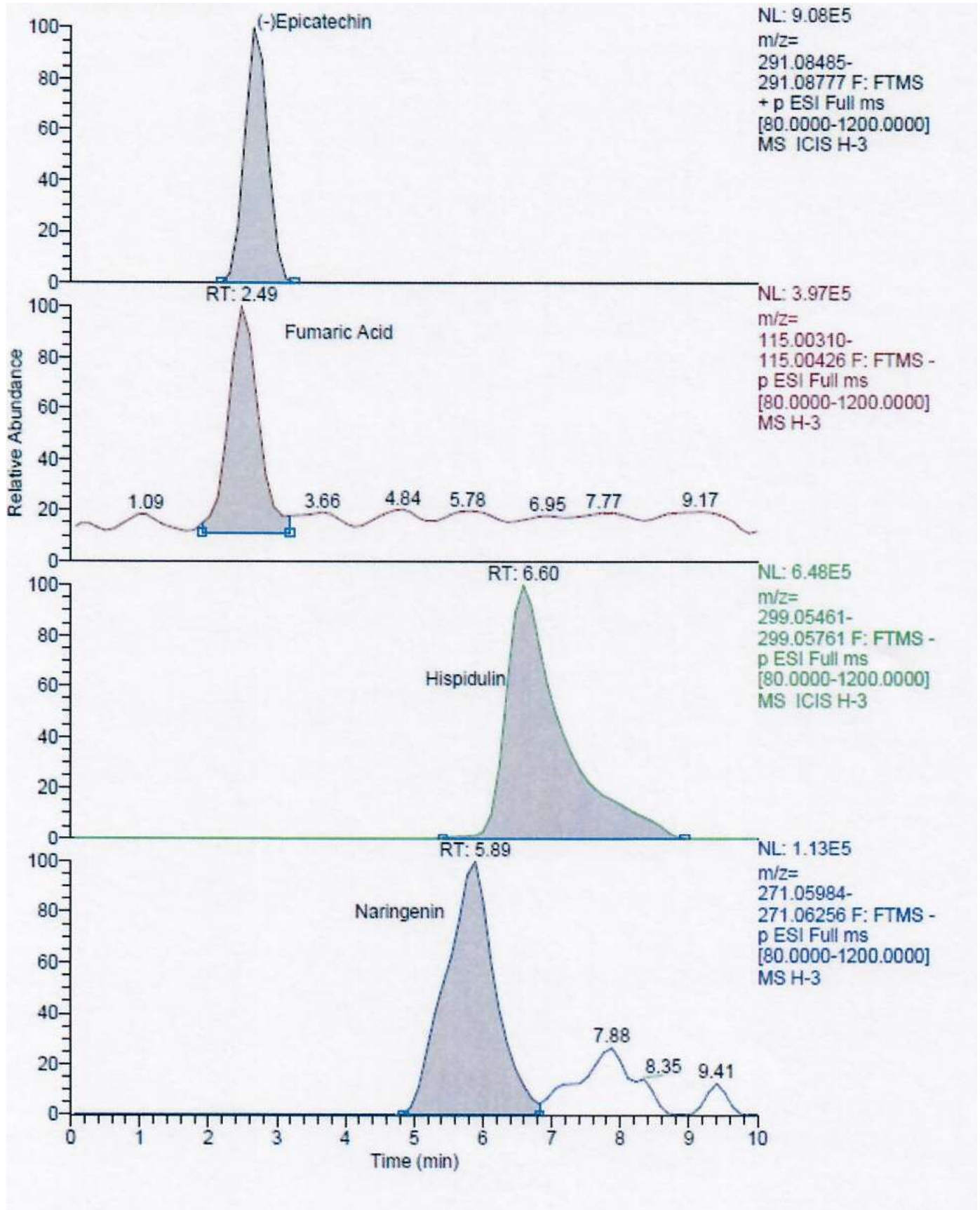
On the other hand, acacetin was produced at only 0.01 mg L^{-1} ABA. Acacetin, which has various therapeutic effects such as anticancer, antidiabetic, antipyretic, antiperoxidative, anti-inflammatory, antiplasmodial, and antiproliferative activities, stops the cell cycle by inducing apoptosis. As well as, the root part of *Scrophularia kakudensis* was detected in *in vitro* samples ($19.52 \mu\text{g} \cdot \text{g}^{-1}$ FW) more than *ex vitro* ($18.23 \mu\text{g} \cdot \text{g}^{-1}$ FW) (Manivannan et al., 2015). Similar to acacetin, naringenin was determined at just 0.05 mg L^{-1} ABA. It has been reported that the naringenin compound has various effects such as antidiabetic, anticancer, antimicrobial, antiobesity, gastroprotective, immunomodulatory, cardioprotective, nephroprotective, and neuroprotective (Uçar & Göktaş, 2023). Low amounts of ABA elicitation appear to be more effective for hispidulin production. Furthermore, it has been reported that hispidulin, mostly found in *Artemisia* and *Salvia* species and has antioxidative, antifungal, anti-inflammatory, and antimutagenic effects (Chulasiri et al., 1992; Gil et al., 1994; Kavvadias et al., 2004).



Şekil 1. 0.1 mg L⁻¹ ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 1. Chromatogram of compounds obtained by LCHR-MS analysis in 0.1 mg L⁻¹ ABA-treated calli



Şekil 2. 0.01 mg L⁻¹ ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 2. Chromatogram of compounds obtained by LCHR-MS analysis in 0.01 mg L⁻¹ ABA-treated calli



Şekil 3. 0.05 mg L⁻¹ ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 3. Chromatogram of compounds obtained by LCHR-MS analysis in 0.05 mg L⁻¹ ABA-treated calli

Table 2. Statistical comparison of total phenolic compounds obtained from callus extracts by ABA elicitation

Çizelge 2. ABA elisitasyonu ile kallus ekstratlarından elde edilen toplam fenolik bileşiklerin istatistiksel karşılaştırılması

	Amount of Compound (mg L ⁻¹)		
	Average±SD	F	p
0.1 mg L ⁻¹ ABA	0.71±0.95	0.192	0.828
0.01 mg L ⁻¹ ABA	0.42±0.62		
0.05 mg L ⁻¹ ABA	0.59±0.55		

SD: Standard Deviation, a,b,c Values within a row with different superscripts differ significantly p>05

Different letters show significant p>.05 from ANOVA and KW.

Statistical results and comparisons of total phenolic content of *H. perforatum* calli are given at Figure 4 and Table 2 and all groups of ABA ($p > 0.05$) were not found to be significant. The average of the compounds obtained from 0.1 mg L⁻¹ ABA was 0.71±0.95; 0.01 mg L⁻¹ ABA was 0.42±0.62; and 0.05 mg L⁻¹ ABA was 0.59±0.55 ($p > 0.05$), respectively. The highest total compound rate was determined in calli containing 0.1 mg L⁻¹ ABA. In the literature, no study has been found regarding the effect of ABA on *H. perforatum* calli. When other elicitation studies were examined in *H. perforatum* that investigated the effect of 50, 100, and 250 µM jasmonic acid in cell suspension for 21 days and established that 50 µM JA was more effective in biomass production (Gadzovska et al., 2007). Furthermore, it was reported that total phenolic production in the presence of 100 µM salicylic acid (SA) was 4 times higher than the control calli (Gadzovska et al., 2013). In another study, *in vitro* plantlets of *H. heterophyllum* produced chlorogenic acid and kaempferol compounds only in medium containing 0.01 mg L⁻¹ ABA. In addition, hyperoside, quercetin, and catechin compounds were determined at the highest level in 0.01 mg L⁻¹ ABA medium. Furthermore, low ABA (0.01 mg L⁻¹) and SA (0.01 mg L⁻¹) induced higher catechin contents in *in vitro* plantlets and callus cultures, respectively (Önlü et al., 2025).

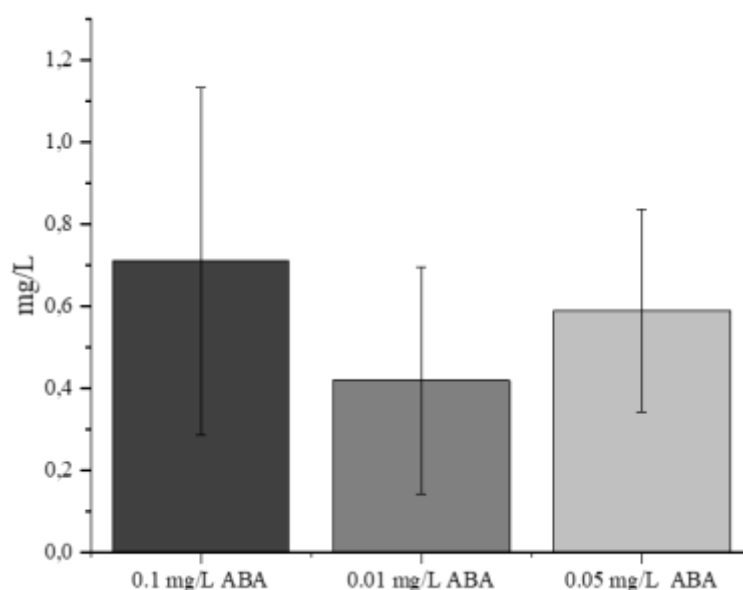


Figure 4. Comparison of total phenolic compounds from *H. perforatum* L. calli. Respectively; 0.1 mg L⁻¹ ABA, 0.01 mg L⁻¹ ABA, 0.05 mg L⁻¹ ABA. Each bar represents the percentage ± standard deviation (SD) for indicated total compounds.

Şekil 4. *H. perforatum* L. kalluslarının toplam fenolik bileşiklerin karşılaştırılması. Sırasıyla; 0.1 mg L⁻¹ ABA, 0.01 mg L⁻¹ ABA, 0.05 mg L⁻¹ ABA. Her çubuk belirtilen toplam bileşikler için yüzde ± standart sapmayı (SD) temsil eder.

However, there are few reports on the production of bioactive components in *H. perforatum* by direct callus culture that such as; production of pseudohypericin using MS/B5 and 4.0-5.0 mg L⁻¹ BA (Gadzovska et al., 2005); pseudohypericin, flavanols and flavonols with 50 µM salicylic acid (SA) (Gadzovska et al., 2013); and hypericin with perlite nanoparticles (25 or 100 L⁻¹) (Ebadollahi et al., 2019; Shasmita et al., 2023). On the other side, several studies have increased the amounts of hypericin, hyperforin, quercetin, rutin, and other phenolic compounds by adding different growth regulators, acetic acid, methyl jasmonate to MS medium through callus-mediated organogenesis (Gjureci et al., 2025; Shasmita et al., 2023).

In a recent work, phenylpropanoid and naphthodianthrone production of *H. perforatum* transgenic shoot clones regenerated from related hairy root lines; it was determined that transgenic shoots produced 2.4 times hypericin and 3 times more pseudohypericin than non-transgenic shoots (Tusevski et al., 2024). Moreover, in extracts of *H.*

perforatum treated separately with 100 µM SA and 100 µM JA in hairy root culture, SA promoted the production of quercetin-6-C-glucoside and epicatechin (Gjureci et al., 2025).

Anticholinesterase activity of Calli's total compounds

As shown in Table 3, the best anticholinesterase activity was exhibited against acetylcholinesterase 0.207±0.012 and butyrylcholinesterase 0.243±0.019 with 0.1 mg L⁻¹ ABA in enzyme studies performed on extracts. According to (Altun et al., 2013); due to the high phenolic compounds contained in the methanol extract of the aerial part of *H. perforatum*, its antioxidant and anticholinesterase activities were highest. On the other hand, *H. neurocalycinum* (85.78±4.11) and *H. malatyanum* (62.24±1.81) exhibited stronger AChE inhibitory activity (Ozkan et al., 2018). In a recent hairy root study, compared to non-transformed shoots, transformed shoots were reported to have a superior capacity to inhibit AchE (Tusevski et al., 2024). Furthermore, *H. heterophyllum in vivo* leaves were extracted with methanol, ethanol, acetone, and chloroform solvents. The IC₅₀ ratio of AChE enzyme showed the highest value in methanol extracts (Yaman et al., 2024). Similarly, in this study, methanol was used as an extraction solvent. Consequently, in this research, epicatechin, fumaric acid, and hispidulin may be effective in AChE and BchE enzyme inhibition at 0.1 mg L⁻¹ ABA. There is no statistically significant difference between the means of the extracts.

Table 3. *Acetylcholinesterase and butyrylcholinesterase activities of H. perforatum calli elicited by ABA*
Çizelge 3. *ABA tarafından elisite edilen H. perforatum kalluslarının asetilkolinesteraz ve bütirilkolinesteraz aktiviteleri*

Extracts	AChE (IC ₅₀ value)	BChE (IC ₅₀ value)
0.1 mg L ⁻¹ ABA	0.207±0,012	0.243±0,019
0.01 mg L ⁻¹ ABA	0.212±0,012	0.256±0,021
0.05 mg L ⁻¹ ABA	0.234±0,017	0.289±0,022
*Galantamine	0.072±0,009	0.196±0,010

Values were the means of three replicates ±SD. *Galantamine was used as reference compound. AChE: Acetylcholinesterase, BchE: butyrylcholinesterase, IC₅₀: Half maximal inhibitory concentration.

CONCLUSION

Chemical composition results showed that ABA applications can change phenolic compounds. In particular, 0.1 mg L⁻¹ ABA resulted in enriched high levels of fumaric acid and hispidulin. For acacetin production, only 0.01 mg L⁻¹ ABA dose should be used. Besides, epicatechin and naringenin contents have the potential to increase by 0.05 mg L⁻¹ ABA. The results showed that 0.1 mg L⁻¹ ABA exhibited AChE and BChE activity after galantamine. Consequently, the highest amount of phenolic compound production was induced in the medium containing 0.1 mg L⁻¹ ABA. It has been shown that if the abscisic acid elicitor is 0.1 mg L⁻¹ or around in calli, the total phenolic amount, especially fumaric acid and hispidulin, can be increased. Further studies, with different elicitor treatments, can be applied to calli to increase cholinesterase activity. This research may be a guide for *in vitro* elicitation studies to be carried out in different species of *Hypericum*.

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Conflict of Interest

The authors declare that they have contributed equally to the article.

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Antioxidant Properties of Methanolic Extract of *Sigesbeckia orientalis* L. from Rize

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ABSTRACT

Sigesbeckia orientalis Linnaeus is a traditional herbal medicine that has been used for many years to treat inflammatory diseases. The genus *Sigesbeckia* comprises three species: *Sigesbeckia pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG), and *Sigesbeckia orientalis* L. (SO). *S. orientalis* has been used in Eastern medicine to alleviate rheumatic disorders, improve joint mobility, and provide protection against toxicity. This plant is naturally found in countries such as China, the Philippines, and Vietnam. In Türkiye, particularly in the Black Sea region, *S. orientalis* is known as Sarıteçan. In this study, the antioxidant capacity of the methanolic extract of *S. orientalis* was evaluated by determining the total phenolic content (TPMC) and total flavonoid content (TFC). The antioxidant activity was assessed using Ferric Reducing Antioxidant Power (FRAP), Cu (II) Ion Reducing Antioxidant Capacity (CUPRAC), and 2,2-Diphenyl-1-picrylhydrazyl Free Radical Scavenging Capacity (DPPH) assays. The total phenolic content (TPMC) of *S. orientalis* extract was determined to be 18 ± 0.57 mgGAE/g dry weight, and the total flavonoid content (TFC) was 8.55 ± 0.21 mg QE/g dry weight. The DPPH (SC₅₀) was found to be 380 ± 12 µg/mL, while the CUPRAC and FRAP activities were 74 ± 1.99 and 19 ± 1.38 mg TE/g dry weight, respectively. These results demonstrate that *S. orientalis* grown in the Rize İkizdere region possesses strong antioxidant properties. Therefore, *S. orientalis* may have significant potential not only in traditional medicine but also in modern complementary medicine.

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ÖZET

Sigesbeckia orientalis Linnaeus, uzun yıllardır enflamatuvar hastalıkların tedavisinde kullanılan geleneksel bir bitkisel ilaçtır. *Sigesbeckia* cinsine ait üç türü bulunmaktadır: *Sigesbeckia pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG) ve *Sigesbeckia orientalis* L. (SO). *S. orientalis* doğu tıbbında romatizmal rahatsızlıkları hafifletmek, eklemlerin hareketliliğini iyileştirmek ve toksisiteye karşı koruma sağlamak için kullanılmıştır. Bu bitki Çin, Filipinler ve Vietnam gibi Asya ülkelerinde doğal olarak yetişmektedir. Türkiye’de özellikle Karadeniz Bölgesinde yetişen *S. orientalis* Sarıteçan olarak adlandırılmaktadır. Bu çalışmada, *S. orientalis*’in metanollü ekstresinin antioksidan kapasitesi toplam fenolik madde miktarı (TPMA) ve toplam flavonoid miktarı (TFA) belirlenerek, antioksidan aktivitesi Ferrik İndirgeyici Antioksidan Güç (FRAP), Cu (II) İyonu İndirgeyici Antioksidan Kapasite (CUPRAC) ve 2,2-difenil-1-pikrilhidrazil Serbest Radikalleri Temizleme Kapasitesi (DPPH) analizleri yapılarak değerlendirildi. *S. orientalis* ekstresinin toplam fenolik madde miktarı (TPMA) 18 ± 0.57 mgGAE/g kuru ağırlık olarak belirlenirken toplam flavonoid miktarı (TFA) ise 8.55 ± 0.21 mg QE/g kuru ağırlık olarak belirlendi. DPPH (SC₅₀) 380 ± 12 µg/mL bulunurken CUPRAC ve FRAP aktiviteleri

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Anahtar Kelimeler

Sigesbeckia orientalis

Antioksidan kapasite

Antioksidan aktivite

sırasıyla 74 ± 1.99 ve 19 ± 1.38 mg TE/g kuru ağırlık bulundu. Bu sonuçlar, Rize İkizdere bölgesinde yetişen *S. orientalis* bitkisinin güçlü antioksidan özelliklere sahip olduğunu ortaya koymaktadır. Bu nedenle, *S. orientalis* bitkisi, geleneksel tıbbın ötesinde modern tamamlayıcı tıpta da araştırılmaya değer bir potansiyele sahip olabilir.

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INTRODUCTION

Plants have been essential to human survival since antiquity, fulfilling various roles including nourishment, shelter, protection, warmth, and medicinal uses (Tohma et al., 2016; Yılmaz et al., 2023; Karageçili et al., 2023). Throughout history, people have relied on plants for nutrition and for finding remedies for various health problems (Kendir & Güvenç, 2010). The prevalence of synthetic pharmaceuticals, characterized by a singular active ingredient and frequent adverse effects, has prompted a transition towards natural medicines in contemporary healthcare. The utilization of plants, referred to by different terms like alternative medicine and phytotherapy, in healthcare services has recently gained prominence (Dave, 2019). The genus *Sigesbeckia* comprises three species: *Sigesbeckia pubescens* Markino (SP), *Sigesbeckia glabrescens* Makino (SG), and *Sigesbeckia orientalis* Linnaeus (SO). These species are part of the Asteraceae family of plants. The aerial components of these species are utilized in the treatment of various ailments, particularly inflammatory conditions, referred to as Xi-Xian in traditional Chinese medicine. *Sigesbeckia* is a genus of annual plants indigenous to tropical, subtropical, and temperate climates globally (Chen et al., 2022; Tao et al., 2021). *S. glabrescens* is native to countries in East Asia, including China, Japan, and Korea. *S. pubescens* is found in parts of Korea, China, and around Kocaeli, Türkiye. *S. orientalis* is primarily found in the eastern Black Sea region of Türkiye, including Rize, Artvin, and Trabzon. This herbaceous plant, referred to by locals as the Sariteçan, blooms naturally between April and July, with peak flowering occurring during this period. (Ejder, 2020).

Plants can synthesize a wide variety of organic chemicals, with secondary metabolites including polyphenols, volatile compounds, and alkaloids being crucial for their adaptability and defense mechanisms (Güven et al., 2024; Celik et al., 2024; Bayrak et al., 2023). *Sigesbeckia* is acknowledged for its variety of bioactive substances that exhibit health-promoting qualities, including phenolics (flavonoids), carotenoids, capsaicinoids, fatty acids, vitamin C, and vitamin E. There is an increasing emphasis on investigating the functions and applications of many physiological bioactive chemicals, particularly kireanol, recognized as one of the most important bioactive ingredients (Ren et al., 2020; Sukanya et al., 2022). Plants possess antioxidant, antibacterial, anti-inflammatory, and antiviral capabilities owing to the bioactive compounds they contain. Phenolic acids and flavonoids are the predominant categories of polyphenols (Zagoskina et al, 2023). Phenolics and flavonoids, present in nearly all plants, including fruits and vegetables, function as natural antioxidants (Apak et al., 2007). Free radicals exhibit high reactivity and instability owing to the presence of unpaired electrons within their structure. These radicals tend to react with proteins, lipids, nucleotides, DNA, and coenzymes, causing harmful effects. These include diseases of the cardiovascular system, cancer, nervous system disorders like diabetes, Parkinson's, and Alzheimer's, and rheumatoid arthritis. The antioxidant systems within the body act to prevent the damage caused to cells by free radicals, which can lead to the development of various diseases (Topal & Gulcin, 2022; Karageçili et al., 2023; Gulcin, 2020). Antioxidant substances react with free radicals, playing a crucial role in preventing the damage caused by these radicals (Alpkent & Demir, 2006; Öğretmen, 2022). When the antioxidant defense system is inadequate, it is necessary to consume natural antioxidants externally to reduce the effects of free radicals. Plants possess natural antioxidant properties attributed to flavonoids and phenolic acids, which are secondary metabolites produced as part of their metabolism (Bayrak et al., 2023).

S. orientalis grows spontaneously in many areas, from temperate to tropical regions, and grows best where the temperature is in the range of 10-27°C during the growing season. *S. orientalis* can bloom year-round in warm climates, but flowering is at its highest during the rainy season. Propagation of flowers can occur under moist conditions. Phytochemical analysis of *S. orientalis* leaves indicates that they contain high amounts of saponins, alkaloids, flavonoids, oxalates, tannins, phytates, and phenolics. Additionally, the leaves of *S. orientalis* contain significant amounts of vitamins A and C, which act as antioxidants, reducing the harmful effects of free radicals

by donating electrons to various anti-oxidative enzymatic and non-enzymatic reactions (Sukanya et al., 2022). It is recognized that the active metabolite content of *S. orientalis* varies depending on several factors such as altitudinal differences, different parts of the plant, environmental conditions, growing conditions, plant development stages, genotypes, etc. (Pradhan et al.2018).

S. orientalis is reported to possess pharmacological properties with anti-inflammatory, antiallergic, antithrombotic, anti-atherosclerotic, microcirculation-enhancing, wound-healing, and anticancer effects. Research on the plant is primarily focused on cancer and inflammation. While the phenolic content of the plant has been analyzed, there is limited research on its antioxidant content and capacity (Tao et al.,2021).

These findings may enhance our comprehension of the health benefits linked to *S. orientalis*, offering significant insights for the pharmaceutical and food industries. These discoveries could facilitate the development of functional foods, dietary supplements, or medications. This study aims to examine the antioxidant content and activity of the methanolic extract of the *S. orientalis* (Sariteçan) collected from the İkizdere area of Rize.

MATERIALS and METHODS

Preparation of Extracts:

The plant was collected on April 15, 2017, from a location in the İkizdere district of Rize, specifically in the area between Ovit Mountain and its surrounding region, at an altitude of approximately 1800-2000 meters (Figure 1). The plant's species identification was carried out by Prof. Dr. Serdar Makbul, a faculty member from the Biology Department, Faculty of Arts and Sciences, Recep Tayyip Erdogan University.

The aerial components of *S. orientalis* samples were detached, weighed (15-20 g), and pulverized using a mortar and pestle. The plant material was subsequently treated with 100 mL of methanol (MeOH) and maintained in a magnetic stirrer for 48 hours. Due to its elevated polarity, methanol facilitates the effective dissolution of polar phytochemicals, including phenolic and flavonoid molecules. Therefore, methanol was favored as a solvent during the extraction process. The extracts were filtered using filter paper, and the solvents were evaporated in an evaporator at 40°C. The extracted samples were prepared in DMSO and preserved in the freezer for experimental usage (Ejder, 2020).

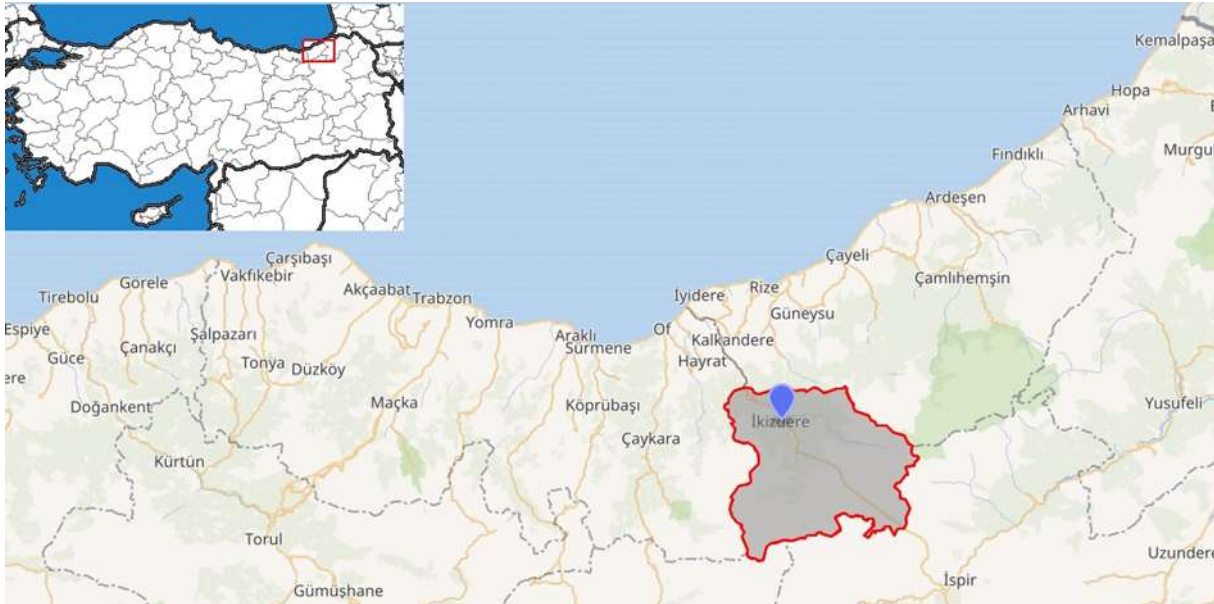


Figure 1. The location of the İkizdere district of Rize province on the map of Türkiye.

Resim 1. Rize ili İkizdere ilçesinin Türkiye haritası üzerindeki konumu.

Total phenolic matter analysis (TPMA):

The Folin-Ciocalteu assay (Folin & Ciocalteu, 1927) was employed to assess the total phenolic content (TPC) of the samples. This method involved the formation of colored complexes with Folin reagents in an alkaline environment, leading to the development of a characteristic purple-violet complex. Gallic acid was employed as the reference standard. The results are expressed as mg GAE/g dry.

Total flavonoid (TFA) analysis:

The total flavonoid content in the extracts was determined spectrophotometrically utilizing the aluminum

chloride/potassium acetate method (Christ & Müller, 1960). Quercetin (QE) served as the reference standard. The results are reported as mg QE/g dry sample.

Methods for Determination of Antioxidant Activity Based on Electron Transfer

Copper ion-reducing antioxidant capacity (CUPRAC) activity:

The cupric reducing antioxidant capacity (CUPRAC) method works by creating a Cu(I)-neocuproine chelate, which can only happen when an antioxidant substance is present. This chelate is produced from the Cu(II)-neocuproin complex generated by Cu (II) of 2,9-dimethyl-1,10-phenanthroline, displaying a peak absorbance at 450 nm (Apak et al., 2008). Trolox served as the reference standard. The results were expressed as grams of Trolox equivalent per gram of dry extract (TECA/g dry sample).

Ferric Reducing Antioxidant Power (FRAP) activity:

The samples' total antioxidant capacity was assessed using the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996). This approach entails the reduction of the Fe (III)-TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) molecule to its blue Fe (II)-TPTZ form in the presence of antioxidants, with absorbance measured at 595 nm. A standard curve was established utilizing different concentrations of Trolox, spanning from 1000 to 31.25 µmol/mL. The results are presented as mg TEFA/g dry extract.

Free Radical Scavenging (DPPH) Activity:

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) employed in this work was obtained from a commercial supplier. The antioxidant activity of the samples was evaluated using the modified Coumout method (Blois, 1958). The result was denoted as SC₅₀, with lower SC₅₀ values indicating greater radical scavenging activity.

RESULT and DISCUSSION

In this study, TPMA and TFA levels were determined in *S. orientalis* plant. In addition, antioxidant activity was investigated by CUPRAC, FRAP, and DPPH measurements in methanolic extracts.

The TPMA results were investigated, and it was found that the *S. orientalis* plant extract contained 18 ± 0.57 mg GAE/g dry weight. The TFA content was found to be 8.55 ± 0.21 mg QE/g dry weight. Researchers used CUPRAC and FRAP tests to find out how antioxidant-rich the extract was. The CUPRAC activity was 74 ± 1.99 mg TE/g dry weight, and the FRAP activity was 19 ± 1.38 mg TE/g dry weight. The DPPH activity (SC₅₀) was found to be 380 ± 12 µg/mL. The antioxidant capacity and antioxidant activity results of *S. orientalis* plant extract have been presented in Table 1.

Table 1. Antioxidant capacity and activity results of *S. orientalis* plant extract.

Tablo1. *S. orientalis* bitki ekstresinin antioksidan kapasite ve aktivite sonuçları.

<i>S. orientalis</i> Antioxidant Capacity and Activity	
TPMA (mg GAE/g dw)	18 ± 0.57
TFA (mg QE/g dw)	8.55 ± 0.21
CUPRAC (mg TE/g dw)	74 ± 1.99
FRAP (mg TE/g dw)	19 ± 1.38
DPPH (SC ₅₀ , µg/mL)	380 ± 12

S. orientalis is an herbal medicine that has been traditionally used to treat rheumatism in China for many years, since at least the Tang dynasty (Linghu et al., 2020). It has been reported that extracts collected from the whole plant *S. orientalis* have anti-inflammatory and antirheumatic properties and strong antioxidative activity (Pradhan et al., 2018; Chen et al., 2022). A phytochemical study of *S. orientalis* revealed hundreds of compounds, which have been classified into three main groups: sesquiterpenes, diterpenes, and phenolics (Tao et al., 2021). The HPLC analysis of extracts of *S. orientalis* plant revealed the presence of rutin, quercitrin, chlorogenic acid, caffeic acid, 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid. The plant contains a multitude of phenolic compounds, including kaempferol-3-O-rhamnoside and kaempferol-3-O-rutinoside. (Nguyen et al., 2017; Tran et al., 2023). Research has demonstrated that the antioxidant activity of plant extracts was closely correlated with the concentration of phenolic compounds present. High levels of phenolics have been shown to exhibit strong antioxidant effects by neutralizing free radicals (Teixeira et al., 2017; Asif, 2015).

Recent studies have further expanded our understanding of *S. orientalis*' bioactive properties. A study conducted

by Zhou et al. (2024) demonstrated that diterpenoids isolated from *S. orientalis* exhibited significant antimicrobial activity, particularly against antibiotic-resistant bacteria. This finding highlights the potential of *S. orientalis* not only for its traditional use but also for its relevance in addressing modern health challenges, particularly in combating drug-resistant infections.

A recent study on *S. pubescens* highlighted the isolation of novel diterpenoids with potent anti-inflammatory effects, particularly in the context of diabetic retinopathy. This study demonstrated that these diterpenoids, especially compound 5, effectively reduced oxidative stress and inflammatory responses *in vitro* and *in vivo*. Recent research has identified bioactive compounds with significant anti-inflammatory properties, further emphasizing the potential of *Sigesbeckia* species in the development of natural therapeutic agents (Liu et al., 2025). Moreover, while *S. orientalis* has been used for centuries in traditional medicine, its modern applications are expanding. The strong antioxidant properties of the plant have made it an attractive candidate for research into new therapies, particularly in the treatment of age-related diseases and chronic inflammation. The therapeutic value of *S. orientalis* could be enhanced through further exploration of its bioactive compounds and their roles in reducing oxidative damage and inflammation (Teixeira et al., 2017; Asif, 2015).

In this study, *S. orientalis* extract was prepared with methanol solvent in order to investigate the antioxidant capacities and activities of *S. orientalis* plant growing in İkizdere of Rize. We used TPMA and TFA analyses to determine the antioxidant capacity of the *S. orientalis* plant extract prepared with methanol. Also, CUPRAC, FRAP, and DPPH were analyzed for the determination of antioxidant activity.

Hung et al. (2017) purchased plant materials of the *S. orientalis* species from the Yuanshan Company in Kaohsiung City, Taiwan. They extracted the plant materials using ethanol and subsequently separated the ethanol extract using solvents of varying polarity (hexane, ethyl acetate, and methanol). In this study, it was observed that the total amount of phenolic and flavonoid substances in the ethyl acetate extract was the highest, followed by ethanol, methanol, and hexane. That study by Hung et al. (2017) measured the total amount of flavonoids (1.9 ± 0.1 mg QE/g dry weight) and phenolic substances (15.7 ± 0.4 mg GAE/g dry weight). This study, on the other hand, measured the total amount of flavonoids (8.55 ± 0.21 mg QE/g dry weight) and phenolic substances (18.57 ± 0.57 mg GAE/g dry weight) in the same solvent extract. In the same study, DPPH activity was investigated in extracts prepared with 4 different solvents. They found that DPPH scavenging activities were highest in ethyl acetate and lowest in hexane. They determined the IC_{50} value (346.6 ± 34.7 µg/mL) in methanolic extracts. In the present study, DPPH scavenging activity was found to be ($SC_{50} = 380 \pm 12.0$ µg/mL), compatible with the study by Hung et al. (2017).

A study by Pradhan et al. (2018) looked at diploid and tetraploid parts of *S. orientalis* plants and found that the parts above ground had more total phenolic matter and total flavonoid matter than the parts below ground. In this study, only the above-ground part was used. The TPMA levels were higher than those found by Pradhan et al. (9.70 ± 0.10 mg/g DW) in tetraploid leaves, but the total flavonoid content was the same (7.73 ± 0.02 mg/g DW). Across all three studies, the total flavonoid content remained lower than the total phenol content in *S. orientalis*. Additionally, Pradhan et al. (2018) reported the highest DPPH levels in the leaves of the tetraploid cytotype (16.71 ± 1.68 µg/mL). DPPH activity in this study and that of Hung et al. (2017) was lower than that of Pradhan et al. (2018). The content of bioavailable compounds in plants varies according to the region where they grow. Therefore, it was concluded that the differences between the studies may be due to both the extraction methods and the growing regions. It has been demonstrated that there was a positive correlation between the antioxidant activity (DPPH, FRAP, and CUPRAC) of a plant or fruit extract and the concentration of phenolic compounds present. High levels of phenolic compounds have been shown to exhibit strong antioxidant activity by inhibiting free radicals and donating electrons from phenol groups (Junsathian et al. 2022). The present study has identified a correlation between the phenolic and flavonoid contents of *S. orientalis* and its antioxidant activity. Previous studies have investigated the DPPH free radical scavenging activity of *S. orientalis* (Hung et al. 2017, Pradhan et al. 2018), and the results of the present study are consistent with those of these previous studies. In this study, the CUPRAC activity for *S. orientalis* plant extract was determined to be 300 µmol TE/g DW (0.30 mmol TE/g DW). As a result of the literature review, no study on the CUPRAC activity of *S. orientalis* was found. This shows that our study provides an important contribution to the literature in this field.

There is limited data on the FRAP activity of *S. orientalis*. A study looked at the antioxidant power and total phenolic content of 223 medicinal plant infusions (Li et al., 2013). For *S. orientalis*, the total phenolic content was found to be 11.61 mg GAE/g, and the FRAP activity was found to be 192.82 µmol Fe (II)/g. The total phenolic content of the *S. orientalis* extract in our study was higher (18.57 mg GAE/g DW), but the FRAP activity levels were different from those in this study (192.82 µmol Fe (II)/g). It is thought that this difference may be due to the extraction method, analysis conditions, or ecological factors in which the plant grows.

Prior research on the antioxidant properties of *S. orientalis* has predominantly utilized the DPPH technique (Yang et al., 2016). This study's thorough assessment of the methanol extract using CUPRAC, FRAP, and DPPH

techniques offers a distinctive contribution to the field. In the literature, there is no research on CUPRAC analysis, and just one study utilizing the FRAP approach exists. This work is among the first to investigate the antioxidant capabilities of *S. orientalis* from a broader perspective.

These findings emphasize the importance of further research into the bioactive compounds of *S. orientalis*, as they could play a critical role in the development of modern complementary therapies. By combining its traditional uses with modern scientific findings, *S. orientalis* has the potential to be integrated into contemporary medical treatments.

The presence of bioactive compounds in *S. orientalis* and other plants indicates that these species have potential applications in the food and pharmaceutical industries (Sacan, 2019; Choi et al., 2021). In this context, the results of our study are expected to provide a basis for further research on *S. orientalis* and comparative analysis with other species.

CONCLUSIONS

In recent years, there has been a global increase in interest in herbal products as a means of maintaining optimal health, in part due to the increasing toxicity of synthetic drugs. Furthermore, this interest has facilitated the investigation of plants that are found in their natural habitat and not cultivated. *S. orientalis* is a plant that has been known for its anti-inflammatory effect for many years and is found growing spontaneously in nature. The present study provides useful information about the antioxidant content and capacity of the *S. orientalis* plant from İkizdere, Rize. The study's findings revealed that the methanolic extracts of this plant were rich in antioxidants. This was shown by checking the levels of TFA, TPMA, DPPH, FRAP, and CUPRAC. Notably, this study is the first to evaluate the antioxidant properties of *S. orientalis* using the CUPRAC method, highlighting the contribution of this research to the existing body of knowledge. While previous studies on this plant have typically employed different solvents, such as ethanol or water, our work with methanol provides a more thorough exploration of its bioactive compounds. The bioactive compounds in *S. orientalis* have shown promising therapeutic potential, particularly for their antioxidant effects. These findings confirm that *S. orientalis*, a plant native to the Rize İkizdere region, possesses significant antioxidant properties. Already utilized in traditional medicine for various ailments, these properties further supplement the plant's potential for integration into modern complementary medicine.

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Contribution of Authors

The authors state that they have contributed equally to the article.

Conflict of Interest

The authors declare no conflict of interest.

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Characterization of Red Poppy (*Papaver rhoeas* L.) Extract: An Alternative Food Colorant

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ABSTRACT

The growing interest in natural products has prompted researchers and the food industry to seek out clean additives. The red petals of the poppy plant offer a strong alternative to synthetic additives, thanks to their bioactive compounds and vibrant color. This study extracted anthocyanins from poppy petals using an acid-ethanol-water mixture and examined the nutritional properties and color stability of the resulting pigment. It was found that the poppy extract (PE) is rich in sodium and potassium minerals and contains major phenolic compounds, such as quercetin and kaempferol. The total anthocyanin content was determined to be 17.11 ± 0.60 mg cyanidin-3-glucoside g⁻¹. The antioxidant activity of PE was evaluated using DPPH, CUPRAC, and FRAP assays, with results of 0.92 ± 0.08 µg mL⁻¹, 155.98 ± 8.73 mM TE g⁻¹, and 581.94 ± 12.09 µmol TE g⁻¹, respectively. Antimicrobial activity of PE was determined on 4 pathogenic microorganisms, *Salmonella enteridis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcus aureus*, with the highest inhibition order. The anthocyanins were found to decrease by approximately 15% after 60 minutes of heat treatment at 100°C, and oxidative degradation (H₂O₂) increased over time but did not exceed 13%. Additionally, under different pH levels, the anthocyanins exhibited characteristic behavior, shifting in color from red to purple and then to a yellowish green. In conclusion, poppy extract has the potential to be used as a functional colorant in food products subjected to heat treatment and oxygen exposure. Furthermore, its ability to change color with pH paves the way for its application in the development of smart packaging.

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Gelincik Çiçeği (*Papaver rhoeas* L.) Ekstraktının Karakterizasyonu: Alternatif Bir Gıda Boyası

ÖZET

Doğal içerikli ürünlere olan ilginin günümüzde artması, araştırmacıları ve gıda endüstrisini temiz katkı maddeleri bulmaya yönlendirmektedir. Gelincik bitkisinin kırmızı taç yaprakları, biyoaktif bileşenleri ve rengi sayesinde sentetik katkı maddelerine güçlü bir alternatif sunar. Bu çalışmada, gelincik yapraklarından asit:etanol:su karışımı ile antosiyaninler ekstrakte edilmiş ve elde edilen renk maddesinin besleyici özellikleri ile renk stabilitesi incelenmiştir. Gelincik ekstraktının (GE) sodyum ve potasyum mineralleri açısından zengin olduğu, ayrıca majör fenolik bileşenler olarak kuersetin ve kamferol içerdiği tespit edilmiştir. Toplam antosiyanin miktarı 17.11 ± 0.60 mg siyanidin-3-glukozid g⁻¹ olarak belirlenmiştir. GE'nin antioksidan aktivitesi DPPH, CUPRAC ve FRAP testleri ile değerlendirilmiş olup, sonuçlar sırasıyla 0.92 ± 0.08 µg mL⁻¹, 155.98 ± 8.73 mM TE g⁻¹ ve 581.94 ± 12.09 µmol TE g⁻¹ olarak bulunmuştur. GE'nin antimikrobiyel aktivitesi 4 patojen mikroorganizma üzerinde incelenmiş ve en yüksek inhibisyon sırasıyla *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*

Gıda Bilimi

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Anahtar Kelimeler

Gelincik çiçeği
Antosiyanin
Doğal renklendirici
Renk kararlılığı
Fenolik bileşik

O157:H7 ve *Staphylococcus aureus* olarak tespit edilmiştir. Antosiyaninlerin 100°C'de 60 dakikalık bir sıcaklık uygulaması sonucunda yaklaşık %15 oranında azaldığı, oksidatif bozulmanın (H₂O₂) zamanla arttığı fakat maksimum %13 olduğu belirlenmiştir. Farklı pH değerlerinde ise antosiyaninler karakteristik davranışını göstererek kırmızıdan mora, ardından sarımsı bir yeşile doğru renk değişimi sergilemiştir. Sonuç olarak, gelincik ekstraktı, ısıl işlem gören ve oksijen maruziyetine uğrayan gıda ürünlerinde fonksiyonel bir renk maddesi olarak kullanılma potansiyeline sahiptir. Ayrıca pH ile renk değiştirme özelliği, bu renk maddesinin akıllı ambalajların geliştirilmesinde kullanımının önünü açmaktadır.

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INTRODUCTION

The trend toward nature is replacing synthetic additives with natural constituents as consumer awareness increases. The most commonly used edible flowers in Turkey are saffron (*Crocus sativus*), rose (*Rosa damascena*), lavender (*Lavandula angustifolia*), violet (*Viola odorata*), hibiscus (*Hibiscus sabdariffa*), verbena (*Verbena officinalis*), poppy (*Papaver rhoeas*), pumpkin (*Cucurbita pepo*), and borage (*Borago officinalis*) (Bayram et al., 2014). Among these plants, the poppy flower stands out as a beneficial alternative to synthetic additives due to its bioactive components and vibrant color. The color of *Papaver rhoeas* L. is primarily derived from its main component, cyanol, which belongs to the red anthocyanins group (Bujak et al., 2021).

The genus *Papaver* (family *Papaveraceae*) is a plant that grows in the temperate climates of Eurasia, Africa, and North America, with a general distribution in the Mediterranean region. (Mohammed et al., 2023). The *P. rhoeas* (poppy) plant is known to contain high concentrations of primary and secondary metabolites, including amino acids, carbohydrates, fatty acids, vitamins, phenolic compounds, essential oils, flavonoids, alkaloids, coumarins, organic acids, and other compounds (Hmamou et al., 2022). The stems, seeds, and petals of the poppy plant are consumed as food (Yüksel et al., 2022). The seeds are used to flavor cakes, biscuits, salads, and breads, while the green stems and leaves are used in the traditional cuisine of the Italian region, mostly in cooked products (Montefusco et al., 2015; Grauso et al., 2020). The red petals are used to make sherbet in Turkey (Yüksel et al., 2022). It is also used as a colorant in beverages (Marsoul et al., 2020). The commercial use of red petals has not been found in the literature.

The poppy (*P. rhoeas* L.) has been utilized in alternative medicine since antiquity for its bioactive compounds, which assist in promoting sleep, alleviating thoracic pain, and mitigating inflammation of the throat and tongue (Grauso et al., 2020). *P. rhoeas* extracts possess numerous pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, anti-ulcerogenic, cytotoxic, cough suppressant, antispasmodic, anti-genotoxic, anti-mutagenic, and anti-carcinogenic effects (Grauso et al., 2020; Marsoul et al., 2020). Poppy is extensively utilized in traditional Turkish medicine as a cough syrup, a depressant, analgesic, remedy for diarrhea, treatment for skin irritation, and as a sleep tea for children (İpek et al., 2023). Rhoeadine and benzyloquinoline compounds, alkaloids derived from poppy flowers, exhibit moderate analgesic and sedative effects (Grauso et al., 2020). Poppy leaves possess alkaloids with notable anticancer properties, including those present in buprenorphine and codeine, alongside alkaloids utilized as narcotics and analgesics, such as morphine (Mohammed, et al., 2023). The dark region surrounding the capsule of the poppy flower harbors thebaine, a potent toxic alkaloid, necessitating careful removal of this section, particularly in beverage manufacturing (Ekici, 2014; Mohammed et al., 2023). Nonetheless, it has been documented that the efficacy of these highly potent alkaloids is diminished by the bioactive compounds present in the poppy flower (Bujak et al., 2021).

Coloring additives are frequently used to prevent color loss during food processing, to make food more attractive, and to improve quality. Increasing awareness of the toxicity of synthetic dyes leads manufacturers to use natural colorants. These natural colorants also attract attention with their therapeutic and medicinal properties. Natural dyes are derived from naturally occurring sources such as plants, insects, animals, and minerals (Chaitanya-Lakshmi, 2014). Plant-based colorants are preferred due to their medicinal value. The poppy flower also draws attention as a natural food coloring with its color and composition. The color of the poppy flower comes from the anthocyanins it contains, and the most abundant anthocyanin was determined as cyanidin (Ekici, 2014).

Natural colors of plant origin used in the food industry are yellow-orange (Marigold, β -carotene, lycopene, gentian, turmeric, saffron, *Sanguinaria canadensis*), brown (*Camellia sinensis*, *Lawsonia inermis*), red (Annatto, *Beta vulgaris*, paprika, grapes, *Vitaceae polistiformis*, *Alkanna tinctoria*), and purple-blue (*Centaurea cyanus*, *Indigofera tinctoria*, *Vaccinium myrtillus*) (Chaitanya-Lakshmi, 2014).

Synthetic antioxidants and preservatives are frequently used in industrial processing to extend the storage stability of food. However, it has been found that synthetic additives can lead to nutrient loss and even create toxic effects (Zhang et al., 2013). At the beginning of the 20th century, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were restricted due to their carcinogenic properties (Shi et al., 2005). In recent years, natural antioxidants and preservatives obtained from edible materials, edible by-products, and waste sources have become intriguing because they can protect the human body from free radicals and delay the progression of many chronic diseases (Zhang et al., 2013).

Antioxidants are bioactive components that protect molecules and biological systems from free radicals, such as reactive oxygen (Montefusco et al., 2015). When endogenous defense system enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the human body are insufficient against free radicals, antioxidant molecules such as phenol, flavonoid, carotenoid, vitamin C and E, and reduced glutathione should be taken externally (Montefusco et al., 2015). Wild poppy, an annual herbaceous plant belonging to the *Papaveraceae* family, is a beneficial source of antioxidants (Montefusco et al., 2015). The aqueous extract of *P. rhoeas* contains phenolic compounds such as caffeic acid, quinic acid, gallic acid, rutin, and quercetin, which are known for their antioxidant properties (Bujak et al., 2022). In addition, vitamin C, known as an antioxidant, is also present in poppy flowers (Bujak et al., 2022).

Since anthocyanins used as natural colorants have high polarity, their extraction from plant matrices is usually carried out using organic solvents (Bleve et al., 2008; Bridgers et al., 2010). It is also known that hydrochloric acid added to the extraction solvent is effective in terms of the extraction efficiency of anthocyanins (Oancea et al., 2012; Patil et al., 2009). In addition, modern extraction techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) have limitations such as the heat sensitivity of anthocyanins and the fact that the SFE technique is particularly suitable for non-polar solvents. For this reason, solvents such as ethanol, methanol, and acetone are still used in the extraction of natural colorants today (Patil et al., 2009). However, since methanol and acetone are toxic, ethanol, which is more environmentally friendly, was used in this study.

In this study, the usability of poppy flower extract, which contains bioactive components and natural coloring substances such as anthocyanin, as a natural food coloring was evaluated. The main objective of the study was to demonstrate the potential of poppy flower extract not only for coloring but also for providing nutritional and functional properties to food products with its bioactive component composition and activity. In this direction, physicochemical properties, total phenolic and anthocyanin content, phenolic compound composition, antioxidant and antimicrobial activities, and mineral composition of the extract were investigated in detail. Color stability and degradation of anthocyanins were also evaluated. The findings indicate that poppy flower extract can be used as a functional additive as well as a coloring agent, and thus has the potential as a versatile additive.

MATERIAL and METHOD

Material

Poppy leaves of the same color and maturity were collected from Kırklareli University Kayalı Campus (41°47'23.7 'N 27°09'32.4 'E) in the first week of May 2024. The collected red leaves were separated from the black parts on the same day and subjected to extraction to minimize deterioration in quality.

Preparation of Poppy Color Extract (PE)

In the extraction of anthocyanin pigments, a mixture of acidified ethanol (0.01% HCl v/v) (Merck, Darmstadt, Germany) and distilled water (1:1) was used as the solvent. The sample-solvent ratio in the extraction process (Figure 1) has been set to 1:20 (weight/volume). It has been left to macerate in the dark and at room temperature for 72 hours. Extracts purified from the solvent in a rotary evaporator (Scilogex, RE100, USA) have been stored in amber bottles at -18 °C (Bayram et al., 2015).

Physicochemical Properties of Poppy Color Extract

The pH value of the poppy color extract was determined using a pH meter (Hanna, HI 2211-02, USA). The determination of dry matter and ash content has been established according to the AOAC (2006) method. The color parameters L* (lightness), a* (red-green), b* (yellow-blue), C*, h°, and Y values were measured using the Konica Minolta Chromo Meter device (Konica Minolta Sensing Inc., CR-5, Japan).

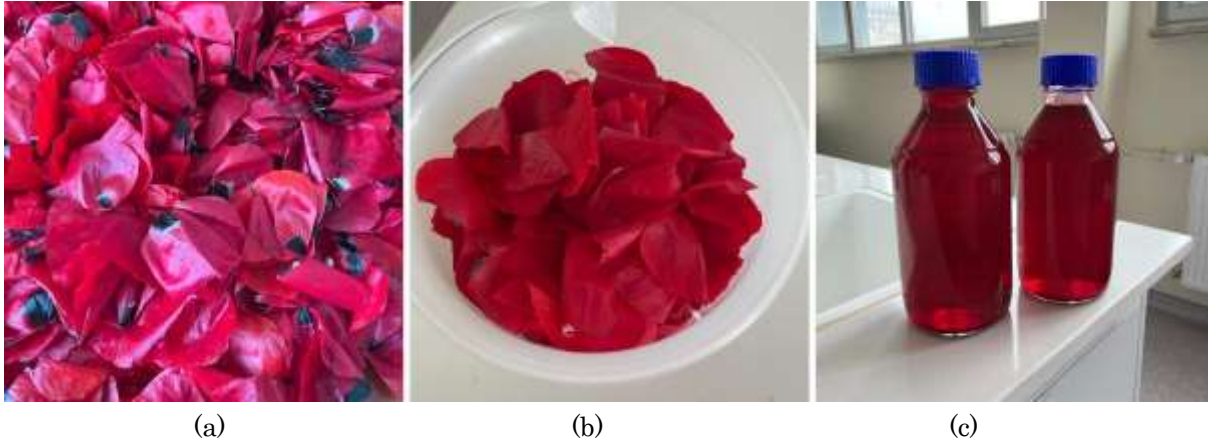


Figure 1. (a): freshly collected poppy leaves, (b): leaves separated from seeds and black part, (c): poppy leaf extract
Şekil 1. (a): taze toplanmış gelincik çiçeği yaprakları, (b): tohumlarından ve siyah kısmından ayrılmış yapraklar, (c): gelincik çiçeği özütü

As a result of color measurements, the Hunter L*, a*, and b* values do not represent the color phenomenon directly perceived by consumers in the market. Instead, the "Hue Angle" (h°) has been determined, where red-purple colors fall within the 0°-270° angle range, yellow colors are within the 60° angle range, and blue and green colors are within the 240°-120° angle range, along with the "Chroma" value. Chroma (C*), a measure of intensity or saturation, is used to determine the degree of difference of a hue compared to a gray color with the same hue angle (h°) and is considered a quantitative quality of colorfulness. The chroma parameter according to the CIELab system has been calculated as follows:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{Eq. 1}$$

The h° value, which expresses the tone angle according to the CIELab system, was calculated as follows:

$$h^0 = \arctan \frac{b^*}{a^*} \quad \text{Eq.2}$$

Mineral Composition of Poppy Color Extract

The sample was incinerated in a microwave incineration system (Mars 6, CEM, USA) at 200 °C for 45 minutes by adding 65% (v/v) HNO₃. The sample was subjected to elemental analysis by Agilent Technologies 7700 ICP-MS (Inductively coupled plasma-mass spectrometer) against a blind sample after removal of organic compounds and inorganic compounds in acid solution. The results are given in ppb.

Bioactive Compounds

450 µL of phenolic extract (5 times diluted) was subjected to a reaction by adding 2.25 mL of distilled water, 1:9 (v/v) diluted Folin-Ciocalteu reagent, and 1.8 mL of Na₂CO₃ solution (75 g L⁻¹). After being kept in a dark room at room temperature for 2 hours, the absorbance of the color formed during the reaction was read against a blank at a wavelength of 760 nm using a spectrophotometer. The total phenolic content is expressed as mg GAE g⁻¹ (Atacan & Yanık, 2017).

The total anthocyanin content (TAC) of PE is based on the pH differential method. (Wrolstad, 1993). Samples were diluted in the same ratio after being passed through a 0.45 µm filter, using potassium chloride (pH 1.0) and sodium acetate buffer (pH 4.5) to ensure that the absorbance values remained below 1.2. Absorbance was measured at 510 and 700 nm. The total anthocyanin content of PE has been calculated based on cyanidin-3-glucoside, which has a molecular weight of 449.2 and a molar absorptivity (ε) of 26900 L cm⁻¹ mg⁻¹ (Küskü and Karaman, 2023).

The phenolic profile of PE's clear supernatant was determined using an Agilent 6460 system (Agilent Technologies, Santa Clara, California, USA) equipped with an electrospray ionization (ESI) interface in triple quadrupole liquid chromatography-tandem mass spectrometry (LC-MS/MS). The Agilent Zorbax SB-C8 column (150 mm × 3.0 mm, 3.5 µm particle size) was used for separation (Bayram et al., 2022).

Antioxidant Capacity

The antioxidant activity of poppy extract has been determined in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC), and ferric-reducing ability of plasma (FRAP).

The extracts of PE were determined by adding 600 µL of 1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution and incubating the mixture with a final volume of 6000 µL in the dark for 30 minutes, followed by measuring the

absorbance at 517 nm. The IC₅₀ value has been calculated for the radical sweeping activity ($\mu\text{g mL}^{-1}$) (Yıldız et al., 2019).

For the CUPRAC experiment, a 500 μL sample solution was prepared by mixing 1000 μL of 0.0075 M neocuproine (2,9-dimethyl-1,10-phenanthroline), 1000 μL of 0.01 M copper (II) chloride, and 600 μL of distilled water. The mixture has been incubated in the dark for one hour. Subsequently, absorbance measurements were performed at 450 nm, and the results were calculated as mM Trolox equivalents (mM TE g^{-1}) (Durmuş et al., 2020).

The iron reducing power of the samples was determined using FRAP reagent (obtained by mixing sodium acetate buffer solution, 10 mM TPTZ solution, 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution at a ratio of 10:1:1 (v/v/v), respectively). 100 μL of the extract was reacted with 3.0 mL of FRAP reagent, and after being incubated at 37 °C for 30 minutes, the absorbance was measured at a wavelength of 593 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The antioxidant capacity is expressed as Trolox equivalent ($\mu\text{mol TE g}^{-1}$) (Atacan & Koçak-Yanık, 2017).

Antimicrobial Activity

The disk diffusion method has been used to determine the antimicrobial activity of PE. For the analysis, strains of *Escherichia coli* O157 (ATCC 33150), *Listeria monocytogenes* (ATCC 7644), *Salmonella enteritidis* (ATCC 13076), and *Staphylococcus aureus* (ATCC 25923) were used. Bacterial cultures were prepared by inoculating each strain into Nutrient Broth medium and incubating at 37 °C for 24 hours. After incubation, the cultures blur was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU mL^{-1} . Standardized bacterial suspensions have been spread onto Müller-Hinton agar plates for inoculation. After the plates were dried for 15 minutes, 30 μL of PE was added to the discs with a diameter of 6 mm. Ethanol-imbued discs have been used as a control. The plates were incubated at 37 °C for 24 hours. After the incubation, the diameters of the inhibition zones formed to demonstrate antimicrobial activity were measured using calipers and recorded in millimeters (Kittisakulnam et al., 2017; Reinheimer et al., 1990).

Color Stability and Degradation

Thermal stability

The thermal stability of the flower extracts was determined using the temperature degradation kinetics method. A volume of 1 mL of the aqueous extract was made up to 9 mL with 0.1 M citrate buffer (pH 1), placed in a test tube with a screw cap, and heated in a thermostatically controlled water bath at 60, 80, and 100 °C for 60 min. The test tubes were immediately cooled to ambient temperature under running tap water, and the change in absorbance at 520 nm was measured at 30-, 45-, and 60-min. Initial absorbance values were determined before each experiment (Venter et al., 2022).

Oxidative stability

The oxidative stability of PE was determined using three different H_2O_2 concentrations (31.91, 21.27, and 10.64 mM). The predetermined amounts of H_2O_2 were rapidly added to 1 mL of flower extracts (in citrate-phosphate buffer pH 1) to form 8 mL each of 9.31, 18.61, and 27.92 mM. The change in absorbance at 520 nm was measured at 30, 45, 60, 90, 120, 150, 180, and 240 min for 240 min for each concentration of hydrogen peroxide used. The absorbance value of the sample solution prepared with the same amount of distilled water instead of hydrogen peroxide was determined and recorded as the initial value (Venter et al., 2022).

Anthocyanin degradation

Oxidative and thermal degradation, pseudo-first-order kinetic rate constants (k), were obtained by plotting $\ln(C_t/C_0)$ versus time to estimate the kinetic degradation parameters of anthocyanins. The modelling equation is as follows:

$$C_t = C_0 \exp(-kt) \quad \text{Eq.3}$$

C_t , represents the number of anthocyanins at time t, and C_0 indicates the initial amount (Gümüş et al., 2024).

pH sensitivity

The color change of the extract at different pH values was determined spectrophotometrically. 5 mL of the extract was taken in test tubes, and the pH of the samples was adjusted from 2 to 14 with 0.1 N HCl and 0.1 N NaOH. Samples with different colors were photographed using a smartphone camera. Spectra scans of all samples were then performed using a UV/visible spectrophotometer in the wavelength range from 400 to 700 nm (Kamer et al., 2022).

Statistically Analysis

Statistical evaluations were conducted using one-way analysis of variance (ANOVA). The all experiments were conducted in triplicate, and the differences between the samples were analyzed using Duncan's multiple range tests with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The level of statistical significance is 95% (Erol, 2013).

RESULTS and DISCUSSION

Physicochemical Properties

Color is an important sensory characteristic in determining product quality and is closely related to anthocyanin pigments found in various plants. Minimizing pigment losses in food processing processes is very important (Karasu et al., 2015). The red petals of the poppy plant and the color parameters of the obtained aqueous extract are presented in Table 1. The L^* , a^* , b^* , C^* , h° , and Y values were found to be higher in the leaves. The brightness value (L^*) was low in both samples. This situation may have arisen due to the quite high value of the redness (a^*) parameter. The a^* value of the poppy leaf was only 1.3 times higher than that of the color extract, while the b^* value is 1.8 times greater. The close values of redness indicate that the anthocyanins were well extracted. A high chroma value (C^*) represented a more saturated, vibrant, and pure color, while the opposite indicates a pale, grayish, or pastel color. It was observed that this value is higher in the poppy leaf in parallel with brightness. Hue angle values (h°) represent the degree of redness, yellowness, greenness, and blueness of colors; the maximum values for these hues are 0° , 90° , 180° , and 270° , respectively. C^* and h° values were similar to *Monascus* red (Mapari et al., 2006). In both samples, the Y value was closer to 0 than 100. This was an indication that they reflect less light and support the L^* value results.

Table 1. Comparison of the color parameters of poppy leaves and GE.

Çizelge 1. Gelincik çiçeği yapraklarının ve GE'nin renk parametrelerinin karşılaştırılması.

	GE	Poppy Leaves
L^*	28.89 ± 0.84	38.24 ± 0.14
a^*	49.56 ± 0.14	65.58 ± 0.05
b^*	18.02 ± 0.03	30.92 ± 0.16
C^*	52.72 ± 0.14	72.48 ± 0.16
h°	19.98 ± 0.22	25.25 ± 0.09
Y	38.27 ± 0.22	46.76 ± 0.08

Abbreviations: L^* , lightness; a^* , green-red color; b^* , blue-yellow color; C^* , Chroma; h° , hue angle.

In a study, L^* , a^* , b^* , C^* , and h° values of ethanol: water (20:80) extract of poppy flower were determined as 14.19 ± 0.09 ; 3.04 ± 0.03 ; 2.02 ± 0.01 ; 3.7 ± 0.03 ; and 33.4 ± 0.03 , respectively (Bujak et al., 2021). In another study, these values were determined as 23.23 ± 0.06 ; 2.51 ± 0.10 ; -1.18 ± 0.01 ; 2.8 ± 0.02 ; and -25.2 ± 0.05 for aqueous extract, respectively (Bujak et al., 2022). In another study where the extract was added to skin cream, it was determined that although the C^* value was low, the color was clearly noticeable and maintained its color stability over time (Bujak et al., 2021; Bujak et al., 2022). The color parameters of the aqueous extract of poppy flower were determined as 6.00 ± 0.04 ; 0.45 ± 0.12 ; 0.37 ± 0.08 ; 0.47 ± 0.15 ; and 18.57 ± 5.08 (Çelik & Göncü, 2021). In another study where ferret flower sherbet was concentrated by different methods, the best color values were obtained for the vacuum method, and L^* , C^* , and h° values were determined as 16.3, 1.59, and 307.88, respectively (Ekici, 2014). The elevated color parameters found may be attributed to methodological variances, including the solvent type and ratio, extraction duration, as well as collection timing, cultivation circumstances, and species variations. The utilization of extracts in various matrices (cream, etc.) in certain investigations may elucidate the discrepancies noted in the color parameters. The fact that the C^* value of PE is quite high compared to the literature data shows that it has a more saturated, vivid, and pure color (Fig. 1-c).

The % ash content of ferret extract in dry matter was determined as 1.32 (Table 2). In a study conducted with dried poppy, ash content was determined as 15.33% (Kaya et al., 2004). It can be said that approximately 7% of the mineral substances can be recovered with the extraction method. The brix value of the extract was determined as 8.36. Considering that the total dry matter content of poppy flowers is approximately 95% (Kaya et al., 2004), it can be said that approximately 9% of the dry matter can be recovered. The pH value of the PE had been determined to be 0.58, as it was extracted with acid. According to the study conducted by Çelik and Göncü, (2021), the pH of the aqueous extract of the poppy flower was found to be neutral (7.04 ± 0.69). In another study, the pH of dried weasel was found to be 5.87 (Kaya et al., 2004). The thermal stability of anthocyanins decreases with increasing pH (Ekici, Simsek, Ozturk, Sagdic, & Yetim, 2014). In the study by Liu et al. (2018), which examined the effects of

temperature and pH on the stability of anthocyanins obtained from blueberries, it was observed that anthocyanins rapidly degrade above pH 3. The low pH is important in both the extraction and stability of anthocyanins.

Mineral Compounds

Owing to the crucial roles of mineral substances in metabolism, a mineral composition analysis was performed on PE. Macroelements such as potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), and phosphorus (P) are essential nutrients necessary for maintaining normal body functions (Katarzyna et al., 2021). When the mineral composition of the poppy flower was examined, it was seen that the minerals were found in the following order from most to least: Ca, K, Na, Mg, and P (Grauso et al., 2020).

In PE, analysis was performed for P, Mn, Fe, Ni, Cu, Zn, Na, Mg, Al, K, and Ca minerals, and only Na, K, P, and Fe minerals were detected (Table 2). Mn, Ni, Cu, Zn, Mg, Al, and Ca are below the quantification limit (< 0.00 ppb). Katarzyna et al. (2021) detected K, P, Mg, and Na minerals in aqueous extracts of poppy flower petals from more to less, respectively. The mineral composition in plant extracts can vary significantly depending on environmental conditions. Various environmental conditions, including the mineral content of the soil, water availability, temperature, light level, and stress factors exposed to the plant, can cause this variability (Anjum et al., 2022). Potassium, the most important intracellular cation, is responsible for regulating the normal functioning of neurons and muscles. Along with sodium ions, it is an important part of the enzyme Na⁺/K⁺-ATPase, which helps with things like muscle contraction and nerve cell excitation (Quintaes & Diez-Garcia, 2015). Sodium is an electrolyte that helps the body regulate fluid balance and blood pressure. Na is vital for fluid balance and nerve conduction in the body, but excess intake can increase the risk of high blood pressure and heart disease. P supports bone and tooth health and is essential for energy production and cellular function, but excess can negatively affect kidney health. Fe, which is found in the structure of proteins such as hemoglobin and myoglobin, helps transport oxygen to cells, and this process is vital for energy production. Iron deficiency can lead to anemia (Godswill et al., 2020).

Considering the mineral substances in the poppy extract and its positive effects on health, it will increase the nutritional value of the food used as a natural coloring agent and provide a functional quality.

Table 2. Mineral and physicochemical properties of poppy extract.

Çizelge 2. Gelincik çiçeği özütünün mineral ve fizikokimyasal özellikleri.

pH	0.58 ± 0.01
% Ash*	1.32 ± 0.00
Brix	8.36 ± 0.50
P (mg/kg)*	186.60
Fe (mg/kg)*	186.00
Na (mg/kg)*	9382.77
K (mg/kg)*	5901.07

*Dry weight.

Bioactive Components

The total phenolic content (TPC) of plants used for obtaining medicinal plant extracts and natural food colorants varies between 8.9-299.5 mg GAE g⁻¹ extract and 2.96-125.18 mg GAE g⁻¹ dry matter, respectively (Table 4). The phenolic content of the poppy flower (45.10 ± 0.84 mg GAE g⁻¹ dry matter) is comparable to that of other medicinal plants (Table 3). According to a study, the basal green leaves of the poppy plant had a total phenolic content of 1.348±0.006 mg GAE g⁻¹ and an antioxidant activity of 13.26 ± 0.23 µmol TE g⁻¹ (ABTS) (Montefusco et al., 2015). A study on the concentration of poppy flower syrup using various methods revealed that the vacuum approach yielded the highest results, with total phenolic content and total anthocyanin values measured at 1022.84 mg GAE kg⁻¹ and 774.49 mg cyanidin-3-glucoside kg⁻¹, respectively. In contrast to our study, Marsoul et al. (2020) and Kazazic et al. (2016) found higher levels of phenolic compounds, whereas and Bayram et al. (2015); Katarzyna et al. (2021); Kostic et al. (2010) found lower levels. This situation is thought to be due to differences in extraction method, solvent and harvesting region. The examined extract's substantial polyphenol content suggests it is a potent source of bioactive compounds. Polyphenols are widely recognized for their numerous health benefits, including antioxidant, antimicrobial, anti-inflammatory, prebiotic, and anticancer properties (Katarzyna et al., 2021).

Table 3. Bioactive compound content and antioxidant capacity of poppy extract.

Çizelge 3. Gelincik çiçeği özütünün biyoaktif bileşik içeriği ve antioksidan kapasitesi

TPC*	45.10 ± 0.84 mg GAE g ⁻¹
TAC*	17.11 ± 0.60 mg cyanidin-3-glucoside g ⁻¹
DPPH (IC ₅₀)*	0.92 ± 0.08 µg mL ⁻¹
CUPRAC*	155.98 ± 8.73 mM TE g ⁻¹
FRAP*	581.94 ± 12.09 µmol TE g ⁻¹

*Dry weight

Anthocyanins are flavonoids classified as water-soluble natural pigments. They exhibit strong absorption in the UV-visible region of the electromagnetic spectrum and are responsible for the red-blue color and its derivatives in the plant kingdom (Enaru et al., 2021). Cyanidin, pelargonidin, delphinidin, petunidin, peonidin, and malvidin are the most commonly found types of anthocyanins. Anthocyanin is a biologically active compound known for its ability to neutralize free radicals, inhibit angiogenesis, and its anticancer, antidiabetic, antimicrobial, and neuroprotective effects. Due to these properties, it is widely used in functional foods, traditional medicine, and as a food additive (Roy & Rhim, 2021). The red color of the poppy flower is due to anthocyanins, primarily cyanidin (Grauso et al., 2020). The anthocyanin content in the poppy extract was determined to be 17.11 ± 0.60 mg cyanidin-3-glucoside g⁻¹ on a dry matter basis. Kostic et al. (2010) found the anthocyanin content in poppy extract lower than in our study. Bayram et al. (2015) and Ekici (2014) reported higher total anthocyanin content in poppy extract compared to our findings. The reason why our findings differ from the literature can be explained by the difference in the extraction methods applied and the different harvesting regions of the poppy flower. The anthocyanin content of poppy flowers is higher than plum, red onion, red radish, strawberry, red raspberry, red cabbage and cranberry, but lower than blueberry, blackberry and grape (Bridgers et al., 2010). Since anthocyanins have high polarity, their extraction from plant matrices is usually carried out using organic solvents such as methanol, ethanol, acetone etc. (Bleve et al., 2008; Bridgers et al., 2010). Traditional extraction of anthocyanins is usually carried out in acetone or acidified methanol solutions to obtain the stable flavylum cation with red color. It is known that hydrochloric acid added to the extraction solvent is effective in terms of the extraction efficiency of anthocyanins, and this effect is higher in ethanol: water (1:1) solvent compared to water (Oancea et al., 2012; Patil et al., 2009). In fact, a study determined that acidified solvents yielded 10-45% and 16-46% more anthocyanins than non-acidified ethanol and methanol solvents, respectively (Bridgers et al., 2010).

Phytochemical studies have revealed that the leaves of *P. rhoeas* L. contain various flavanols such as quercetin, kaempferol, myricetin, and isorhamnetin (Katarzyna et al., 2021). In our study, quercetin, kaempferol, isorhamnetin, and protocatechuic acid were detected at levels of 283.01, 125.72, 47.49, and 20.69 mg kg⁻¹, respectively. As shown in Figure 2, the poppy flower also contains trace amounts of phenolic compounds, from most to least, luteolin, rutin, hesperidin, p-coumaric acid, naringenin, caffeic acid, chlorogenic acid, myricetin and phlorizin. The poppy extract, which predominantly contains quercetin and kaempferol, is highly valuable due to the antioxidant, anti-osteoporotic, anti-inflammatory, anti-neuroprotective, thermogenic, cellular signaling modulation, gene expression, and immune response, as well as antihistaminic, anticancer, antidiabetic, hepatoprotective, neuroprotective, cardioprotective, antimicrobial, analgesic, and antihypertensive properties of these compounds (Guven et al., 2019).

The maximum suggested dosage of quercetin, the main phenolic component in poppy extract (PE), is around 1000 mg/day in dietary supplements. However, when used in combination with other ingredients like bromelain, doses of 1200-2400 mg/day have been reported (Andres et al., 2018). Human trials involving doses of 500-1000 mg/day over 3-12 weeks have not identified any pro-oxidative effects of quercetin. The long-term effects of extended exposure remain unclear (Andres et al., 2018). A study of healthy individuals demonstrated that quercetin supplementation, given at gradually increasing doses (up to 150 mg per day) over two weeks, resulted in a dose-dependent elevation of plasma quercetin levels; yet, it did not significantly affect antioxidant status, oxidized LDL, inflammation, or metabolism (Egert et al., 2008). In conclusion, the high level of bioactive components in a natural colorant is significant for both nutritional value and its capacity to prolong the shelf life of the food it improves. Still, the long-term health implications of these chemicals are still insufficiently investigated.

Studies on the phenolic composition of poppy flowers are quite limited. In a study, *Papaver rhoeas* L. contained the phenolic compounds quercetin (263 mg/kg), kaempferol (23 mg/kg), myricetin (11 mg/kg), isorhamnetin (11 mg/kg), luteolin (1 mg/kg) and apigenin (1 mg/kg), from most to least (Trichopoulou et al., 2000). When the results of Trichopoulou et al. (2000) are compared with our study, it is seen that the quercetin results are similar, our kaempferol and luteolin contents are 5.5 and 6.5 times higher, respectively, and our myricetin content is 20 times lower.

Table 4. Total phenolic content (TPC) and antioxidant activity (DPPH and FRAP) of plants (dry weight) used for obtaining medicinal plant extracts and natural food colorants.

Çizelge 4. Tıbbi bitki ekstraktları ve doğal gıda renklendiricileri elde etmek için kullanılan bitkilerin toplam fenolik madde içeriği (TPC) ve antioksidan aktivitesi (DPPH ve FRAP) (kuru ağırlık).

Herbs	TPC (mg GAE g ⁻¹)	DPPH (%)	FRAP (mmol Fe ²⁺ g ⁻¹)	References
Medicinal herbs extracts				
Rosemary leaves	50.0–178.8	19.8–64.7	2.2–10.3	Ulewicz-Magulska & Wesolowski, 2019
Sage leaves	16.2–173.6	6.4–64.5	2.2–7.9	Ulewicz-Magulska & Wesolowski, 2019
Thyme herbs	42.4–240.8 (a)	12.4–79.3 (a)	0.6–15.4 (a)	Ulewicz-Magulska & Wesolowski, 2019
	46.7–59.0 (b)		16.0 mg GAE/g (b)	Vábková & Neugebauerová, 2012
Oregano herbs	104.3–325.1	28.6–87.2	6.8–22.3	Ulewicz-Magulska & Wesolowski, 2019
Basil herbs	28.2–47.5 (a)	13.9–28.3 (a)	0.9–2.4 (a)	Ulewicz-Magulska & Wesolowski, 2019
	147.0 (b)	0.49 (IC ₅₀) (b)	0.54 mmol AAE/g (b)	Hinneburg et al., 2006
Melissa leaves	54.9–299.5	25.7–87.5	9.7–26.9	Ulewicz-Magulska & Wesolowski, 2019
Peppermint leaves	18.3–284.3	14.5–185.6	4.1–18.7	Ulewicz-Magulska & Wesolowski, 2019
Caraway seed	8.9–26.9 (a)	1.3–21.1 (a)	0.1–0.2 (a)	Ulewicz-Magulska & Wesolowski, 2019
	37.4 (b)			Hinneburg et al., 2006
Lovage roots	13.8–26.4	1.8–22.7	0.2–0.4	Ulewicz-Magulska & Wesolowski, 2019
Angelica roots	10.2–21.6	1.7–22.8	0.1–0.2	Ulewicz-Magulska & Wesolowski, 2019
Marjoram	51.8–236.0 (a)	6.9–53.04 (a)	1.8–7.2 (a)	Ulewicz-Magulska & Wesolowski, 2019
	67.2 (b)		16.6 mg GAE/g (b)	Vábková & Neugebauerová, 2012
Tarragon	59.5–253.5	25.6–64.7	1.9–6.3	Ulewicz-Magulska & Wesolowski, 2019
Summer savory	41.6–64.4	-	14.2 mg GAE/g	Vábková & Neugebauerová, 2012
Parsley	29.2	12.0 (IC ₅₀)	0.05 mmol AAE/g	Hinneburg et al., 2006
Laurel	92.0	0.49 (IC ₅₀)	0.46 mmol AAE/g	Hinneburg et al., 2006
Juniper	18.5	2.2 (IC ₅₀)	0.18 mmol AAE/g	Hinneburg et al., 2006
Cardamom	24.2	7.8 (IC ₅₀)	0.051 mmol AAE/g	Hinneburg et al., 2006
Aniseed	20.8	2.9 (IC ₅₀)	0.11 mmol AAE/g	Hinneburg et al., 2006
Fennel	30.3	2.1 (IC ₅₀)	0.12 mmol AAE/g	Hinneburg et al., 2006
Plants from which natural food dyes are obtained				
<i>Centaurea cyanus</i> (Cornflower)*	7.19	83.42	78.01(mg TE/mL)	Marian et al., 2017
<i>Indigofera tinctoria</i> *	69.7	-	-	Prakash et al., 2009
<i>Lawsonia inermis</i> *	75.8	-	-	Prakash et al., 2009
<i>Curcuma longa</i> (Turmeric)*	36.6	-	-	Prakash et al., 2009
Annatto (<i>Bixa orellana</i> L.)	0.73–1.81	29.88–94.20	-	Viuda-Martos et al., 2012
<i>Alkanna tinctoria</i> (ext.)	18.27	76.86 (IC ₅₀)	-	Das et al., 2024
<i>Camellia sinensis</i> (tea)*	56.63–80.27	0.03–0.04 (IC ₅₀)	12.40–14.83(μmol Fe ₂ SO ₄ ·7H ₂ O/mL)	Nor Qhairul Izzreen & Mohd Fadzelly, 2013
<i>Tagetes erecta</i> (Mexican Marigold)*	57.52–125.18	32.5–113.32 (IC ₅₀)	-	Youssef et al., 2020
<i>Crocus sativus</i> (Saffron)*	2.96–6.17	248–280 (IC ₅₀)	-	Ghanbari et al., 2019

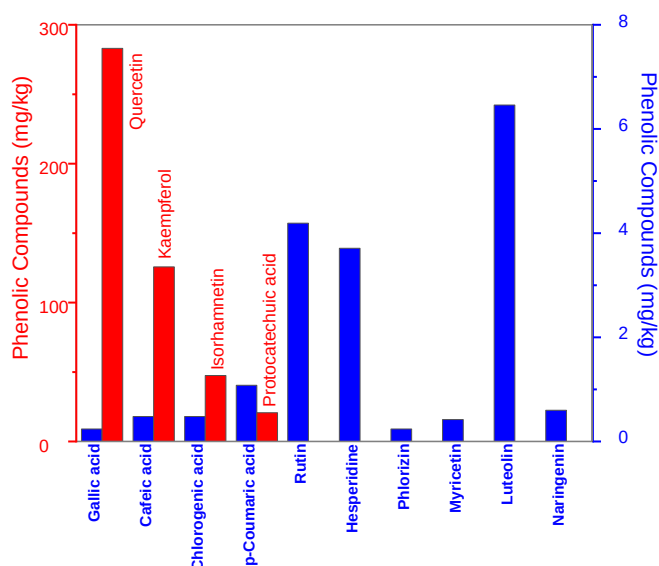


Figure 2. Quantitative distribution of the phenolic compound profile of poppy flower extract (dry matter basis, mg/kg). Highly detected compounds are indicated by red columns (left axis), lowly detected compounds by blue columns (right axis).

Şekil 2. Gelincik çiçeği özütünün fenolik bileşik profilinin kantitatif dağılımı (kuru madde bazında, mg/kg). Yüksek miktarda tespit edilen bileşikler kırmızı sütunlarla (sol eksen), düşük miktarda tespit edilen bileşikler mavi sütunlarla (sağ eksen) gösterilmiştir.

In their study, Bujak et al. (2021) determined the amount of quercetin in the poppy flower extract as 18 times lower (16 mg/kg), the amount of gallic acid as similar (0.2 mg/kg), and the amount of caffeic acid as 3 times (1.21 mg/kg) higher than our study. It is thought that the method they applied in extraction (10 g dry flowers/100 g ethanol: water (20:80) solution, 25 C, 20 min) is not sufficient in the recovery of phenolic compounds. In their study on poppy flower sherbet, Aydoğdu et al. (2023) found the amount of gallic acid 92 times (24 mg/kg) higher, while the amounts of protocatechuic acid (1.15 mg/kg) and rutin (1.35 mg/kg) were found 18 and 3 times lower, respectively. According to our study, quercetin, the dominant phenolic component, could not be detected in poppy flower sherbet. These results show that gallic acid can be extracted at a higher rate with water, but ethanol is important in the extraction of other phenolic compounds.

Antioxidant Capacity

The poppy plant exhibits significant antioxidant capacity, attributed to its many bioactive components and the presence of vitamins (Grauso et al., 2020). A study by Maurizi et al. (2015) evaluated the antioxidant activity of an acetone: water: acid (70:29.5:0.5 v/v/v) extract from the green leaves of the poppy plant, yielding a value of 43.89 mmol TE g⁻¹ by the ORAC method. A study conducted by Haspova et al. (2011) indicated that the DPPH free radical scavenging activity of the pure methanol extract (25 mg/mL) from poppy flowers was 85%. Table 2 presents the DPPH (IC₅₀), CUPRAC, and FRAP results, demonstrating the antioxidant activity of poppy extract. Dhiman et al. (2017) reported significantly lower CUPRAC and FRAP values for poppy, at 8.75 µmol TE g⁻¹ and 2.07 µmol g⁻¹, respectively, compared to our study. Bayram et al. (2015) determined the IC₅₀ value to be 55.9 µg mL⁻¹. It is thought that the difference between the literature results and the result of our study is due to the differences in the harvest regions of the poppy flower, extraction methods, and solvents. When examining the phenolic content, it can be suggested that the strong antioxidant effects of the major identified components contributed to these analysis results. The red pigment derived from poppy was found to have a considerably higher antioxidant content compared to alternative colorant sources such as red carrot (FRAP; 2.30 µmolTE/g and CUPRAC; 3.31 µmolTE/g) (Singh et al., 2018), red beet (FRAP; 63-302 µmolTE/g and CUPRAC; 62-143 µmolTE/g) (Carrillo et al., 2019), red pepper (FRAP; ~0.112 µmol TE/g) (Anaya-Esparza et al., 2021), and hibiscus (DPPH higher than 25 mg/g, FRAP lower than 90 µmolTE/g and CUPRAC lower than 110 µmolTE/g) (Nguyen et al., 2022). Additionally, when examining the medicinal plants in Table 4, the highest antioxidant activity in terms of IC₅₀ was observed in *Camellia sinensis* (0.03-0.04 µg mL⁻¹), while the lowest was found in saffron (*Crocus sativus*, 248-280 µg mL⁻¹). The IC₅₀ value for the poppy extract (PE) was determined to be 0.92 µg mL⁻¹ (Table 3), indicating significantly higher antioxidant activity compared to many medicinal plants.

Antimicrobial Activity

The antimicrobial activity of poppy extract (PE) was evaluated against two Gram-positive and two Gram-negative pathogenic bacteria using the disk diffusion method to investigate its broad-spectrum antibacterial potential. The results, presented in Table 5, show that for Gram-positive bacteria, inhibition zones were 11.32 ± 0.25 mm for *Listeria monocytogenes* and 9.14 ± 0.14 mm for *Staphylococcus aureus*. In the case of Gram-negative bacteria, inhibition zones were 13.21 ± 2.10 mm for *Salmonella* Enteritidis and 10.29 ± 0.41 mm for *Escherichia coli* O157:H7. PE demonstrated the highest antimicrobial activity against *S. enteritidis* followed by *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*. These findings indicate that Gram-negative bacteria may be more sensitive to PE, possibly due to their more permeable outer membrane. Conversely, the thicker peptidoglycan layer in Gram-positive bacteria may reduce the efficacy of PE against these strains.

Table 5. Antimicrobial activity of poppy extract.

Çizelge 5. Gelincik çiçeği ekstraktının antimikrobiyal aktivitesi.

Microorganism	Inhibition zone (mm)
<i>Escherichia coli</i> O157:H7 ATCC 33150	10.29 ± 0.41^{bc}
<i>Listeria monocytogenes</i> ATCC 7644	11.32 ± 0.25^{ab}
<i>Salmonella</i> Enteritidis ATCC 13076	13.21 ± 2.10^a
<i>Staphylococcus aureus</i> ATCC 25923	9.14 ± 0.14^c

^{a-c} Statistically significant differences between the means in the same column are indicated ($p < .05$).

The antimicrobial activity of the poppy (*Papaver rhoeas* L.) flower can be attributed to the presence of phenolic compounds and flavonoids in its extracts. These phytochemicals demonstrate antimicrobial properties by mechanisms such as damaging bacterial cell walls and disrupting intracellular metabolic processes (Hmamou et al., 2022). A study by Hmamou et al. (2022) reported that the total phenolic content in *Papaver rhoeas* extracts was strongly correlated with significant antimicrobial activity against various bacterial strains, suggesting that these compounds play a key role in inhibiting bacterial growth.

Bayram et al. (2015) reported that poppy flower extract did not exhibit antimicrobial activity against several strains (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *Enterobacter cloacae* ATCC 13047, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 23897, *Escherichia coli* O157:H7 ATCC 33150, *Yersinia enterocolitica* ATCC 27729, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 33019, *Bacillus subtilis* ATCC 6630, and two yeasts: *Saccharomyces cerevisiae* BC 5461 and *Candida albicans* ATCC 1223), including *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, *S. aureus*, *E. cloacae*, *Y. enterocolitica*, *B. cereus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. The minimum inhibitory concentration (MIC) of *Papaver rhoeas* green leaves against *S. aureus* was determined to be $39.06 \mu\text{g mL}^{-1}$ (Ünsal et al., 2009). In another study, chloroform extracts from the aerial parts of the poppy plant showed significant activity against *S. aureus* (MIC = $1.22 \mu\text{g mL}^{-1}$), *S. epidermidis*, and *K. pneumoniae*, but no activity against *P. mirabilis* (Çoban et al., 2017). The MIC value of poppy flower extract against *Enterobacter faecalis* was reported to be 0.11 mg/mL (Marsoul et al., 2020). Furthermore, extracts from the green leaves of *P. rhoeas* were used in silver nanoparticle synthesis, and the MIC values for *S. aureus*, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* were 1.50, 0.75, 3.00, 6.00, and 0.37 mg mL⁻¹, respectively (İpek et al., 2023). This difference may be largely due to the extraction methods used. Bayram et al. carried out the extraction at 35 °C for 2 hours, whereas in our study, it was left to macerate at room temperature for 72 hours. This longer time and low-temperature extraction process may have made it possible to extract not only anthocyanins, but also alkaloids and other phenolic compounds with potentially antimicrobial effects. In addition, the observed differences may also be explained by the variation in phytochemical composition depending on the region and conditions in which the plant grows.

A study by Katarzyna et al. (2021) demonstrated that alkaloids extracted from poppy flowers significantly inhibited the growth of *S. enteritidis*, likely through disruption of the bacterial cell membrane or interference with metabolic processes. Similarly, Hmamou et al. (2022) found that phenolic compounds in poppy flower extract exhibited notable antimicrobial effects against *L. monocytogenes* by disrupting bacterial cell walls and altering membrane permeability. Çoban et al. (2017) also reported that poppy phytochemicals showed significant antimicrobial activity against *E. coli* O157:H7, suggesting that this effect may result from the synergistic actions of flavonoids and alkaloids. These phytochemicals were found to reduce bacterial viability by inhibiting protein synthesis and disrupting cellular functions. The antimicrobial activity observed in this study aligns with these previous findings, highlighting the potential of bioactive compounds from poppy flowers as natural antimicrobial agents. These results provide a strong foundation for the application of poppy flower extracts in the food and health industries.

Color Stability and Degradation

Anthocyanins are among the primary water-soluble pigments responsible for the wide range of colors in fruits and vegetables. Changes during food processing and storage can significantly affect the stability or degradation of these pigments (Karasu et al., 2015). The changes in anthocyanin content and the percentage of anthocyanins retained in poppy extract after exposure to different temperatures and durations are presented in Figure 3-A and 4-A, respectively. The extract was subjected to temperatures of 60 °C, 80 °C, and 100 °C, and the changes in anthocyanin content were measured at 30-, 45-, and 60-minutes relative to the initial levels. The results indicated that exposure to 60 °C caused less loss of anthocyanins compared to higher temperatures, while 60 minutes of exposure to 100 °C reduced the anthocyanin content by only 15.49%. A significant time-dependent decrease in anthocyanin content was observed across all temperature treatments ($p < .05$). The most substantial decline occurred during the first 30 minutes at 100 °C, while no statistical difference was found between 60 °C and 80 °C treatments during this period ($p < .05$). Overall, no significant difference was observed between the 60 °C and 80 °C thermal processes over the entire duration. At the end of 60 minutes, the percentage of retained anthocyanins in the extract was 87.60%, 85.22%, and 84.51% for 60 °C, 80 °C, and 100 °C, respectively. These findings suggest that the poppy extract demonstrates stability under low temperature long time (LTLT), high temperature long time (HTLT), and even extreme pasteurization or sterilization conditions. This opens the possibility for the use of poppy extract as a colorant in thermally processed products. Accordingly, PE has the potential to be used as a natural colorant and/or antioxidant additive in various heat-treated food products such as fruit juices, jams and bakery products. This potential, considering both the high thermal stability of PE and the functional properties of the phenolic compounds it contains, provides a basis for further research on its application.

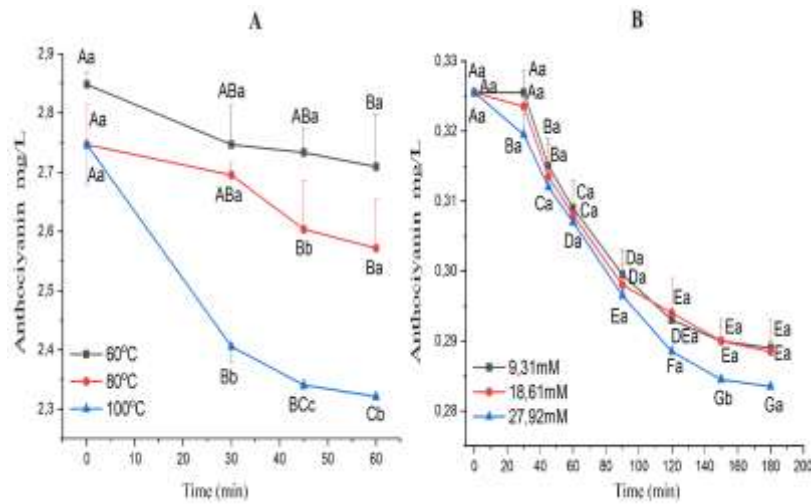


Figure 3. Changes in anthocyanin content of poppy color extract under different temperature treatments (A) and varying concentrations of hydrogen peroxide (B). (a-c Indicates statistically significant differences between different temperatures and hydrogen peroxide concentrations at the same time point ($p < .05$). A-C Indicates statistically significant differences between time points at the same temperature or hydrogen peroxide concentration ($p < .05$)).

Şekil 3. Farklı sıcaklık uygulamaları (A) ve değişen hidrojen peroksit konsantrasyonları (B) altında gelincik çiçeği özütünün antosiyanin içeriğindeki değişimler. (a-c Aynı zaman noktasında farklı sıcaklıklar ve hidrojen peroksit konsantrasyonları arasındaki istatistiksel olarak anlamlı farklılıkları gösterir ($p < .05$). A-C Aynı sıcaklık veya hidrojen peroksit konsantrasyonundaki zaman noktaları arasındaki istatistiksel olarak anlamlı farklılıkları gösterir ($p < .05$)).

Bahreini et al. (2015) studied the thermal stability of anthocyanins obtained from eggplant peel and carried out analyses at temperatures of 20, 30, and 40 °C for 1, 3, 6, 9, and 15 days. The percentage of retained anthocyanins for temperatures of 20, 30, and 40 °C was determined as 92, 93, and 92, respectively, at the end of the first day, and 64, 43, and 2 at the end of the fifteenth day. Fernández-López et al. (2013) studied the thermal stability of anthocyanins obtained from elderberry, red cabbage, hibiscus, red beet, Opuntia fruits, and red cochineal. The anthocyanin percentages of the aforementioned natural colorant extracts remaining after 1 hour at 50, 70 and 90 °C were determined as 95, 90, 90, 90, 82, 100 (50 °C); 85, 75, 70, 43, 30, 98 (70 °C); 72, 65, 50, 43, 3, 99 (90 °C), respectively. In this study, the retention of anthocyanins above 84% even at higher temperatures reveals that poppy flower extract exhibits remarkable stability against heat treatment, and in this respect, our study is superior compared to many natural sources in the literature.

The first-order kinetic model for anthocyanin degradation in poppy extract subjected to thermal treatment at

various temperatures over specific time periods was confirmed by high R^2 values ranging from 0.91 to 0.97 (Figure 4-A). Anthocyanin degradation increased across all temperature conditions over time. When examining the degradation rate constant (k), the highest rate was observed at 80 °C ($15.60 \times 10^{-4} \text{ min}^{-1}$), followed by 100 °C ($11.90 \times 10^{-4} \text{ min}^{-1}$), with the lowest at 60 °C ($4.60 \times 10^{-4} \text{ min}^{-1}$). It can be inferred that the degradation at 80 °C was approximately 3.4 times greater than at 60 °C over time. At the end of the temperature treatments, degradation percentages of 4.88%, 6.37%, and 15.49% were recorded at 60 °C, 80 °C, and 100 °C, respectively.

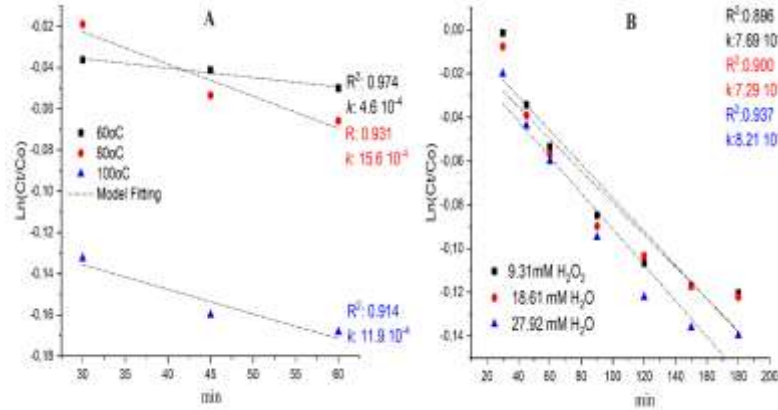


Figure 4. Degradation curves of poppy color extract under different temperature treatments (A) and varying concentrations of hydrogen peroxide (B).

Şekil 4. Gelincik çiçeği özütünün farklı sıcaklık uygulamaları (A) ve değişen hidrojen peroksit konsantrasyonları (B) altında bozunma eğrileri.

Aztatzi-Ruggerio et al. (2019) observed similar color loss when they exposed betanin, a colorant derived from beets, to heat at 75 °C for 60 minutes. In comparison, only 70% of the total anthocyanin content in microencapsulated color components was retained under light at 70 °C (Sarabandi et al., 2023). Our study demonstrates that poppy extract is heat-resistant and that food processing involving thermal treatments does not significantly affect its stability. These findings suggest that the colorant derived from poppy extract is suitable for use in the production of thermally processed food products.

Hydrogen peroxide (H_2O_2) is a biological compound found in low concentrations in air, water, plants, microorganisms, and foods (Ma et al., 2011). Hydrogen peroxide and its radicals can bind to the carbon ring of anthocyanins, causing structural breakdown and degradation (Satake & Yanase, 2018). The resistance of the plant extract to oxidation and its degradation kinetics are shown in Figure 3-B and 4-B, respectively. When examining the oxidative degradation kinetics of the pigmented poppy extract in the presence of H_2O_2 , the R^2 values were found to range between 0.90 and 0.93, indicating a high correlation between the experimental data and the linear regression analysis. The rate constants (k) for anthocyanin degradation over time were $7.69 \times 10^{-4} \text{ min}^{-1}$, $7.29 \times 10^{-4} \text{ min}^{-1}$, and $8.21 \times 10^{-4} \text{ min}^{-1}$ for the extract reacted with hydrogen peroxide concentrations of 9.31, 18.61, and 27.92 mM, respectively. The highest degradation occurred at the 27.92 mM concentration. As shown in Figure 5-B, increasing doses of H_2O_2 over time decreased the percentage of anthocyanins retained in all samples. After 30 minutes, the lowest H_2O_2 concentration (9.31 mM) retained the highest percentage of anthocyanins (99.84%). However, as the concentration increased, the retention decreased. After 3 hours, the anthocyanin retention rates were 88.65%, 88.49%, and 86.96% for the 9.31, 18.61, and 27.92 mM concentrations, respectively. While there was no significant difference between the 9.31 and 18.61 mM concentrations, the retention at 27.92 mM showed a more substantial decrease (13.04%).

The application of different doses of H_2O_2 did not cause significant changes in the anthocyanin content of poppy extract ($p < .05$). However, a statistically significant decrease in anthocyanin content occurred over time across all doses. In the first 30 minutes, no significant decrease was observed with the 9.31 and 18.61 mM H_2O_2 treatments, but after 45 minutes, all concentrations showed a statistically significant reduction ($p < .05$). Quercetin, kaempferol, and isorhamnetin are the primary phenolic compounds in poppy extract, known to be effective antioxidants against oxidative stress (Kim et al., 2014). The relatively low degradation observed is likely due to the hydrogen peroxide scavenging capacity of these phenolic compounds, which is attributed to their antioxidant properties.

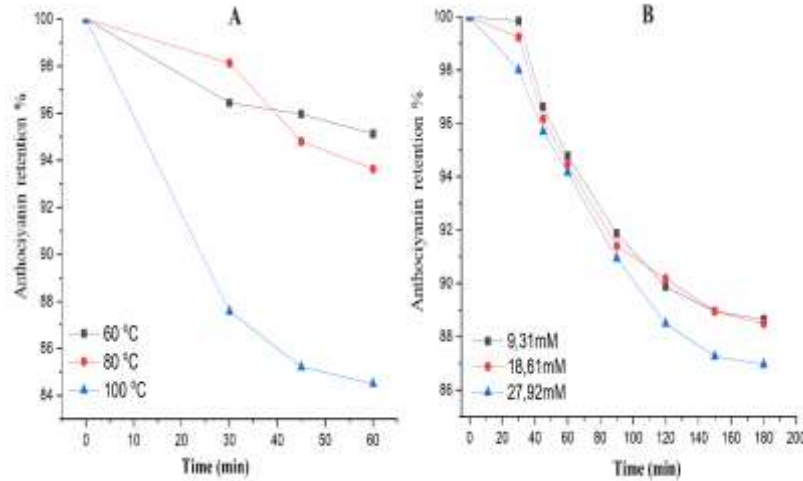


Figure 5. Percentage of anthocyanins retained in poppy color extract after exposure to different temperatures (A) and varying concentrations of hydrogen peroxide (B).

Şekil 5. Farklı sıcaklıklara (A) ve değişen konsantrasyonlarda hidrojen peroksit (B) maruziyetinden sonra gelincik çiçeği özütünde tutulan antosiyaninlerin yüzdesi.

Anthocyanins, which have a central core structure in the form of 2-phenylbenzopyrylium or flavylium cation, are classified as polyphenols and secondary metabolites (Enaru et al., 2021). Various colors such as crimson, indigo, violet, purple, and pink in flowers, fruits, and vegetables are attributed to anthocyanins. These pigments are known to change color in response to pH variations. This pH-dependent color-changing property is crucial for potential applications in monitoring food quality, determining shelf life, enhancing consumer interest, and using anthocyanins as color indicators in food packaging (Roy & Rhim, 2021).

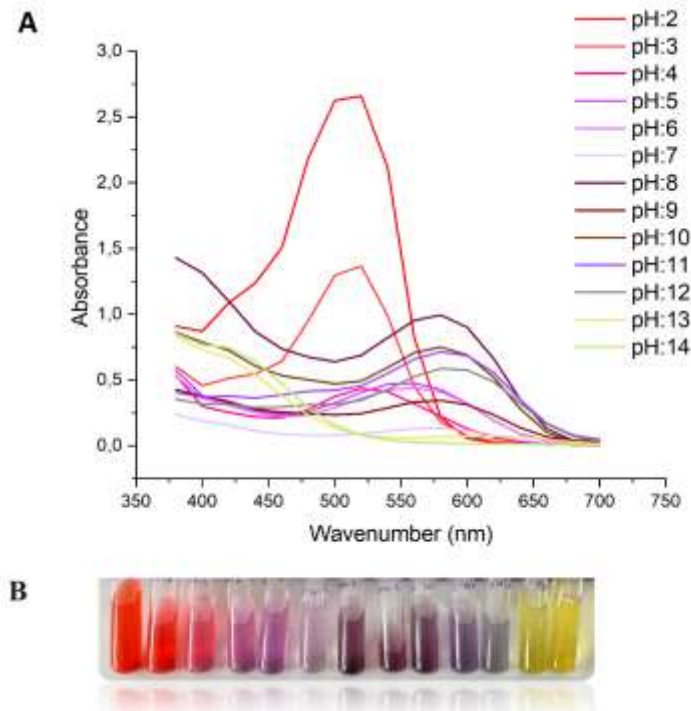


Figure 6. Spectrum scans (A) and color changes (B) of poppy color extract at different pH values.

Şekil 6. Gelincik çiçeği özütünün farklı pH değerlerinde spektrum taramaları (A) ve renk değişimleri (B).

Poppy extracts exhibited a red-pink color at pH 2-4, a more purplish (magenta) hue at pH 5-6, colorlessness at neutral pH, a brownish color at pH 8-10, a bluish tint at pH 11-12, and a greenish-yellow color at pH 13-14 (Figure 6). The red color observed at pH 2-4 is attributed to the presence of the flavylium cation, while the blue quinoidal

forms dominate at pH 5-6. At neutral pH, anthocyanins shift to the quinonoidal base form, which typically shows purple and blue hues. The quinonoidal base form becomes more prominent at pH 8-10, with increasing alkalinity darkening the colors. In highly alkaline conditions, anthocyanins shift towards yellow tones, a result of further deprotonation and structural changes in the molecule. These yellow shades are due to the formation of the chalcone form (Roy & Rhim, 2021). The ability of poppy extract to maintain its stable color in acidic environments makes it suitable for use as a colorant in acidic foods. Additionally, it has potential applications as a spoilage indicator in various food packaging systems. Studies by Bakhshizadeh et al. (2023), Tavassoli et al. (2024) and Tavassoli et al. (2023) have explored the use of poppy extract as a spoilage indicator in food packaging, with promising results.

CONCLUSION

This study assessed the potential application of poppy flowers as a natural food colorant, along with their bioactive chemical composition, activity, and mineral content. The TPC and TAC content of the poppy flower were determined as 45.10 ± 0.84 mg GAE g⁻¹ and 17.11 ± 0.60 mg cyanidin-3-glucoside g⁻¹, respectively. The antioxidant activity was determined as 0.92 ± 0.08 µg mL⁻¹ for DPPH (IC₅₀), 155.98 ± 8.73 mM TE g⁻¹ for CUPRAC, and 581.94 ± 12.09 µmol TE g⁻¹ for FRAP. In terms of antimicrobial activity, the highest inhibition was observed in *S. enteritidis*, followed by *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*, respectively. The dominant phenolic compounds in the poppy flower were identified as quercetin, kaempferol, isorhamnetin, and protocatechuic acid. To emphasize the nutritional properties of the poppy flower, an analysis was conducted on 11 minerals. The most abundant minerals were sodium (9382.77 mg kg⁻¹), potassium (5901.07 mg kg⁻¹), phosphorus (186.6 mg kg⁻¹), and iron (186.0 mg kg⁻¹). The color stability results of the poppy flower indicate that, even at the maximum temperature and duration examined (100 °C for 60 minutes), 84.15% of the anthocyanins were maintained. Although no statistically significant differences were observed among the results for the H₂O₂ concentrations tested, 86.96% of the anthocyanins were still preserved after a treatment duration of 180 min. The results demonstrate that poppy flower extract has considerable antioxidant capacity and a significant concentration of phenolic components. The extract exhibited high color stability, demonstrating resistance to thermal treatment and oxidative conditions. The extract's color-changing characteristics in relation to changes in pH highlight its potential application in food packaging as a spoilage indicator. Since the red poppy is not yet cultivated in our country, the limited feasibility studies constrain the ability to perform cost analysis and assess its commercial scalability. However, future studies focusing on this area could significantly contribute to unlocking the economic potential of the red poppy, promoting its agricultural and industrial applications. Consequently, poppy flower extract can be regarded as both a cost-effective and practical substitute for the natural colorant companies. Moreover, given the limited studies on the phenolic composition of poppy flower extract, this research will provide a valuable contribution to the literature.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Ethics Statement

This study does not involve any human or animal testing.

Conflict of Interest

The authors declare no conflicts of interest.

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Investigating The Effect of Technological Features on Tractor Preference: Case of Konya Province

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ABSTRACT

Tractors are the most important vehicle of the variety of mechanization used in the agricultural sector. Different fractions can be preferred by the manufacturer because of the many advantages it provide. Preferences that differ according to land type, land size, quality of production, and soil characteristics have necessitated a close examination of tractor markets. When researching tractor markets, it is very important to determine the expectations of the market in question. In this study, the preferences of manufacturers in Konya province in terms of technological features in choosing tractors were examined. The results of the survey conducted with 340 producers through proportional sampling were evaluated using conjoint analysis. According to the analysis results, the technological features of the tractors most preferred by the manufacturers are those with a power range of 71-100 HP, a traction feature of 4WD, price range 1-2 million TL (32,000-62,000\$), automation, hydraulic system, air conditioning, heating, and sound insulation. It was determined that they were tractors with a large cabin volume and GPS and mapping systems. Planning the organization of the Turkish tractor market in line with producer expectations and regional/local market segmentation according to producer expectations is considered important for tractor manufacturers and efficiency in agricultural production.

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Traktör Tercihine Teknolojik Özelliklerin Etkisinin Araştırılması: Konya İli Örneği

ÖZET

Traktörler, tarım sektöründe kullanılan mekanizasyon çeşitliliğinin en önemli bileşenidir. Sağladığı birçok avantaj nedeniyle üretici tarafından farklı fraksiyonlar tercih edilebilmektedir. Arazi türü, arazi büyüklüğü, üretim kalitesi ve toprak özelliklerine göre farklılık gösteren tercihler, traktör piyasalarının yakından incelenmesini gerekli kılmıştır. Traktör piyasaları araştırılırken söz konusu piyasanın beklentilerinin belirlenmesi oldukça önemlidir. Bu çalışmada, Konya ilindeki üreticilerin traktör seçiminde teknolojik özellik açısından tercihleri incelenmiştir. Oransal örnekleme yoluyla 340 üretici ile yapılan anket sonuçları, konjoint analizi kullanılarak değerlendirilmiştir. Analiz sonuçlarına göre, üreticiler tarafından en çok tercih edilen traktörlerin teknolojik özellikleri; güç aralığı 71-100 BG, çekiş özelliği 4WD, fiyat aralığı 1-2 Milyon TL (32.000-62.000\$), otomasyon, hidrolik sistem, klima, ısıtma ve ses yalıtımıdır. Bu traktörler, kabin hacmi büyük, GPS ve haritalama sistemlerine sahip traktörler olduğu belirlenmiştir. Türkiye traktör pazarının organizasyonunun üretici beklentileri doğrultusunda planlanması ve bölgesel/yerel pazar segmentasyonunun üretici beklentileri doğrultusunda yapılması, traktör üreticileri ve tarımsal üretimde verimlilik açısından önemli görülmektedir.

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INTRODUCTION

Agricultural machinery markets are shaped by economic and political movements on a national, regional, and global scale. The products in the market in question have some differences from other markets and products according to their features and qualities specific to the field (Gazzarin, 2019). These differences are harsh working conditions, minimum working hours despite its potential, seasonal use, high need for security, long economic life, and operation in rugged terrains. On the other hand, many handicaps arising from the nature of agricultural production (energy costs, input-intensive production techniques, high risk, low return, etc.) can lead to the problem of narrowing access to capital for the acquisition of agricultural machinery (Okonkwo et al., 2023). However, policymakers and other stakeholders are struggling with factors that directly affect economic phenomena, such as the shrinkage of arable agricultural lands, deterioration of land quality, and the effects of global climate change. However, because this process is dynamic, the agricultural machinery industry continues to develop in a certain motion (Daum and Birner, 2020; Srivastava, 1993). Modern agricultural machinery has begun to become widespread in the agricultural production systems of developing countries worldwide, and this has caused an increase in the demand for agricultural machinery and tractors (Mottaleb, 2018). In every agricultural land, several tillage operations are carried out after harvest to prepare the desired soil conditions for planting the next crop (Singh et al., 2019a; Singh et al., 2019b). They do this with the help of tractors. From this perspective, tractors can be seen as the backbone of the agricultural sector. Meeting the increasing demand can only be achieved with increased productivity and correct production techniques and achieving this is almost impossible without tractors (Singh et al., 2021; Singh et al., 2022a; Singh et al., 2022b; Singh et al., 2022c). However, although tractors and related machines play an important role in increasing agricultural production, they may cause some problems that will cause discomfort and health problems to their drivers due to the lack of comfort and technological opportunities. At this point, it is considered very important to investigate the existence of technological opportunities that are effective in increasing agricultural production and to determine the preferences of the users (Servadio et al., 2007). Another important element is the cost of agricultural production. Soil tillage in agricultural production is a high-cost input because of both the energy used and its low portfolio (<0.4 ha/day). If a rough calculation is made, considering that field agriculture is carried out on an area of 15.5 million hectares in Türkiye, the average annual fuel consumption is 620 million liters, depending on the soil processing methods. This amount constitutes 2.5% of the total diesel used in Türkiye (24 million tons). 1% savings in fuel consumption can contribute approximately 6.2 million dollars to the country's economy (Marakoglu and Çarman, 2010). In order to increase the fuel efficiency, traction performance, and stability of tractors, systems have been designed and implemented that will provide simultaneous easy and precise measurement of slip and traction resistance by providing visual and auditory warnings to the operators (Raheman and Jha, 2007, Kumar et al., 2016, Kumar et al., 2017). These systems can provide information to operators. In addition, the systems are designed to ensure continuous control of the traction force depending on the highest traction force produced by the tractors. It is known that traditionally operators had to constantly adjust the depth to obtain the required traction power, and it would be correct to state that this situation has negative effects on productivity (Dwyer et al., 1974, Cowell and Milne, 1977). In the calculations, it was determined that the operating frequency of a hydraulic control level is 3.13 times/min, and only 40% of these operations prevent excessive shifts (Ismail et al., 1981, Ismail et al., 1983).

The existence of support systems that determine demand-side decisions in the acquisition of agricultural tools and machinery should be multifaceted. Because it is stated that approximately 150 different tools, machines, and devices are used in a modernized farm. Therefore, market expectations need to be analyzed well when marketing agricultural machinery. Thus far, various studies on agricultural machinery and tractors have been revealed in research on agricultural machinery. Although the research was aimed at filling the gap in the literature, it allowed for ranking the studies in a general framework (Durczak, 2011).

The main purpose of this research is based on the following motivation: evaluation of agricultural machinery is important from every aspect. The need to evaluate the quality/qualities/features of agricultural machinery and tractors is especially noticed by farmers who use these machines. In this research, we examined how the differences in tractor technologies, which are considered essential in terms of both comfort and work efficiency, are included in manufacturer preference decisions. The results determined according to the multi-dimensional preference criteria will shed light on policymakers both in terms of the tractor market and sectoral efficiency projections.

MATERIAL and METHOD

Method Used in Obtaining Data

The research was carried out in the Konya province. In Konya, there are 107 thousand agricultural enterprises (TOB, 2023) and 74,628 tractors in the Farmer Registration System (TUIK, 2024). Konya has characteristics representing Turkish agriculture, considering its diversity in production, sociodemographic structure, and five agroecological production regions. Within the scope of this study, the preference for technological features of tractors with 35 horsepower was investigated. The number of tractors with 35 horsepower and above in Konya is 69,121 (TUIK, 2024). The presence of tractors by district in Konya is given in Table 1.

Table 1. Tractor presence by district
Çizelge 1 İlçelere göre traktör varlığı

Districts	Number of tractors	Tractor Ratio	Number of Surveys
Çumra	7500	12.08	41
Karatay	6561	10.57	36
Altınekin	5129	8.26	28
Cihanbeyli	4650	7.49	25
Ereğli	4332	6.98	24
İlgın	4323	6.96	24
Akşehir	3804	6.13	21
Beyşehir	3730	6.01	20
Seydişehir	3250	5.23	18
Kadınhanı	3200	5.15	18
Karapınar	2972	4.79	16
Meram	2887	4.65	16
Yunak	2631	4.24	14
Selçuklu	2618	4.22	14
Kulu	2549	4.11	14
Sarayönü	1952	3.14	11
Total	62,088	100.00	340

In Konya, 16 districts with tractors of 35 horsepower and above were purposely determined, and the main mass was created. The presence of tractors in these 16 districts constitutes 89.83% of the total tractor existence of 35 horsepower and above. The Proportional Sampling Method was used to determine the number of businesses to be surveyed (Newbold et al., 1995).

$$n = \frac{N(pq)}{(N-1)D^2 + (pq)}$$

In the formula, n= sample size, N = number of businesses in the population (62,088), $D^2 = (d/t)^2$, d = the amount of error allowed in the population average, and t = the value of the allowed confidence limit (t) in the distribution table.

p = Ratio of tractor presence that maximizes the sample volume. In a study conducted in Konya Çumra district (Keleş & Haciseferoğulları, 2016), the presence of tractors per enterprise was determined to be 1.04 and in the Kadınhanı district to be 0.81 (Yalman, 2008). In this study, $p = 0.5$ was used to reach the maximum number of samples. Since $q = 1-p$, $q = 0.5$ is taken.

Using the formula given above, the number of businesses to be surveyed was determined to be 338 with a 99% confidence interval and a 7% margin of error. But it was made 340. In the distribution of the enterprises to be surveyed by districts, the ratio of the districts' tractor assets to the total tractor assets was considered (Table 1).

Method Used to Analyze Data

In this study, conjoint analysis was used to determine the factors affecting tractor preference. Conjoint analysis follows the decomposition approach. It assumes that the overall preference for a product (or service) is determined by preferences for specific features of the product, that these preferences come together, and that the participant maximizes his/her own utility when deciding on the features of the product (Heck et al., 2024). When applying conjoint analysis, participants are asked questions indirectly, assuming that they cannot express their preferences reliably. Participants are presented with various sets of features with different characteristics and are asked to rate them. Based on the participants' overall ratings, their relative preferences (partial value utilities) for different levels are inferred. The determined partial value utilities can be used in simulations to investigate which feature

combinations are most preferred in the relevant product choice (Orme, 2010). Conjoint models are constructed according to the additive part-value rule, which is generally preferred (Ness, 1997; Hair et al., 1990). In the additive part-value model, it is assumed that the part-value of each attribute level is independent and that the total utility of any product profile is the sum of the part-values of the attribute levels of that product profile. Assuming that S_{ij} represents the consumer's level of preference for any alternative product profile (for one of the sixteen choices presented to him), W_{iat} represents the utility or part-value corresponding to level t of attribute a for person i , and Y_{jat} represents a variable expressing the existence of level t of attribute a in product profile j , the additive part-value can be formulated as follows:

$$S_{ij} = \sum_{a=1}^n W_{iat} Y_{iat}$$

The aim of conjoint analysis is to calculate W_{iat} values for each feature level.

In addition to traditional conjoint analysis, the most widely used conjoint analysis today is choice-based conjoint analysis (Sawtooth Software, 2017). In choice-based conjoint analysis, participants are presented with different concepts and asked to choose one of them; In traditional conjoint analysis, participants rate the alternatives. Selecting attributes and attribute levels is a critical step in choice-based conjoint analysis. Additionally, manufacturers' preferences are categorized by the size of their business based on the average scores they give to their conjoint cards.

RESULTS

The average age of the producers participating in the research was determined as 42.32 years, 37.05% of the education level had a high school, the average irrigated land size was 338.66 da, and the average dry land size was 128.42 da. In a study conducted in Erzurum province, the average age of producers was calculated as 41.39, and the amount of irrigated land was 98.89 da (Aksoy et al., 2019). In a similar study conducted in the region, the average dry land size was calculated as 128.42 da (Baybas & Aksoy, 2021). When the production pattern in the region is examined, sugar beet, calf corn, silage corn, alfalfa, potatoes, tomatoes, watermelons, melons, and onions are produced in irrigated areas. It can be stated that in dry areas, wheat, barley, sunflower, chickpeas, and lentils are produced. Within the scope of the research, the average number of tractors per enterprise was determined to be 1.77 units, and the average horsepower was determined to be 100.96 hp. The results of the conjoint analysis applied to determine the technological features effective in the tractor preference of the participating producers are given in Table 2. The results obtained reflect the benefit scores and average importance levels of the technological features. Pearson's R and Kendall's tau values, which reflect the suitability of the model, were found to be statistically significant ($p < 0.01$). When the features are examined in terms of tractor technology, the most important feature is the presence of a cabin (20.79%). GPS (13.80%), Security (12.89%), Control (12.17%), Hydraulics (11.26%), Horsepower (10.18%), Price (9.46%), Emission (9.11%), and Propulsion (0.30%). When the lower levels are examined in terms of benefits, it can be stated that tractors with 1-2 million TL in terms of price, tractors with 101-150 hp in terms of horsepower, and tractors with 2WD in terms of propulsion come to the fore.

Preferences in different criteria and total benefit scores obtained because of the combinations created from the preference cards presented to the producers in determining the technological features that affect tractor preference are given in Table 3.

Table 3 shows the tractor features preferred by manufacturers and their related benefit scores. When the 16 profiles created were ranked in terms of benefit scores, the most preferred profile was profile 16, and the least preferred profile was profile 1. Accordingly, the most preferred tractor type by producers in Konya province in terms of technological features has a power range of 71-100 hp, 4 WD in terms of Propulsion, a price of 1-2 million TL, an automated hydraulic system, air conditioning, heating, sound insulation, and cabin volume. It was determined that they were large tractors equipped with GPS and mapping systems. The least preferred tractor type in terms of technological features is the tractors that have 40-70 hp engine power, 2WD in terms of drive, a price between 1 and 2 million TL, and do not have any other technological features. When Table 4 is examined, according to the average scores of 16 preference cards on the 5-point Likert scale, the card in the 16th row is compatible with Table 3.

DISCUSSION

Using farm equipment in agriculture and updating it according to developing technologies provides many advantages. These advantages include labor saving, resource efficiency, output efficiency, saving time in tillage and land management, and obtaining several crops per year in the case of suitable irrigation facilities and climatic factors. The most important of these farm tools is the tractor. The development of tractor technologies is extremely

important for agricultural production. While the presence of GPS systems enables automatic steering, air conditioning, and cabin systems enable labor efficiency because they are comfort oriented. While appropriate tractor power provides work efficiency, optimal capital and fuel consumption provide economic advantages. Therefore, manufacturers' preference criteria for tractor acquisition form the basis of national policy. For example, Ethiopia publicly announced its decision to encourage the acquisition of tractors to reduce postharvest losses and increase the mechanical strength index (ENPC, 2015). In addition, an agricultural mechanization strategy has been proposed to increase the level of advanced agricultural mechanization by supporting middle-income producers and to ensure national food production and security (ATA, 2017). Various methods have attracted attention in the literature for determining tractor preference. Mehta et al., (2011) developed a decision support system to determine tractors and suitable equipment working in different field conditions, and Zou et al., (2011) developed a hybrid system that allows selection using neural networks and a support vector machine. Grisso et al., (2014)'s selection method, developed with tractor test data, and Osman, (2011)'s methods for determining selection criteria with linear programming can be listed. In this research, tractor options with different technological features were presented to the manufacturer, and the manufacturer selected the most suitable ones among 16 different selection criteria. And then the data was analyzed with Conjoint Analysis. The research was conducted in the Konya province of Türkiye. One of the most important regions where intensive agriculture is practiced in Türkiye is Konya. Konya province, which consists of five agro-ecological zones and 31 districts, has an agricultural structure that can represent the whole of Türkiye in terms of both business scale size and production pattern.

Table 2. Utility Scores and Average Importance Values Resulting from the Conjoint Analysis

Çizelge 2. Konjoint Analizinden Elde Edilen Fayda Puanları ve Ortalama Önem Değerleri

Technological Features Affecting Tractor Preference		Utility Estimate	Std. Error	Average importance score (%)
Horsepower	40-70	-.184	.095	10.187
	71-100	-.019	.095	
	101-150	.151	.095	
	150-+	.052	.095	
Propulsion	2-WD	.005	.055	0.304
	4-WD	-.005	.055	
Price	between 1 Million and 2 Million	.156	.073	9.463
	between 2 Million and 4 Million	-.001	.085	
	more than 4 Million	-.155	.085	
Hydraulics	None	-.185	.055	11.262
	Automated Hydraulic System	.185	.055	
Cabin	None	-.342	.055	20.794
	Air conditioning, heating, sound insulation, and cabin volume	.342	.055	
GPS	None	-.227	.055	13.808
	GPS and Mapping Systems	.227	.055	
Control	None	-.200	.055	12.173
	Intelligent speed control, autopilot systems, and data collection features	.200	.055	
Security	None	-.212	.055	12.897
	Emergency Braking System, Collision Prevention System, Rear View Camera, Airbag	.212	.055	
Emission	None	-.150	.055	9.112
	A system designed to reduce pollutants released into the atmosphere from exhaust gasses	.150	.055	
(Constant)		2.850	.058	
Correlation of Observed and Expected Values				
		Value	Sig.(p)	
Pearson's R		.987	.000	(p<.05)
Kendall's tau		.917	.000	(p<.05)

Table 3. Total and Ranking of the Utility Values of All Profiles

Çizelge 3. Tüm Profillerin Fayda Değerlerinin Toplamı ve Sıralaması

Row	Horsepower	Propulsion mechanism	Price	Automated Hydraulic System	Cabin Design and Comfort (Air Conditioning, Heating, Sound Insulation and Cabin Volume)	GPS and Mapping Systems	Control Systems (Smart speed control, automatic pilot systems and data collection features)	Security Technology (Emergency Brake System, Collision Prevention System, Rear View Camera, Airbag)	Emission Control Systems (Systems designed to reduce pollutants released into the atmosphere from exhaust gasses)	Total Score
1	between 40 and 70 hp	(2-WD)	between 1 million and 2 million	None	None	None	None	None	None	-1.341
2	between 101 and 150 hp	(4-WD)	between 2 million and 4 million	None	None	Yes	None	None	None	-0.718
3	between 71 and 100 hp	(4-WD)	More than 4 million	Yes	None	None	None	None	Yes	-0.514
4	between 40 and 70 hp	(4-WD)	More than 4 million	None	None	None	Yes	Yes	None	-0.466
5	between 71 and 100 hp	(2-WD)	between 2 million and 4 million	None	Yes	Yes	None	Yes	None	-0.207
6	More than 150 hp	(4-WD)	between 1 million and 2 million	None	Yes	None	None	Yes	None	-0.005
7	between 101 and 150 hp	(2-WD)	between 1 million and 2 million	Yes	Yes	None	None	Yes	Yes	0.05
8	between 101 and 150 hp	(4-WD)	More than 4 million	None	Yes	None	Yes	None	Yes	0.059
9	between 71 and 100 hp	(2-WD)	between 2 million and 4 million	None	None	None	Yes	Yes	Yes	0.077
10	More than 150 hp	(2-WD)	between 2 million and 4 million	Yes	Yes	None	Yes	None	None	0.194
11	More than 150 hp	(2-WD)	More than 4 million	Yes	None	Yes	None	None	Yes	0.394
12	More than 150 hp	(4-WD)	between 1 million and 2 million	None	None	Yes	Yes	Yes	Yes	0.466
13	between 40 and 70 hp	(2-WD)	between 1 million and 2 million	None	Yes	Yes	Yes	None	Yes	0.499
14	between 101 and 150 hp	(2-WD)	between 1 million and 2 million	Yes	Yes	Yes	None	Yes	None	0.646
15	between 40 and 70 hp	(4-WD)	between 2 million and 4 million	Yes	Yes	Yes	None	Yes	Yes	0.726
16	between 71 and 100 hp	(4-WD)	between 1 million and 2 million	Yes	Yes	Yes	Yes	None	None	0.726

Table 4. Average Score of the Preference Criteria by Business Size Groups

Çizelge 4. İşletme Büyüklük Gruplarına Göre Tercih Kriterlerinin Ortalama Puanı

	Agribusiness Size Groups (da)			Total
	0-100	101-300	301-+	
1	3.584	3.694	3.589	3.621
2	2.232	1.963	2.084	2.100
3	2.424	2.056	2.290	2.265
4	2.384	2.176	2.458	2.341
5	2.912	3.037	2.822	2.924
6	2.808	2.815	2.757	2.794
7	3.584	3.694	3.589	3.621
8	3.152	2.981	2.879	3.012
9	2.800	2.630	2.636	2.694
10	3.112	2.991	2.991	3.035
11	2.704	2.602	2.523	2.615
12	3.544	3.398	3.196	3.388
13	3.112	3.194	3.215	3.171
14	3.392	3.565	3.495	3.479
15	3.472	3.778	3.729	3.650
16	3.664	3.685	3.692	3.679

CONCLUSION

When the research results obtained are examined, it can be stated that there is a holistic demand for tractor preference in terms of technological features, apart from safety and emission criteria. It has been concluded that tractor power, which is sized according to the average business size, is sufficient if it is double-wheel drive and in the range of 71-100 hp. Considering the agricultural structure of Konya, which is the study area, it can be stated that tractors are mostly used in soil cultivation and in transportation operations. Therefore, it can be said that safety and emission preferences are not considered among the primary criteria by the manufacturer. These results are important in terms of taking regional dynamics into consideration when making simulations for the tractor market, diversifying financing opportunities/financing range, and creating marketing systems. However, it is important to detail the findings obtained on a national and regional basis. Although Konya province has a profile that can represent Turkey in terms of agriculture, it can be stated that the business structures in Turkey vary from region to region and even in different provinces in the same region. Therefore, it is recommended to expand this study in order to ensure the correct selection of tractors, which are the most important tools of agricultural production suitable for the conditions of the age, and to increase agricultural productivity accordingly.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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Dağıtılmış Gecikme Modelleri: Koyck ve Almon Modeli: Tarım Verileri Üzerine Bir Uygulama

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ÖZET

Mısır, sıcak iklim tahılları grubuna giren tek yıllık bit bitki olup, dünyada tahıllar içinde üretim açısından buğdaydan sonra ikinci sırada yer almaktadır. Mısır, hayvan yemi ve insan yiyeceği olarak çok değişik alanlarda kullanılmaktadır. Mısır bitkisi, Türkiye’de buğday ve arpadan sonra en fazla üretilen hububat bitkisidir. Tarla bitkileri sınıfına giren mısır bitkisinin üretim miktarı ve fiyat arasındaki ilişki Koyck ve Almon yöntemleri kullanılarak analiz edilmiştir. Çalışma verileri Türkiye İstatistik Kurumu (TÜİK)’ndan alınan 2000-2023 yılları arası dönemi kapsamaktadır. Koyck modelinden elde edilen sonuçlara göre mısır üretimi, geriye dönük olarak en çok son üç yıllık fiyattan etkilenmektedir. Mısır fiyatlarında oluşacak olan değişim 6.194 yıllık süreçte üretim bazında dikkat çeken bir etkiye sebep olmuştur. Analizden elde edilen bir diğer sonuç, t döneminde görülen mısır fiyatlarındaki bir TL artma miktarı, üretim miktarını 302283.6 ton arttırmıştır. Bunun yanında t-1 dönemde fiyatta görülen bir TL artma üretim miktarını 2602666.2 ton artırırken, t-2 dönem fiyatındaki bir TL artma 224089.2 ton, t-3, t-4, t-5 ve t-6 dönem fiyat için bir TL artma, üretim üzerinde sırasıyla 192940.8, 166122, 143031.1 ve 123149.7 ton artışına sebep olmaktadır. Mısır bitkisinin fiyat bazındaki gecikmeli değerlerindeki değişimler üretim miktarı üzerinde azalan yönde pozitif etki oluşturmuştur. Modelin uygunluğu için determinasyon katsayısı (R^2), düzeltilmiş determinasyon katsayısı ($\bar{R}^2$), Bayesci Bilgi Kriteri (BIC) ve Akaike Bilgi Kriteri (AIC) uyum iyiliği istatistikleri incelenmiştir. Uyum iyiliği testleri incelendiğinde Koyck modeli için $R^2=0.940$, $\bar{R}^2=0.925$ ve BIC=29.340 sonuçlarına ulaşılmıştır. Almon modeli için $R^2=0.935$, $\bar{R}^2=0.924$ ve BIC=29.374 sonuçlarına ulaşılmıştır. Dolayısıyla her iki modelin de uygun olduğu görülmüştür. Ancak, araştırma bulguları bize, mısır üretimi-fiyat ilişkisini Koyck Modelinin biraz daha iyi sonuçlar verdiğini göstermiştir.

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Distributed Lag Models: Koyck and Almon Model: An Application on Agricultural Data

ABSTRACT

Corn is an annual plant that falls into the warm-weather cereal group and ranks second in terms of production among cereals in the world after wheat. Corn is used in many different areas as animal feed and human food. Corn is the most produced cereal plant in Turkey after wheat and barley. The relationship between the production amount and the price of corn plant, which is classified as a field crop, was analyzed using the Koyck and Almon methods. The study data covers the period between 2000-2023, obtained from the Turkish Statistical Institute (TSI). According to the results obtained from the Koyck model, corn production is retrospectively affected by the last three years' prices. The change in corn prices has caused a remarkable effect on production in a period of 6.194 years. Another result obtained from the analysis is that a one TL increase in corn prices in period t increased the production amount by 302283.6 tons. In addition, while a TL increase in price in period t-1 increases the production amount by 2602666.2 tons, a TL increase in price in period t-2 increases the production amount by 224089.2 tons, a

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TL increase in price in periods t-3, t-4, t-5 and t-6 increases the production amount by 192940.8, 166122, 143031.1 and 123149.7 tons, respectively. Changes in the lagged values of maize prices have a positive effect on production in a decreasing direction. For the suitability of the model, the coefficient of determination (R^2), the adjusted coefficient of determination ($\bar{R}^2$), the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC) goodness of fit statistics were examined. When the goodness of fit tests is analyzed, $R^2=0.940$, $\bar{R}^2=0.925$, and BIC=29.340 for the Koyck model. The Almon model has resulted in $R^2 = 0.935$, adjusted $R^2 = 0.924$, and BIC = 29.374. Therefore, it is observed that both models are suitable. However, the research findings indicate that the Koyck model provides slightly better results in explaining the corn production-price relationship.

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GİRİŞ

İnsanların beslenme sürecinde ana unsur hayvansal besinler olarak görülse de hayvan yemi olarak da kullanıldığı için bitkilerin birinci sırada olduğunu söylemek mümkündür. İnsanların besin kaynağı olarak görülen hayvan yetiştiriciliğinin çoğunluğu bitkilerle ilişkili olduğundan, bitki üretimini geliştirmek önem taşımaktadır. Türkiye'nin halihazırda, tarımdaki durumuna bakılırsa, mısır, nohut, patates, kuru fasulye vb. ürünlerde verimliliğin yüksek olduğu görülmekle birlikte daha ileri seviyeye çekilme gerekliliği, artan nüfusla birlikte zorunluluk haline gelmiştir. Bitkisel üretimin verimliliğine etki eden faktörlerin sosyal, çevre ve ekonomik faktörler olarak sıralanması mümkündür (Tuğay, 2012).

Küresel gıda fiyatları 2008 yılından itibaren artma eğilimi göstermiş ve birçok gıda türünün fiyatında yaklaşık olarak katı kadar artış gözlemlenmiştir. Bu süreçten sonraki yıllarda gıda fiyat enflasyonunun kontrol altına alınması mümkün olmamıştır. FAO'ya (Birleşmiş Milletler Gıda ve Tarım Örgütü) göre 2003 yılı için gıda fiyat endeksi 97,7 olurken, bu değer 2008 yılında 201,4 olarak belirlenmiştir. 2011 yılında 211,9 olarak belirlenmiş ve daha sonra gerileyerek 2017 yılında 174,6'lara düşmüştür. Ocak 2021 başında gıda fiyat endeksi Aralık 2020 değerine göre 4,3 puan daha yüksek olan 113,3 ortalama puana sahiptir. Gıda fiyatlarında görülen sürekli artış, tüm tahıllarda olduğu gibi mısır ürününde de görülmüştür (Sun ve ark., 2023).

Mısır bitkisinin bir başka özelliği de benzersiz enerjilendirme özelliklerine sahip başlıca tahıl ürünlerinden biri olmasıdır. Ham petrol gibi fosil yakıtların yenilenemeyen doğası sebebiyle çevreyi koruma gereksinimi günden güne artmış bu da biyokütle enerjisine yönelik küresel talebin ilgisine yol açmıştır. Bu süreçte, biyoetanol üretimi için ana hammadde olarak kullanılan mısır, kaçınılmaz olarak ham petrol piyasasından etkilenmektedir. Ek olarak, mısırın spot fiyatı 2020'den bu yana yükselmektedir. Nispeten vadeli fiyat dalgalanma bilgisi sağlayan yüksek oranda ilişkili olan mısır, vadeli işlem piyasası da yoğun ilgi görmüştür (Feng ve ark., 2023).

Gıda güvenliği, insan yaşamının ve ulusal güvenliğin temelini oluşturmaktadır. Gıda ve Tarım Örgütü'ne (FAO) göre, 2020 yılında dünya nüfusunun yaklaşık %10'u açlık çekmiştir. Artan küresel nüfusla birlikte, küresel gıda talebi 2005'e kıyasla 2050 yılında yaklaşık olarak %100-110 civarında artacağı tahmin edilmektedir. Bu durumun da gıda güvenliğini önemli ölçüde zorlayabileceği düşünülmektedir. Mısır, dünyada en çok ekilen tahıllardan biridir. Sürdürülebilir mısır üretimi, hızla artan gıda talebini karşılamada ve ulusal ve küresel gıda güvenliğini sağlamada önemli bir rol oynamaktadır. Mısır, insanlar ve hayvanlar için önemli bir gıda kaynağı ve endüstriyel hammaddedir (Peng ve ark., 2023).

Buğday, mısır ve pirinç dünyanın önde gelen temel tahılları arasında yer almaktadır. Bu bitkilerin her biri yaklaşık olarak 200 milyon ha alanda yetiştirilmektedir. Mısır bitkisi, 9.000 yıldan daha önceki süreçte Güney Meksika'da evcilleştirilmiştir. Bu süreç, buğdayın Yakın Doğu'daki ve pirincin Çin'deki yaklaşık 10.000 yıl önce evcilleştirilmesinin ardından gerçekleşmiştir. Mısırın biraz daha geç evcilleştirilmesine ve Avrupalıların Amerika'ya yerleşmesine kadar göreceli izolasyonuna rağmen, mısır o zamandan günümüze kadar hızlı bir şekilde dünyaya yayılmıştır. Yıllık üretimi 1 milyar metrik tonu aşarak önde gelen küresel temel tahıl haline gelmiştir (Erenstein ve ark., 2022). Mısır, 2001'den bu yana dünyanın en büyük ikinci tahılı olarak birinci de geride bırakmıştır (Peng ve ark., 2023).

Yeryüzünde insanların beslenmesinde önemli rolü olan altı tahıldan biri olarak görülen mısır, ekilen tarım alanları olarak değerlendirildiğinde buğdaydan sonra 2. sıradaki tahıl olarak dünya sıralamasına girmiştir. Mısır bitkisi çok yönlü kullanım alanına sahip olması, adaptasyon kabiliyetinin yüksek olması ve verimlilik gibi

sebeplerle Dünyanın en çok üretilen tahılı olarak karşımıza çıkmaktadır. Dünya genelinde üretim yapılan 1,5 milyar ha tarım alanlarının 721 milyon ha'nı tahıllar oluşturmaktadır. Tüketim miktarlarına bakıldığında ilk sırada buğday, peşi sıra mısır ve çeltiktir. Mısır, buğdaydan sonra 2. sırada görülürken toplam üretim olarak bakıldığında Dünya birincisidir. Mısır bitkisinin popüler olmasının sebepleri arasında insan gıdası olarak kullanılması, hayvan yemindeki katkısı ve sanayide ham madde olması da sıralanabilir. Mısır bitkisini gelişmiş ülkelerin çoğunluğu hayvansal yem şeklinde kullanırken, gelişmekte olan ülkeler insanların tüketiminde kullanmaktadır (Turhal, 2021).

Mısır bitkisine dünya konjonktüründeki artan talep sayesinde, gelişmiş ekonomiler işleme endüstrisinin gelişimini teşvik edecek ve destekleyecektir. Çünkü tarımsal ürünlerin işleme süreci ülke ekonomisi açısından da birçok avantaj getirmektedir. İlk akla gelenler daha fazla işgücüne ihtiyaç, ödenen vergiler ve yerli ürünlerin tüketimindeki artışlar olarak sıralanabilir (Tanklevska ve ark., 2020).

Mısır mahsulü aslen tropiklerden gelse de yetiştiriciliği ılıman iklimlere sahip bölgelere taşınmıştır (Waqas vd., 2021). Türk tarımında önemli bir yer tutan bitkisel üretim zamana göre farklı olmakla birlikte, son iki yıl için bir değerlendirilme yapıldığında ürünlerin tamamında bir artışın varlığı söz konusudur. 2021 yılı verileri göstermektedir ki, buğday %11,9 oranıyla bir üretim artışı göstererek 19,8 milyon tona ulaşmıştır. Mısır üretimindeki artış %25,9 oranı ile 8,5 milyon ton olurken, arpa üretimi %47,8 bir artışla 8,5 milyon tondur. Çavdar üretimindeki artış ise %36,5 oranı ile 273 bin ton gibi yüksek bir orandadır. Keza yulaf üretimi de çavdarı takip eden yüksek bir orandaki artışla (%32,2 oranında artarak) 365 bin ton olmuştur (TÜİK, 2024).

Tarımsal fiyat değişkenliği gelişmekte olan ülkelerin çoğunda akut bir endişe kaynağıdır. Fiyat değişkenliği hem tüketiciler hem de kırsal üreticiler için önemli ekonomik maliyetlerle ilişkilidir. Aynı zamanda ekonomik büyüme, yerel geçim kaynakları ve gıda güvenliği üzerinde de etkileri vardır. İstikrarlı gıda fiyatları sağlandığında tarımsal yatırımların teşvik edilmesi artar, yoksul üreticiler ve tüketiciler için yoksulluk tuzakları önenebilir. Tüm bunların bir sonucu olarak kırsal göçü caydırabilir. Bu düşünceler, hükümetin yerel emtia piyasalarına fiyat istikrarı amacıyla çeşitli müdahalelerde bulunmasına neden olmuştur. Bu politikalar, üretici fiyat desteği gibi doğrudan fiyat müdahalelerinden, ihracat yasakları ve tampon stok yatırımları gibi daha dolaylı müdahalelere kadar uzanır. Tarımsal fiyat değişkenliğini azaltma politikaları yalnızca gelişmekte olan ülkelere özgü değildir. Gelişmiş ekonomilerdeki tarımsal piyasaların ortak özellikleri arasında ayrıca ürün sigortası, pazarlama ve üretim sözleşmeleri ve stratejik tampon stoklar gibi ikinci derece fiyat istikrar mekanizmaları da bulunmaktadır.

Literatür incelendiğinde konu ile ilgili pek çok çalışma olduğu görülmüştür. You ve ark. (2023) yaptıkları çalışmada erken sezon mısır haritalama yöntemini geliştirmişlerdir. Abokyi vd. (2020) çalışmalarında mısır üreticilerinin çıktı fiyatı desteğinin Gana'daki küçük çiftçilerin gelirleri üzerindeki etkilerini araştırmaktadır. Ge ve Wu (2020) esas olarak mısır fiyatının değişen eğilimini ve mısır fiyatını etkileyen faktörleri analiz etmişlerdir. Faktörlerin belirlenmesinde regresyon analizini kullanılarak, mısır fiyatını tahmin etmek için sırasıyla tek değişkenli doğrusal olmayan ve çok değişkenli doğrusal regresyon modelleri oluşturulmuştur. Analiz sonucunda, mısır fiyatındaki değişikliklerin diğer gıda fiyatları ve mısır stokları gibi birçok başka faktörden de etkilenmiş olduğu sonucuna varılmıştır.

Tarım ürünleri fiyatları incelendiğinde güçlü dalgalanmalara tabii oldukları gözlemlenmiştir. Fiyatta oluşan bu riskli durum, düşük talep fiyat esnekliğiyle ilişkili olan üretim kararı ile hasat zamanlaması arasındaki gecikmeden kaynaklanmaktadır (Assouto ve ark., 2020).

Birçok ekonometrik çalışma, dağıtılmış gecikmeli (örneğin Koyck ve Almon) modeller aracılığıyla başlangıçtaki fiyat darbelerinin birikmiş etkilerini ifade etmek için 'uzun vadeli' esnekliği kullanır; bu modellerde, genellikle etkinin büyük çoğunluğunun (%95'ten fazla) on yıl içinde meydana geleceği tahmin edilir (Bashmakov et al., 2024).

Bu makalenin amacı, Koyck ve Almon modelleri kullanılarak mısır bitkisinin üretim miktarı ve fiyat ilişkisinin incelenmesi ve fiyattaki değişimin üretime olan etkisinin belirlenmesidir.

MATERYAL ve METOD

Araştırmanın materyali Türkiye'de 2000-2023 yılları arası mısır üretim miktarı ve fiyatından oluşmaktadır. Çalışmada Mısır üretim miktarı bağımlı değişken olurken mısır fiyatı bağımsız değişkendir. Veri toplama aracı: www.tuik.gov.tr web sitesinden TÜİK (2024) kayıtlarından sağlanmıştır. Verilerin analizinde gecikmesi dağıtılmış modellerden Koyck ve Almon modelleri kullanılmıştır.

İstatistik Analizler

Koyck Modeli

Koyck tekniği, nicel araştırma yöntemleri içinde yer alan bir zaman serisi analizi yöntemi olduğu için deneysel olmayan desenler içinde yer almaktadır. Koyck modeli ayrıca bağımlı değişken ile bağımsız değişkenler arasındaki ilişkileri incelediği için ilişkisel desen içerisinde yer almaktadır.

Koyck tekniği β parametrelerine sınırlama getiren bir tekniktir. Koyck modelinde, sonsuz sayıda gecikme modelinde yer alan β gecikme katsayıları bir geometrik dizi olarak azalış gösterdiğinden yola çıkılır. Gecikmeli model bu varsayım üzerine kurulur. Koyck modelinin genel hali

$$Y_t = \alpha + \beta_0 X_t + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \dots + \varepsilon_t \quad (1)$$

şeklinde ve sonsuz gecikmeli modeldir (Dikmen, 2006). Bu modelde β_i 'lerin geometrik olarak azaldığı varsayılır.

$$\beta_k = \beta_0 \lambda^k, \quad k = 0, 1, \dots \text{ Geometrik gecikmeli katsayılar}$$

λ : Dağıtılmış gecikmenin azalma oranıdır ve $0 < \lambda < 1$

$1 - \lambda$: Uyum hızı veya intibak hızıdır (Koyck, 1954).

Dağıtılmış gecikme modeli Koyck modeli varsayımı kullanılarak,

$$\beta_k = \beta_0 \lambda^k, \quad k=0, 1 \text{ ve } 2 \text{ için elde edilen sonuçlar aşağıda görülebilir.}$$

$$\beta_1 = \beta_0 \lambda$$

$$\beta_2 = \beta_0 \lambda^2$$

...

$$\beta_k = \beta_0 \lambda^k$$

$$Y_t = \alpha + \beta_0 X_t + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \dots + \varepsilon_t$$

Modelinde β lar yerine eşitleri konduğunda

$$Y_t = \alpha + \beta_0 X_t + \beta_0 \lambda X_{t-1} + \beta_0 \lambda^2 X_{t-2} + \dots + \varepsilon_t \quad (2)$$

Koyck modeli elde edilir.

Koyck modeli dönüşümü: (1) nolu model bir dönem geciktirilerek yazılır.

$$Y_{t-1} = \alpha + \beta_0 X_{t-1} + \beta_0 \lambda X_{t-2} + \beta_0 \lambda^2 X_{t-3} + \dots + \varepsilon_{t-1} \quad (3)$$

$$\lambda Y_{t-1} = \lambda \alpha + \lambda \beta_0 X_{t-1} + \beta_0 \lambda^2 X_{t-2} + \beta_0 \lambda^3 X_{t-3} + \dots + \lambda \varepsilon_{t-1} \quad (4)$$

(1) nolu modelden (3) nolu model çıkartılır.

$$Y_t - \lambda Y_{t-1} = (\alpha + \beta_0 X_t + \beta_0 \lambda X_{t-1} + \beta_0 \lambda^2 X_{t-2} + \dots + \varepsilon_t) - \lambda \alpha + \lambda \beta_0 X_{t-1} + \beta_0 \lambda^2 X_{t-2} + \beta_0 \lambda^3 X_{t-3} + \dots + \lambda \varepsilon_{t-1} \quad (5)$$

$$Y_t = \alpha(1 - \lambda) + \beta_0 X_t + \lambda Y_{t-1} + v_t \quad (6)$$

(5) numaralı model dönüşümlü Koyck modelidir. Burada $v_t = \varepsilon_t - \lambda \varepsilon_{t-1}$ dir.

Ortalama gecikme sayısı gecikmelerin tartılı ortalaması olup aşağıdaki gibi hesaplanır (Güriş ve ark., 2013).

$$\text{Ortalama gecikme} = \frac{\lambda}{1 - \lambda} \quad (7)$$

Almon Modeli

Gecikmesi dağıtılmış modellerden Almon modeli (Almon, 1965)

$$Y_t = \alpha_0 + \beta_0 X_t + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \dots + \beta_k X_{t-k} + \varepsilon_t \quad (8)$$

şeklindeki model çerçevesinde Z_0 , Z_1 ve Z_2 aşağıdaki gibi bulunur.

$$Z_{0t} = X_{t-i} = (X_t + X_{t-1} + X_{t-2} + X_{t-3}) \quad (9)$$

$$Z_{1t} = X_{t-i} = (X_{t-1} + 2X_{t-2} + 3X_{t-3}) \quad (10)$$

$$Z_{2t} = X_{t-i} = (X_{t-1} + 4X_{t-2} + 9X_{t-3}) \quad (11)$$

Almon modelinin ikinci derecede bir polinom olduğu durumu ele alındığında,

$$Y_t = \alpha_0 + \sum_{i=0}^k (a_0 + a_1^i + a_2^{i^2}) X_{t-i} + \varepsilon_t = \alpha_0 + a_0 \sum_{i=0}^k X_{t-i} + a_1 \sum_{i=0}^k i X_{t-i} + a_2 \sum_{i=0}^k i^2 X_{t-i} + \varepsilon_t \quad (12)$$

elde edilir. Burada

$$Z_{0t} = \sum_{i=0}^k X_{t-i}, \quad Z_{1t} = \sum_{i=0}^k i X_{t-i}, \quad Z_{2t} = \sum_{i=0}^k i^2 X_{t-i} \quad (13)$$

tanımlaması ile

$$Y_t = \alpha + a_0 Z_{0t} + a_1 Z_{1t} + a_2 Z_{2t} + \varepsilon_t \quad (14)$$

denklemleri yazılabilir (Gujarati, 2003). α 'lar (13)'ten tahmin edildikten sonra β 'lar (14) nolu eşitlikten (15) nolu ifade tahmin edilir.

$$\beta_i = a_0 + a_1 i + a_2 i^2 \quad (15)$$

$$\begin{aligned}\hat{\beta}_0 &= \hat{a}_0 \\ \hat{\beta}_1 &= \hat{a}_0 + \hat{a}_1 + \hat{a}_2 \\ \hat{\beta}_2 &= \hat{a}_0 + 2\hat{a}_1 + 4\hat{a}_2 \\ \hat{\beta}_3 &= \hat{a}_0 + 3\hat{a}_1 + 9\hat{a}_2 \\ &\dots\dots\dots \\ \hat{\beta}_k &= \hat{a}_0 + k\hat{a}_1 + k^2\hat{a}_2\end{aligned}\tag{16}$$

Modeldeki uygun gecikme için BIC (Bayesci Bilgi Kriteri) kullanılır. En küçük BIC değerini veren model en uygun model olarak seçilir (Kutlar, 2005). BIC istatistiği aşağıdaki gibi hesaplanır (Kasalı ve Adeyemi, 2022).

$$BIC = n. \ln(\sigma_\varepsilon^2) + k. \ln(n) \quad (17)$$

Burada, σ_{ε}^2 : Modele ait hata varyansı, k : Parametre sayısı, n : Gözlem sayısıdır.

BULGULAR ve TARTIŞMA

Bu başlık altında, yalnızca araştırmadan elde edilen bulgular sunularak, konuyla ilgili daha önceden gerçekleştirilmiş benzer ve dolaylı çalışmalarla atıf yapmak kaydıyla bulgular karşılaştırılır. Benzer ve farklı yanlar vurgulanır ve yayına sunulan çalışmada diğer çalışmalara göre neden farklı bir bulgu elde edildiği tartışılır. Sonra da bu tartışma üzerinden araştırmada elde edilen bulgular istikametinde alanın uzmanı olarak yorum yapılır. Bu bölümde, deneysel sonuçların net bir sunumu yapılmalıdır (Çizelge 1).

Mısır fiyatı, üreticilerin ellerine geçtiği düşünülen ortalama kg fiyatı olarak belirlenmiştir. Mısır üretim miktarı ton olarak alınmıştır. Değişkenlerin aldığı değerler yıl olarak, 2000–2023 dönemi verileridir (Çizelge 1).

Türkiye’de 2000-2023 dönemine ait mısır üretim miktarındaki (ton) gelişmeler Şekil 1’de sunulmuştur. Üretim miktarı 2000-2018 yılları arası inişli çıkışlı seyir içindeyken, 2019 yılından itibaren artışa geçmiştir. Aynı dönemdeki mısırın kg fiyatı Şekil 2’de gösterilmiştir. Mısırın fiyatı 2006 ve 2012 yıllarında bir önceki yıla göre düşmüştür. Bir önceki yıla göre 2016 yılında fiyat aynı kalırken, diğer yıllarda sürekli olarak artış göstermiştir. En yüksek artış oranı 2022 yılında olmuştur. 2022’deki artış Türkiye’nin ekonomik durumuyla ilişkili olabilir.

Türkiye'nin mısır üretimi fiyat ilişkisinin belirlenmesinde önce Koyck modeli kullanılmıştır. Koyck modelini oluşturmak için incelenen verilerden mısır fiyatının gecikmeli değerleri belirlenmiştir. Modelin gecikme uzunluğu hesaplanılmasında Schwartz gecikme uzunluğu kriteri belirleyici olmuştur (Yurdakul, 1998). İlerleyen süreçte oldukça büyük q (gecikme uzunluğu) ile başlanmıştır. Ayrıca dağıtılmış gecikme biçimi ile ilgili olarak herhangi bir sınır konulmamıştır. Sürenin kısaltması ile modelin anlamlı bir şekilde bozulup bozulmaması üzerindeki etki değerlendirilerek, süreç sonlandırılmıştır (Davidson ve MacKinnon, 1993). Çizelge 2'de gecikme uzunluklarına göre Schwartz kriteri (BIC) hesaplanmıştır.

Tablo 2 incelendiğinde, mutlak değerce en düşük BIC değerleri karşılaştırılır. Buradaki sonuç 3 gecikme uzunluğudur. Ancak üç yıldan sonra mısırın fiyat ve üretim etkisi sıfır değerine yakınsamaktadır. Hesaplanan gecikme uzunluğu ile mısır üretimi ve fiyatı arasındaki ilişkinin bulunmasında en küçük kareler yöntemi kullanılmıştır. Değerler Çizelge 3'te sunulmuştur ve aşağıdaki eşitlikte olduğu gibi tahmin edilmiştir.

Modelde, değişkenlerin katsayıları istatistiksel olarak anlamlı değildir. Model için F istatistiği 62.348'dir. Bunun yanında modelin açıklanan belirlilik katsayısı $R^2=0.940$ ve $\bar{R}^2 = 0.925$ bulunmuştur. Bu çok yüksek bir değerdir.

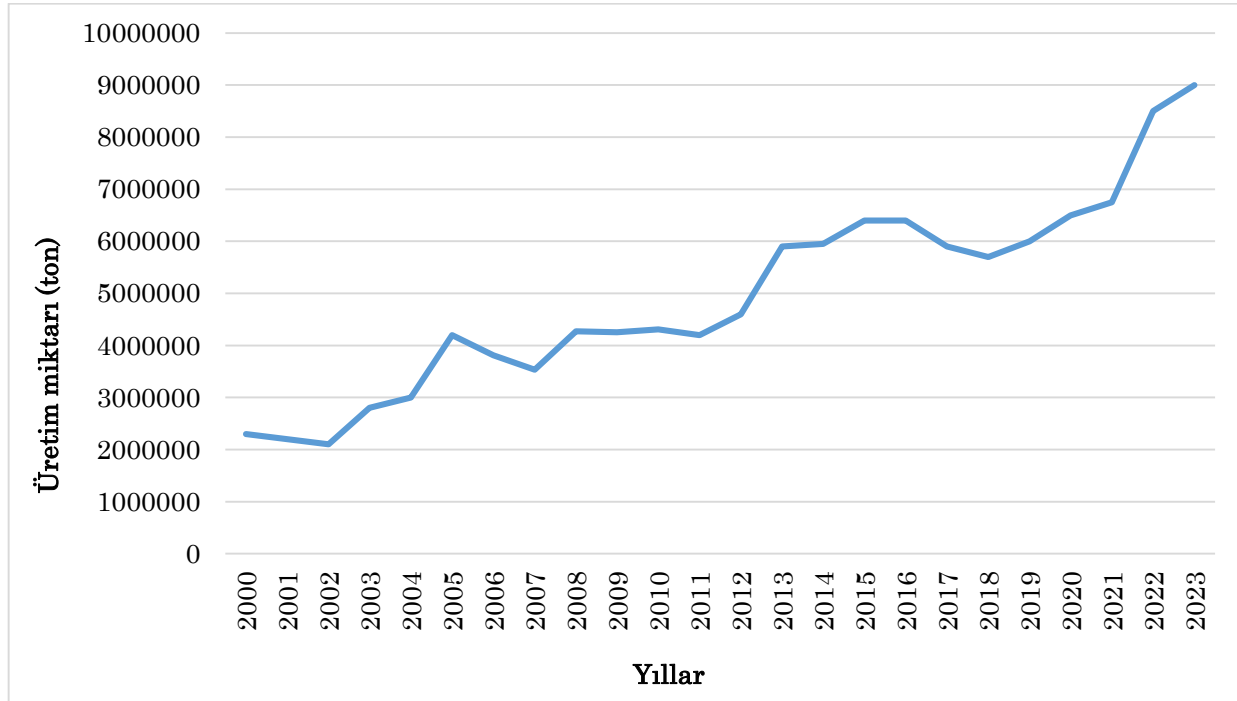
Koçk modeline ilişkin belirleme katsayılarının (R^2) hesaplandığı çalışmalar incelendiğinde, tütün üretimi ve fiyat ilişkisi çalışmasında $R^2=0.87$ (Dikmen 2006), kuru soğanın üretim ve fiyat araştırması için $R^2=0.85$ hesaplanılmıştır (Doğan ve Gürler, 2013). Ayrıca bu değer Doğan ve ark. (2014) çeltik- patates üretim ve fiyat ilişkisi modellerinde bulunan değerden de büyüktür. Tüm bunlar bir arada değerlendirildiğinde, bitkisel ve hayvansal ürünlere ait üretim-fiyat ilişkisi daha yüksek belirlilik katsayısı kullanılarak daha kolay ifade edilebilmektedir.

Model sonuçlarına göre, t ve t-1 dönemdeki fiyatlar mısır üretimini negatif yönde etkilerken, t-2 ve t-3 dönemdeki fiyatlar mısır üretimini pozitif yönde etkilemiştir. Koyck modeli tahminlerine ait regresyon denklemi bulguları Çizelge 4’te verilmiştir. Aşağıda görüldüğü gibi fiyat ve mısır üretimi için gecikmeli değer bağımsız değişken olarak modelde yer almıştır.

Çizelge 1. 2000-2023 dönemi mısır üretim miktarı ve fiyatı

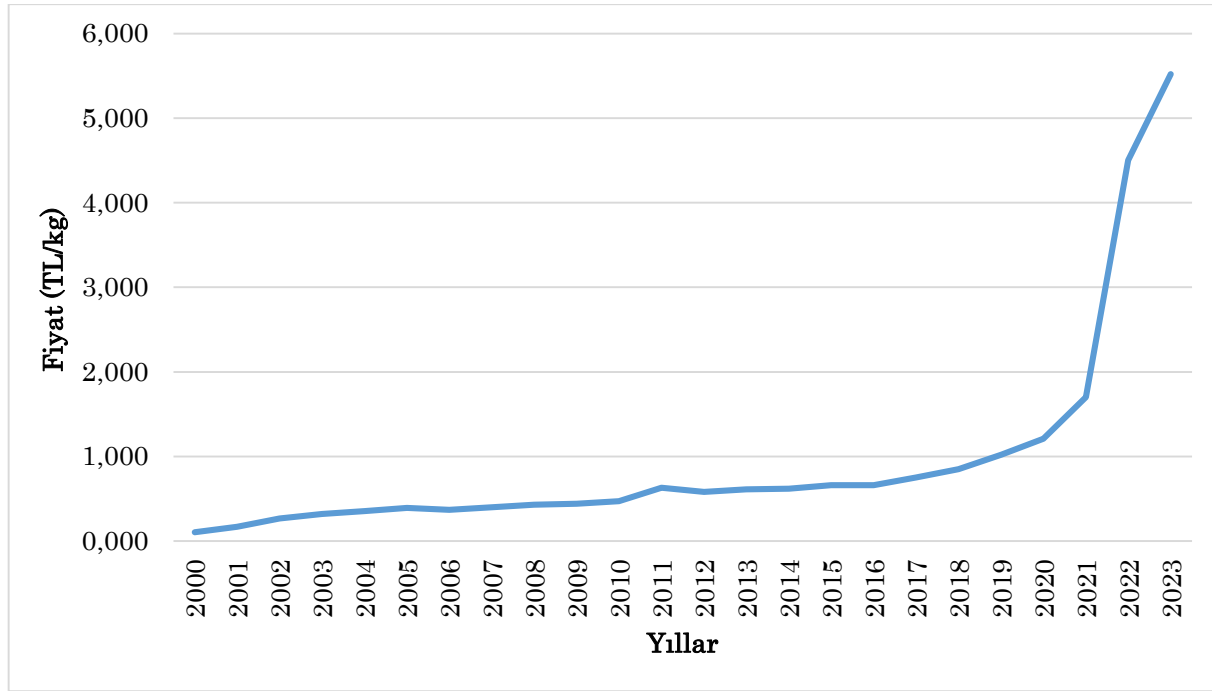
Table 1. Corn production quantity and price in the 2000-2023 period

Yıllar	Üretim (ton)	Fiyat (TL/kg)
2000	2300000	0.103
2001	2200000	0.169
2002	2100000	0.268
2003	2800000	0.321
2004	3000000	0.353
2005	4200000	0.39
2006	3811000	0.37
2007	3535000	0.4
2008	4274000	0.43
2009	4250000	0.44
2010	4310000	0.47
2011	4200000	0.63
2012	4600000	0.58
2013	5900000	0.61
2014	5950000	0.62
2015	6400000	0.66
2016	6400000	0.66
2017	5900000	0.75
2018	5700000	0.85
2019	6000000	1.02
2020	6500000	1.21
2021	6750000	1.7
2022	8500000	4.5
2023	9000000	5.52



Şekil 1. Türkiye’de yıllara göre mısır üretim miktarı (ton)

Figure 1. Corn production in Turkey by years (tons)



Şekil 2. Türkiye’de yıllara göre mısır fiyatlarının seyri
Figure 2. The course of corn prices in Turkey by years

Çizelge 2. Uygun gecikme sayısı
Table 2. Suitable number of delays

Gecikme uzunluğu	Schwartz kriteri (BIC)
K=1	31.103
K=2	29.374
K=3	29.340
K=4	29.543
K=5	29.773
K=6	29.768
K=7	30.038
K=8	29.658
K=9	29.598

Çizelge 3. Gecikme uzunluğuna göre üretim-fiyat ilişkisi analizi
Table 3. Analysis of production-price relationship according to delay length.

Değişken	Katsayı	Std. Hata	t-istatistiği	Olasılık (p)
Sabit	1980344	296809.9	6.672094	0.0000
P _t	-6484.219	224116.2	-0.028932	0.9773
P _{t-1}	-505073.5	387465.3	-1.303532	0.2108
P _{t-2}	2500165	2372759	1.053696	0.3077
P _{t-3}	4156703	2125261	1.955855	0.0682

$R^2=0.940$, $\bar{R}^2 = 0.925$, $F=62.348$, $p=0.001$, $BIC=29.340$

$$Q_t = 1980344 - 6484.219 P_t - 505073.5 P_{t-1} + 2500165 P_{t-2} + 4156703 P_{t-3} \quad (18)$$

(296809.9) (224116.2) (387465.3) (2372759) (2125261)

Çizelge 4. Dönüşümlü Koyck Modeli tahmini
Table 4. The Rotational Koyck Model estimate

Değişken	Katsayı	Std. hata	t-istatistiği	Olasılık (p)
Sabit	652999.0	364307.7	1.792438	0.0882
P _t	302283.6	112447.6	2.688217	0.0141
Q _{t-1}	0.860853	0.087556	9.832006	0.0001

$R^2=0.931$, $\bar{R}^2 = 0.924$, $F=135.194$, $p=0.001$, $BIC=29.358$, $DW=1.99$

Tablo 4'ten elde edilen Dönüşümlü Koyck Modeli aşağıdaki eşitlikle ifade edilebilir.

$$Q_t = 652999 + 302283.6 P_t + 0.860853 Q_{t-1} \quad (19)$$

Koyck Modeli için; Q_t = t dönemindeki mısır üretimi, P_t = t dönemindeki mısırın kg fiyatı, Q_{t-1} : t-1 dönemindeki mısır üretimi olarak yer almaktadır. Kurulan modelin, mısır fiyatı, t testi sonuçları için anlamlıdır ($P < 0.05$). Mısır üretimi için bir gecikmeli değer Q_{t-1} istatistiksel öneme sahiptir ($P < 0.0001$). Bağımlı değişken katsayısı oldukça anlamlı çıkmıştır. Modelin otokorelasyon varlığını belirlemek için Durbin-Watson (DW) testi yapılmıştır ve $DW = 1.99$ bulunmuştur. DW istatistik değeri 1.5 ile 2.5 arasında bir değere sahipse, yani 2'ye yakın ise seride önemli bir otokorelasyon yoktur (Kadılar ve Öncel Çekim, 2020). Mısırın kg fiyatı için 1 TL'lik artış miktarı mısır üretimini 302283.6 ton arttırmıştır. Bir dönem önceki mısır miktarındaki 1 tonluk artış ise mısır üretiminde yaklaşık olarak 0.861 ton (861 kg) artış göstermiştir. Bu durumda

$$\text{Ortalama gecikme} = \frac{\lambda}{1 - \lambda} = \frac{0.861}{1 - 0.861} = \frac{0.861}{0.139} = 6.194 \quad (20)$$

olarak bulunmuştur. Burada 0.861 değeri, fiyat ve mısır üretimi için gecikmeli değer bağımsız değişken olarak model oluşturulduğunda elde edilen modeldeki mısır üretimi gecikmeli değerin katsayısıdır (Çizelge 4). Mısır fiyatı için oluşacak değişim, mısır üretim miktarını büyük ölçüde etkileyebilmesi için gereken süreç 6.194 yıl olarak belirlenmiştir. Mısır üretimindeki değişimin %86,1'i yaklaşık 6.194 yıl içerisinde meydana gelmektedir.

$$Q_t = \alpha_0 + \beta_0 P_t + \lambda Q_{t-1} + \varepsilon_t \quad (21)$$

ve

$$\beta_k = \beta_0 \lambda^k \quad (22)$$

$0 < \lambda < 1$ ise, (7) nolu eşitlik aşağıda sunulan hesaplamalarla elde edilir:

$$\begin{aligned} \lambda &= 0.861; \\ \beta_0 &= 302283.6, \\ \beta_1 &= \beta_0 \lambda = (302283.6) * (0.861) = 260266.2, \\ \beta_2 &= \beta_0 \lambda^2 = (302283.6) * (0.861)^2 = 224089.2, \\ \beta_3 &= \beta_0 \lambda^3 = (302283.6) * (0.861)^3 = 192940.8, \\ \beta_4 &= \beta_0 \lambda^4 = (302283.6) * (0.861)^4 = 166122, \\ \beta_5 &= \beta_0 \lambda^5 = (302283.6) * (0.861)^5 = 143031.1, \\ \beta_6 &= \beta_0 \lambda^6 = (302283.6) * (0.861)^6 = 123149.7 \\ \alpha_0 &= \frac{\alpha}{1 - \lambda} = \frac{652999}{1 - 0.861} = \frac{652999}{0.139} = 4692299 \quad (23) \\ Q_t &= \alpha_0 + \beta_0 P_t + \beta_1 P_{t-1} + \beta_2 P_{t-2} + \dots + \beta_6 P_{t-6} \end{aligned}$$

Denklemine göre uyarlandığında, bu hesaplamalarla, Koyck modeli yardımıyla elde edilen regresyon denklemi yeniden yazılarak Eşitlik (8) elde edilir.

$$Q_t = 4692299 + 302283.6 P_t + 260266.2 P_{t-1} + 224089.2 P_{t-2} + 192940.8 P_{t-3} + 166122 P_{t-4} + 143031.1 P_{t-5} + 123149.7 P_{t-6} \quad (24)$$

Elde edilen modelin gecikmeli fiyatları, mısır üretiminde azalan etki oluşturmuştur. (Eş. 8)'e göre fiyat üzerindeki 1 TL'lik artma, üretimde 302 283.6 ton artışa neden olmaktadır. 1 önceki dönemde fiyattaki 1 TL'lik artma, üretim miktarını 260 266.2 ton, iki dönem önce fiyatlardaki 1 TL artma üretimi 224 089.2 ton, üç dönem önce fiyatın 1 TL artması üretimi 192 940.8 ton, dört dönem önce fiyatın 1 TL artması üretimi 166 122 ton, beş dönem önce fiyatın 1 TL artması üretimi 143 031.1 ton arttırmıştır. Son olarak altıncı dönem önceki fiyatın 1 TL'lik artışı üretim miktarında 123 149.7 ton artışa sebep olmuştur. Etki süresi 6 yıl sürmüştür. Yedinci yılda düşmektedir. Yıllar ilerledikçe bu azalış devam ederek sifıra yaklaşacaktır. Mesela,

30 dönem önceki fiyatta 1 TL'lik artış olursa üretimde,

$$\beta_{30} = \beta_0 \lambda^{30} = (302283.6) * (0.861)^{30} = 3392.416 \text{ ton artış olacaktır.}$$

40 dönem önceki fiyatta 1 TL'lik artış olursa üretimde

$$\beta_{40} = \beta_0 \lambda^{40} = (302283.6) * (0.861)^{40} = 759.523 \text{ ton artış olacaktır.}$$

50 dönem önceki fiyatta 1 TL'lik artış olursa üretimde

$$\beta_{50} = \beta_0 \lambda^{50} = (302283.6) * (0.861)^{50} = 170.048 \text{ ton artış olacaktır.}$$

70 dönem önceki fiyatta 1 TL'lik artış olursa üretimde

$$\beta_{70} = \beta_0 \lambda^{70} = (302283.6) * (0.861)^{70} = 8.524 \text{ ton artış olacaktır.}$$

80 dönem önceki fiyatta 1 TL'lik artış olursa üretimde

$$\beta_{80} = \beta_0 \lambda^{80} = (302283.6) * (0.861)^{80} = 1.908 \text{ ton artış olacaktır.}$$

90 dönem önceki fiyatta 1 TL'lik artış olursa üretimde

$$\beta_{90} = \beta_0 \lambda^{90} = (302283.6) * (0.861)^{90} = 0.427 \text{ ton artış olacaktır.}$$

Görüldüğü gibi üretimdeki artışlar azalan şekilde devam etmiş ve sıfıra yaklaşmıştır. Kısaca mısır üretimi önemli olarak en fazla 6 yıl önceki fiyatlardan etkilenmektedir. Dolayısıyla araştırmalar, mısırın birçok bitkisel üründen daha fazla fiyattan etkilendiği belirlenmiştir. Mısır fiyatlarının belirlenen dönemlere yönelik gecikmeli değerlerindeki değişimler, üretim miktarında pozitif bir etkiye sebep olmakta ve bu etkileşim giderek azalış göstermektedir. Koyck modeline ait determinasyon katsayısı (R^2) katsayısı 0.940 olduğundan mısır üretiminde meydana gelen değişmelerin % 94'ü cari ve gecikmeli mısır fiyatları tarafından açıklanmaktadır.

Almon modeli uygulandığında, “Z” değerleri elde edilmiştir. Z değerleri için ikinci dereceden bir model kullanılmıştır ve Çizelge 5'deki model sonucunda (24) nolu eşitlik yazılabilir.

Çizelge 5. Almon Modeli ile Mısır Üretim Miktarı ve Fiyat İlişkisinin Tahmini

Table 5. Estimation of Corn Production Quantity and Price Relationship with Almon Model

Değişken	Katsayı	Std. Hata	t-istatistiği	P
C	1858714	273192.3	6.804	0.000
Z_0	-126238.7	227508.4	-0.555	0.586
Z_1	-5313297	1036429	-5.127	0.000
Z_2	4473575	617729.6	7.242	0.000

$$Q_t = 1858714 - 126238.7Z_0 - 5313297Z_1 + 4473575Z_2 \quad (25)$$

Çizelge 5'deki Z değerleri elde edildikten sonra Eşitlik (26)-(29) sonuçları elde edilmiştir.

$$\beta_0 = a_0 = -126238.7 \quad (26)$$

$$\beta_1 = a_0 + a_1 + a_2 = -126238.7 - 5313297 + 4473575 = -965960.7 \quad (27)$$

$$\beta_2 = a_0 + 2a_1 + 4a_2 = -126238.7 - 2(5313297) + 4(4473575) = 7141467 \quad (28)$$

$$\beta_3 = a_0 + 3a_1 + 9a_2 = -126238.7 - 3(5313297) + 9(4473575) = 24196045 \quad (29)$$

Elde edilen bu değerlere göre Almon Modeli Eşitlik (30)'daki gibi yazılabilir.

$$Q_t = 1858714 - 126238.7P_t - 965960.7P_{t-1} + 7141467P_{t-2} + 24196045P_{t-3} \quad (30)$$

Almon modeli sonuçlarına göre, t dönemindeki fiyatlarda bir birimlik değişim, üretim miktarında 126238.7 birim azalışa ve t-1 dönemdeki fiyatlarda oluşacak bir birimlik artış üretim miktarında 965960,7 birimlik azalışa neden olmaktadır. Ancak sırasıyla t-2 ve t-3 dönemlerindeki fiyatlarda meydana gelecek bir birimlik artış üretim miktarında 7141467 ve 24196045 birimlik artışa neden olmaktadır. Almon modeline ait $R^2=0.935$, $\bar{R}^2 = 0.924$ ve BIC=29.374 olarak hesaplanmıştır. Mısır üretiminde meydana gelen değişmelerin % 93.5'i cari ve gecikmeli mısır fiyatları tarafından açıklanmaktadır. Almon modeli sonuçlarına göre, mısır üretim miktarının geçmiş 1 yıldaki fiyatlardan negatif olarak etkilendiği, 2. ve 3. yıllardaki fiyatlardan ise pozitif olarak etkilendiği görülmüştür.

Kahramanmaraş'ın Pazarcık ilçesinde 2015 yılında 55 işletmeye uygulanan anket neticesinde, bölgede ortalama dane mısır ana ürün verimi 1080,43 kg/da bulunmuştur. Buna bağlı olarak 1 kg dane mısır satış fiyatı 0,544 TL/kg olarak saptanmıştır (Paksoy ve Ortasöz, 2018). Mısırın kg satış fiyatı bu çalışmada Türkiye verisinde ilgili döneme ait değerlerin biraz altındadır.

Koyck modeli ile ilgili yapılan bir çalışmada, ceviz üretimi ile fiyat ilişkisi analiz edilmiştir. Yapılan analizde ceviz fiyatlarında oluşan değişikliklerin ceviz üretiminde önemli bir değişikliğe sebep olması için gereken zamanın 1.75 yıl olduğu tespit edilmiştir. Cari yılda ceviz fiyatlarındaki bir TL'lik artışın üretim miktarında 0.003178 tonluk bir artışa neden olduğu, bir önceki dönemdeki ceviz fiyatlarındaki bir TL'lik artışın ise ceviz üretiminde 0.002023 ton

artırdığı görülmüştür (Çukur ve ark., 2023). Bu çalışmada elde edilen sonuçlarla farklılık göstermiştir. Zira ürünler ve katma değerleri birbirinden farklıdır.

Diğer bir çalışmada, 1994-2013 dönemine ait a karpuzun üretim ve fiyat ilişkisi gecikmesi dağıtılmış modellerden Almon yaklaşımı ile analiz edilmiş, karpuz üretimi geriye doğru en fazla sekiz yılın fiyatından etkilenmiştir. Cari yıldaki karpuz fiyatlarındaki 1 TL'lik artış, karpuz üretimini 0.181 ton azaltmış, bir önceki dönemde fiyatlarındaki 1 TL'lik artış üretimi 0.107 ton ve sekiz dönem önceki fiyatlarındaki 1 TL'lik artış 1.690 ton arttırmıştır (Özbay ve Çelik, 2016). Fiyat artışının üretimi arttırması açısından bu çalışmada elde edilen sonuçlardan farklılık göstermiştir.

Berk (2017)'in çalışmasında, Türkiye'de 1980-2014 dönemine ait ayçiçeği üretimi ve fiyatı arasındaki ilişki Koyck modeli ile araştırılmıştır. Model tahminlerine göre, ayçiçeği fiyatı sadece önceki yılların fiyatlarını etkilemiş ve 0.1885 dönem gerekli olmuştur. Böylece ayçiçeği fiyatları, ayçiçeği üretimi üzerinde önemli ve kayda değer bir etkiye sahip olabilir.

Erdal ve ark. (2009) çalışmasında, Türkiye'de 1975-2007 döneminde üretilen patates miktarı ile fiyatlar arasındaki ilişki gecikmesi dağıtılmış modellerden Koyck modeli ile incelenmiştir. Analiz sonucunda, Türkiye'de patates üretimi piyasada oluşan ortalama fiyatın gecikme değerinden etkilenmiştir. Türkiye'de patates fiyatlarındaki değişimin patates üretimini etkilemesi için gereken süre 12,33 dönem olmuştur. Bu değer bu çalışmada elde edilen sonuçtan daha yüksektir.

Almon modeli ile ilgili bir çalışmada, Türkiye'de 2000-2014 dönemi fındık toplu meyveliklerin alanı (dekar) ile fiyat ilişkisi araştırılmıştır (Şahinli ve Özçelik, 2016). Modelden gecikmeli fiyatların toplu fındık alanı üzerinde etkisi azalan bir etkiye sahiptir. Bu etkinin bir yıl sürdüğü saptanmıştır. Modele ait $R^2=0.623$ bulunmuştur ve bu çalışmada elde edilen değerin altındadır.

Akgül ve Yıldız (2016)'ın çalışmasında, 1994-2014 dönemi için kırmızı et üretimi ile fiyatlar arasındaki ilişki, gecikmeli dağıtılmış modellerden Almon modeli kullanılarak incelenmiştir. Almon modelinin sonuçlarına göre, katsayılar ve modelin tamamı %10 önem seviyesinde anlamlı bulunmuş olup, kırmızı et üretim miktarının son 6 yıldaki fiyatlardan etkilendiği belirlenmiştir. Analiz, t, t-1, t-2, t-3, t-4, t-5 ve t-6 yıllarındaki fiyatlarla üretim arasında pozitif bir ilişki olduğunu ortaya koymuştur.

Tartışılan çalışmalar ile bu çalışma sonuçları farklı dönemlerde farklı koşullardaki ürün-fiyat ilişkisi nedeniyle farklılık göstermiştir.

SONUÇ ve ÖNERİLER

Bu çalışmada, üretilen mısır üretim miktarı ile mısır fiyatı arasındaki ilişki incelenmiştir. Bu ilişki, dağıtılmış gecikme modellerinden Koyck ve Almon modelleri kullanılarak incelenmiştir. Analiz sonucunda, mısır üretim miktarı-fiyat ilişkisinde korelasyon değeri %99,70 olarak bulunmuştur. Bulunan yüksek korelasyon değeri miktar fiyat ilişkisinin güçlü olduğunu da göstermektedir.

Modeldeki bilinmeyen parametrelerin tahmini için Schwarz kriteri kullanılarak gecikme uzunluğu 3 olarak belirlenmiştir. Bu, incelenen dönemdeki verilere göre, Türkiye'de mısır üretiminin son üç yıldaki fiyatlardan etkilendiği anlamına gelmektedir. Koyck modeli ile elde edilen sonuçlara göre, mısır fiyatı için belirlenen değişim 6.194 yıllık mısır üretim miktarında büyük bir etki düzeyine sebep olmuştur. Koyck modelinde, t yıl için mısır fiyatı bir birim artışla üretim miktarını 302283.6 ton arttırmıştır. Bir dönem önce üretimdeki bir birimlik değişme mısır üretimini ise 0.861 ton (861 kg) arttırmıştır. Bir yıl önce mısır fiyatı 1 birim değişim karşısında üretim miktarında 260266.2 ton artış görülürken, iki yıl önce mısır fiyatı için 1 birim değişim üretim miktarında 224089.2 ton artmaya yol açmıştır. Tüm bu artma miktarları azalış olarak sürmüş ve üç, dört, beş ve altı yıl önce fiyatların 1 birim değişiminde 192940.8, 166122, 143031.1 ve 123149.7 tona ulaşmıştır. Almon modelinde, mısır bitkisinin en fazla 3 yıl önceki fiyatlardan etkilendiğini göstermektedir. Almon modeline göre hesaplanan parametrelere bakıldığında t döneminde mısır fiyatlarında oluşan 1 birimlik artış, mısır üretiminde 126238.7 birim azalmaya yol açmıştır. Aynı şekilde bir dönem öncesi mısır fiyatında kg başına 1 liralık artış, t dönemindeki mısır üretimini 965960.7 ton azaltmıştır. İki dönem öncesi (t-2 dönemi) mısır fiyatında kg başına 1 liralık artış, mısır üretiminde 7141467 ton artışa yol açmıştır. Üç dönem öncesi (t-3 dönemi) mısır fiyatlarının üretimde 24196045 ton artışa neden olmuştur. t-2 ve t-3 dönemleri mısır fiyatları çiftçilerin üretim kararlarını pozitif yönde etkileyen önemli değişkenlerdir. Koyck ve Almon modelleri uyum iyiliği kriterlerine göre birbirine çok yakın sonuçlar verse de R^2 ve $\bar{R}^2$ değerleri daha yüksek ve BIC değerleri daha düşük olan Koyck modelinin daha uygun olduğu tespit edilmiştir.

Sonuç olarak, mısır Türkiye'deki başlıca temel ürünlerden biridir. Ortalama gecikme sayısı göz önüne alındığında, Türkiye'deki mısır üreticilerinin mısır üretme konusunda oldukça istekli olduğu söylenebilir. Ancak, fiyat belirsizlikleri Türkiye'de bu ürünün üretiminde dalgalanmalara neden olmaktadır. Türkiye'de mısır ürünü ihracat

açısından çok önemli ve her yıl ihraç edilmeye devam etmektedir. Ayrıca en çok tüketilen ürünler arasındadır. Tüketim ihtiyacını karşılamak ve ihracattan daha fazla gelir sağlamak için mısır üretiminde devamlılık esastır. Bitkisel üretim ve fiyat ilişkisinde üretimin fiyattan ne kadar zamanda etkilendiği ve bir önceki dönemdeki değişime karşılık etkisinin belirlenmesinde Dağıtılmış Gecikme Modellerinin uygulanabilir yöntemler olduğu ortaya çıkmıştır.

Araştırmacıların Katkı Oranı Beyan Özeti

Planlama: Köleoğlu, N., Şenol, Ç.; Materyal ve Metot: Köleoğlu, N., Şenol, Ç.; Veri toplama ve İşleme: Köleoğlu, N., Şenol, Ç.; İstatistiki Analiz: Şenol, Ç.; Literatür Tarama: Köleoğlu, N., Şenol, Ç.; Makale Yazımı, İnceleme ve Düzenleme: Köleoğlu, N., Şenol, Ç.

Çıkar Çatışması Beyanı

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Examining the Impact of Consumer Innovativeness on Brand Equity Components and Purchasing Behavior: A SEM-Based Field Study in the Agricultural Equipment Industry

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ABSTRACT

Consumer innovativeness is a crucial concept that reflects individuals' tendencies to adopt new products, services, or ideas. Evolving technology and changing living standards continuously transform consumer needs. In the agricultural sector, these innovative tendencies foster the development of technology-based products. Farmers increasingly demand innovative agricultural equipment that enhances efficiency and simplifies work processes. This study examines the relationship between consumer innovativeness and brand equity components. Based on this relationship, the study aims to reveal farmers' innovation adoption and purchasing behaviors. The findings indicate that innovative products not only improve operational efficiency but also enhance consumer brand awareness, strengthen perceived quality, and reinforce brand loyalty. Additionally, the study identifies differences in innovation adoption based on consumers' age and education levels. The results suggest that strategies related to consumer innovativeness can serve as a valuable resource for guiding businesses' marketing activities. Furthermore, they highlight the critical role of consumer innovativeness in achieving competitive advantage and ensuring long-term sustainable production in the agricultural sector

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Tüketici Yenilikçiliğinin Marka Denkliği Unsurlarına ve Satın Alma Davranışı Üzerine Etkisinin İncelenmesi: Tarım Ekipmanları Sektöründe YEM Tabanlı Bir Uygulama

ÖZET

Tüketici yenilikçiliği, bireylerin yeni ürün, hizmet veya fikirleri benimseme eğilimlerini ifade eden önemli bir kavramdır. Gelişen teknoloji ve değişen yaşam standartları, tüketicilerin ihtiyaçlarını sürekli olarak dönüştürmektedir. Tarım sektöründe de bu yenilikçi eğilimler, teknoloji temelli ürünlerin geliştirilmesine yol açmaktadır. Çiftçiler, verimliliği artıran ve iş süreçlerini kolaylaştıran yenilikçi tarım ekipmanlarını daha fazla talep etmektedirler. Yürütülen bu çalışma, tüketici yenilikçiliği ile marka denkliği unsurları arasındaki ilişkiyi incelemektedir. Bu ilişkiden hareketle, çiftçilerin yenilikleri benimseme ve satın alma davranışlarının ortaya konulması amaçlanmaktadır. Firmaların pazarlama stratejileri geliştirmelerine katkı sunmayı hedefleyen bu çalışma sonuçlarına göre yenilikçi ürünlerin yalnızca iş verimliliğini artırmakla kalmayıp, aynı zamanda tüketicinin marka farkındalığını arttırdığı, kalite algısını ve markaya olan bağlılığı güçlendirdiği belirlenmiştir. Tüketicilerin yaş ve eğitim düzeylerine göre yenilikleri benimsemeleri arasında farklılıkların olduğu da ayrıca tespit edilmiştir. Sonuçlar, tüketici yenilikçiliği ile ilgili stratejilerin işletmelerin pazarlama faaliyetlerine yön verecek önemli bir kaynak teşkil edeceğini göstermektedir. Ayrıca tarım sektöründe firmaların rekabet avantajı elde etmeleri ve uzun vadeli sürdürülebilir üretim sağlamaları açısından kritik olduğunu ortaya koymaktadır.

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Marka denkliği
Tarımsal pazarlama
Tarım ekipmanları
Yapısal eşitlik modellemesi

Atıf için

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INTRODUCTION

Although consumers' needs are finite, their desire to satisfy them appears virtually limitless. Advancements in technology, social interactions, and living standards continuously shape these desires. In response to the ever-evolving consumer landscape, companies consistently strive to develop and introduce products that best address consumer expectations. Consumers' desire to satisfy their needs extends beyond improvements to existing products—it reflects an openness to innovation, driven by curiosity, exploration, and a willingness to take risks. This tendency, known as consumer innovativeness, not only drives demand for new, technologically advanced products but also plays a crucial role in shaping brand loyalty. The influence of innovation is particularly significant in technology-driven sectors such as agriculture, where evolving consumer expectations and the adoption of new technologies are transforming both consumption patterns and production processes.

The agricultural sector is deeply affected by rapid technological developments, including the Internet of Things (IoT), artificial intelligence, automation, sensor technologies, GPS, and precision farming tools. For example, the adoption of autonomous GPS-guided tractors has allowed farmers to optimize fuel consumption and enhance field efficiency. Studies show that these tractors reduce fuel use by 15% and increase efficiency by up to 20% (D'Antonio, 2023). Similarly, drone technology for crop monitoring and pesticide application has led to a 30% reduction in pesticide use, while also improving coverage efficiency by 25-40% (Zanin et al, 2022; Subramanian et al, 2021; Liu et al, 2023). These innovations demonstrate how consumer innovativeness can drive the adoption of technology that improves both productivity and sustainability in agriculture. Farmers, as key stakeholders in this sector, continuously evolve their expectations regarding the equipment they use to maintain productivity and efficiency. However, the adoption of such technologies is not merely a matter of availability but also of individual differences in innovativeness (Altuntaş, 2024). In this context, agricultural equipment manufacturers not only focus on developing advanced, efficient tools but also aim to appeal to farmers who are more open to trying and adopting new technologies. As a result, consumer innovativeness in agriculture influences how quickly and effectively such technologies diffuse across farming communities.

Consumers in the market today have access to a broad range of products, including those from well-established brands and new market entrants. Given such a vast array of alternatives, consumers find it easier to switch between brands. Digital platforms, pricing strategies, and perceived quality all play significant roles in shaping consumer preferences and influencing brand-switching behavior. Consumers now access product information, user experiences, and compare alternative products in real-time. In response, manufacturing firms focus on strengthening their market presence by building brand loyalty and trust—two outcomes closely associated with the consumer's openness to innovation. Recognizing the need to align their marketing strategies with target market expectations, firms increasingly emphasize differentiation strategies to stand out in a highly competitive environment. Among the most critical actions companies can take to maintain a competitive edge and capture consumer interest is fostering innovation.

Management thinker Peter Drucker identifies two core functions of business: marketing and innovation (Cohen, 2011). In line with this perspective, firms aiming to expand their market share must not only innovate but also strategically communicate their innovations to appeal to consumers' innovative tendencies. While many previous studies have explored the relationship between innovativeness and demographic traits, there is a growing need to investigate how consumer innovativeness shapes brand perceptions and purchasing behavior, particularly in under-researched markets such as agricultural machinery.

When consumer innovativeness is paired with brand loyalty, it becomes a powerful determinant of purchasing behavior. For instance, an innovative agricultural machine not only enhances efficiency but also reinforces trust in the brand. This trust, in turn, influences consumers' purchasing decisions and ultimately strengthens their brand loyalty. Studies conducted by Truong (2013) and Wang and Cho (2012) demonstrate how innovative products reinforce brand loyalty and how this, in turn, affects consumer purchasing behavior. In the agricultural equipment market, farmers' trust and loyalty toward certain brands are often shaped by both perceived innovation and peer influence within their communities.

In the national and international literature, several studies have examined agricultural mechanization and equipment adoption across regions (Işık et al., 2003; Bilim et al., 2014; Aybek et al., 2021; Altuntaş et al., 2025). Others have explored behavioral aspects of producers, such as Adnan et al. (2019) on the use of green fertilizers, Damalas and Koutroubas (2018) on pesticide use, and Valizadeh et al. (2020) on drip irrigation adoption. Zarei et al. (2022) investigated Iranian farmers' behavioral intentions toward agricultural e-commerce using the technology

acceptance model (TAM). However, the domestic literature still lacks focused research on the role of *consumer innovativeness* in the agricultural sector. This study aims to contribute to closing that gap.

Moreover, achieving optimal levels of mechanization requires aligning tractors, tools, and equipment with the scale and production patterns of farms (Abdikoglu, 2019). To do so effectively, it is essential to understand producers' behavior—how they adopt and utilize new technologies, and the factors shaping these decisions. This research aims to contribute to that understanding by focusing on the role of innovativeness among farmers.

The main aim of this study is to explore the impact of innovative products on the components of brand equity—brand loyalty, perceived quality, and brand awareness—and to examine how these factors influence consumer purchasing behavior. By focusing on Türkiye's rapidly growing agricultural equipment market, this study aims to measure consumer innovativeness and identify its relationship with brand loyalty, perceived quality, and brand awareness. Additionally, the research aims to explore whether consumers' adoption of innovations varies with demographic characteristics such as age and education level. Observations of farmer behavior indicate that those who are satisfied with their agricultural equipment often recommend and influence other farmers' purchasing decisions. This highlights how brand preferences are shaped among farmers and why differentiation is essential in a highly competitive market. As a result, the agricultural equipment sub-sector emerges as a research-worthy field.

According to the Turkish Statistical Institute (TÜİK, 2024), the agricultural equipment market comprises 99 different product categories. Given the intense competition in this market, analyzing consumer expectations regarding brand preferences and the factors influencing their purchasing behavior is expected to offer valuable insights into how innovation-oriented perspectives influence decision-making. For companies, innovative products not only drive sales but also strengthen brand loyalty, playing a crucial role in ensuring long-term success. Therefore, it is essential to align marketing strategies and brand management with an innovation-oriented approach.

Conceptual Framework

Consumer innovativeness

Consumer innovativeness is a crucial dimension of consumer behavior that reflects individuals' tendencies to try and adopt new products, services, or ideas. This concept encompasses both individual differences and societal trends, playing a significant role in businesses' new product development processes and marketing strategies.

The concept of consumer innovativeness, which relates to consumers' adoption of innovations, was classified based on the adoption criteria of innovations through the research conducted by Everett Rogers in 1962. Rogers, who observed the development of new ideas and changing life standards with technological advancements, developed the Diffusion of Innovations theory (Cheng, 2004). Since its emergence, this theory has been widely used in studies on the adoption of technological innovations. Rogers (1976) defines innovation as an idea, practice, or object that an individual perceives as new. This definition renders the concept of innovation relative, meaning that what is considered an innovation for some may not be perceived as innovative by others (Karaarslan & Akdoğan, 2013).

Consumer innovativeness includes psychological, sociological, and demographic factors that shape individuals' attitudes and behaviors toward new products or services. It is essentially the tendency of consumers to adopt innovations (Tellis et al., 2009). There are two main approaches regarding consumer innovativeness. The first approach conceptualizes consumer innovativeness as personal innovativeness, treating it as a personality trait (Midgley & Dowling, 1978). The second approach suggests that a consumer's level of interest in a specific product determines whether they are an innovative consumer; high interest makes the person an innovative consumer, whereas low interest does not (Goldsmith & Hofacker, 1991).

Identifying innovative consumers is important for various reasons. Understanding consumers' tendencies to adopt innovations provides valuable insights for businesses in their marketing decisions (Karaarslan & Akdoğan, 2015). Since the behavior of innovative consumers directly influences businesses' product development, positioning, and communication strategies, developing strategies aligned with consumer innovativeness can provide a competitive advantage.

Brand equity elements

Brand equity is a multidimensional concept that defines a brand's market value and competitive advantage. In the literature, it is typically described as a structure linked to brand loyalty, perceived quality, brand awareness, brand associations, and other brand-related assets. This concept plays a crucial role in shaping consumers' brand perceptions and influencing companies' economic positions in the market.

Aaker (1996), a key contributor to brand equity research, defines it as the assets and liabilities linked to a brand, brand name, or symbols, which either enhance or detract from the value a business delivers through its products and services. This concept is shaped by how consumers perceive and evaluate a product. Brand equity typically represents intangible assets and significantly impacts consumer behavior. Keller (1998) considers brand equity as the leading factor in the brand-building process. The key elements of brand equity play a fundamental role in the formation of brand value. These elements include:

1. Brand awareness: This refers to the level of consumer recognition of a brand. A brand's recognizability is achieved only when consumers notice it or when firms create awareness (Onurlubaş, 2018). Brand awareness determines the position a brand holds in consumers' minds compared to competitors. It plays a crucial role in the purchasing process (Aktepe & Baş, 2008) as it significantly influences the first impressions and decision-making processes of consumers. It consists of two dimensions: brand recall and brand recognition (Erdil & Uzun, 2009). Brand recognition occurs at the point of sale, while brand recall occurs before the purchasing process (Kaptanoğlu et al., 2019). Another sub-dimension of brand awareness is brand familiarity, which includes the physical attributes of a brand, such as color, size, shape, and packaging. Therefore, product design, packaging, logos, and symbols play a key role in increasing brand familiarity (Kızgın & Benli, 2018:). Without brand awareness, consumers cannot develop perceptions of brand quality or brand associations, making brand loyalty impossible (Albar & Oksuz, 2013). Brand awareness plays a significant role in consumer product selection and purchasing decisions.

2. Perceived quality: This refers to consumers' general perceptions of how well a product meets their expectations. It is a critical factor in purchasing decisions and is directly related to brand loyalty. Perceived quality includes both intangible emotional impressions and tangible functional attributes of a product. As a result, factors such as a brand's reliability, performance, and durability come to the forefront. Thus, perceived quality is not merely about an emotional connection with a brand but also emphasizes the product's functional and practical aspects. Products perceived as high quality by consumers tend to have higher brand value (Ayas, 2012). Consumers are more likely to consider high-quality products successful.

3. Brand loyalty: This refers to the emotional and rational connections consumers establish with a brand. It is observed when consumers are willing to pay a higher price for a specific brand within the same product category and recommend it to others (Giddens, 2002). It represents either a purchase intention or actual purchase behavior, or both simultaneously (Matzler et al., 2008). Brand loyalty, which influences repeat purchases and word-of-mouth recommendations, is an essential marketing tool in today's highly competitive markets for fostering long-term consumer relationships (Torlak et al., 2014). As brand loyalty increases, the brand becomes less vulnerable to competitors and gains a stronger market position, ultimately leading to greater profitability (Aaker, 2010). Brand loyalty is known to enhance profitability in both the short and long term (Rowley, 2005).

4. Brand associations: These are the emotional and cognitive connections consumers make with a brand. These associations significantly influence the brand's identity and positioning. Brand association is a struggle to create a mental link between the brand and consumers. In recent years, companies in the agricultural sector have intensified their efforts to develop such associations due to increasing competition. Since firms aim to differentiate themselves, marketers use brand associations for differentiation, positioning, brand extension, and creating positive consumer attitudes and emotions (Low & Lamb, 2000). Brand associations form the foundation of brand loyalty.

5. Purchasing behavior: Purchasing behavior is a complex psychological and social phenomenon encompassing consumers' processes of selecting, buying, and using a product or service. Consumer decision-making and purchasing behavior have been extensively researched for years and remain a relevant topic. The purchasing process begins when a consumer recognizes a need and continues until that need is satisfied, passing through several stages. As Taşkın (2003) states, "Every consumption leads to production." Purchasing behavior is shaped by the interaction of individual and environmental factors in consumers' decision-making processes. Research on consumer behavior aims to understand these fundamental elements and guide businesses in adjusting their marketing strategies accordingly. The core of purchasing behavior lies in the emergence of needs and the evaluation of available options to satisfy those needs. Before making a purchase, consumers typically compare alternatives and assess factors such as price, quality, and brand reputation. This evaluation process is based on various conscious or subconscious analyses. Several factors influence consumers' purchasing decisions, including psychological aspects, personal preferences, social environment, cultural influences, and economic conditions. For example, while one consumer may prioritize affordability, another may prefer a prestigious, high-quality brand. This highlights how individual differences shape purchasing behavior. Additionally, environmental factors play a crucial role. Consumers often take into account the recommendations of family, friends, or their social circle.

MATERIAL and METHOD

Scope and Objective of Research

Innovation remains a relevant and significant concept. The main goal of this study is to investigate the impact of consumer innovativeness on specific elements of brand equity and to explore how these elements affect consumer purchasing behavior. Additionally, the study aims to identify whether there are differences in consumers' acceptance of innovations based on age and education variables.

The agricultural sector was selected as the focus of this study to achieve these objectives. Due to its strategic importance, agriculture is considered a valuable field for research. In this sector, producers simultaneously act as consumers. Understanding farmers' behaviors has become increasingly significant for companies in recent years. Compared to the past, farmers have become more conscious, knowledgeable about their needs, and willing to research and experiment with innovations in their production processes. Furthermore, technological advancements have had a substantial impact on the sector.

The agricultural sector consists of multiple sub-sectors, and this study specifically focuses on agricultural equipment. This sub-sector is characterized by intense competition. Therefore, while firms aim to enhance the loyalty of existing customers, they also develop strategies to raise awareness among potential new customers. The findings of this study are expected to contribute to firms' strategy development and provide guidance for new market entrants. Moreover, the results are anticipated to assist agricultural machinery manufacturers in understanding the innovation perceptions of local farmers and their brand loyalty. These insights could help companies formulate target market strategies, optimize resource allocation, and guide customer-oriented marketing activities.

Limitations, Hypotheses, and Model of Research

As the research is limited to the Kırşehir province, the findings cannot be generalized to all farmers in Türkiye. The perception of innovation among farmers has been examined through their preferences and purchasing decisions concerning specific agricultural machinery (plows, disc harrows, cultivators, and seed drills). The primary reason for this choice is that these machines are both widely used and offer significant brand- and model-based variety. Moreover, as they are utilized in the early stages of the production cycle, they have a more direct impact on farmers' perceptions of product performance and quality. Due to the noticeable price differences in the market, they also possess strong potential to influence consumer preferences. With these characteristics, the four selected types of equipment offer a high level of representativeness in measuring innovation adoption and brand equity components. Therefore, evaluating these specific equipment categories was expected to yield more accurate and comparable insights into farmers' brand perceptions and openness to innovation. Sector-specific dynamics such as sales frequency, variety of innovative product options, and regional usage prevalence were also taken into account when selecting machinery types. Tractors, irrigation equipment, harvesting machines, and other agricultural tools were excluded from the study.

The study was conducted based on consumers' experiences with a specific brand, which constitutes a limitation in terms of the adaptation of scale statements. The research model employed in this study is based on Aaker's (1991) brand equity components. However, only measurable and behaviorally relevant variables—such as brand awareness, perceived quality, and brand loyalty—were included in the model. More abstract and interpretive elements, such as brand image and associations, were excluded due to the need for a broader theoretical framework and more extensive data collection in the agricultural context. This decision was made to maintain a clear and focused investigation into how consumer innovativeness influences specific brand equity dimensions. Brand image and brand associations, while undoubtedly important, are broader constructs that warrant separate investigation due to their complex and multidimensional nature. Including these elements would require a broader conceptual framework and additional variables that fall outside the current research objectives.

By narrowing the focus to brand loyalty, perceived quality, and brand awareness, this study aims to offer a deeper understanding of how consumer innovativeness interacts with these components, particularly in the context of the agricultural equipment market. Although the role of brand image and associations is acknowledged, their impact on purchasing behavior and brand equity will be explored in future studies, ensuring that each factor is given the attention it deserves. This focused approach allows for more detailed analysis within the limits of the research scope and ensures that the study's objectives are aligned with the underlying research questions.

Another limitation of the study pertains to the exclusion of certain socioeconomic variables such as farm size and producer income. Although these factors are known to influence technology adoption in agriculture by affecting a farmer's investment capacity and access to innovation, they were not included in the current research model. This decision was made to maintain a focused and manageable study scope.

Various hypotheses were developed to address the research questions:

- H1: There is a difference between individuals' age and their acceptance of innovations.
- H2: There is a difference between individuals' education level and their acceptance of innovations.
- H3: Consumer innovativeness has a positive impact on brand awareness.
- H4: Consumer innovativeness has a positive impact on brand loyalty.
- H5: Consumer innovativeness has a positive impact on perceived quality.
- H6: Brand awareness has a positive impact on purchasing behavior.
- H7: Perceived quality has a positive impact on purchasing behavior.
- H8: Brand loyalty has a positive impact on purchasing behavior.

Based on these hypotheses, the research model was developed, as illustrated in Figure 1.

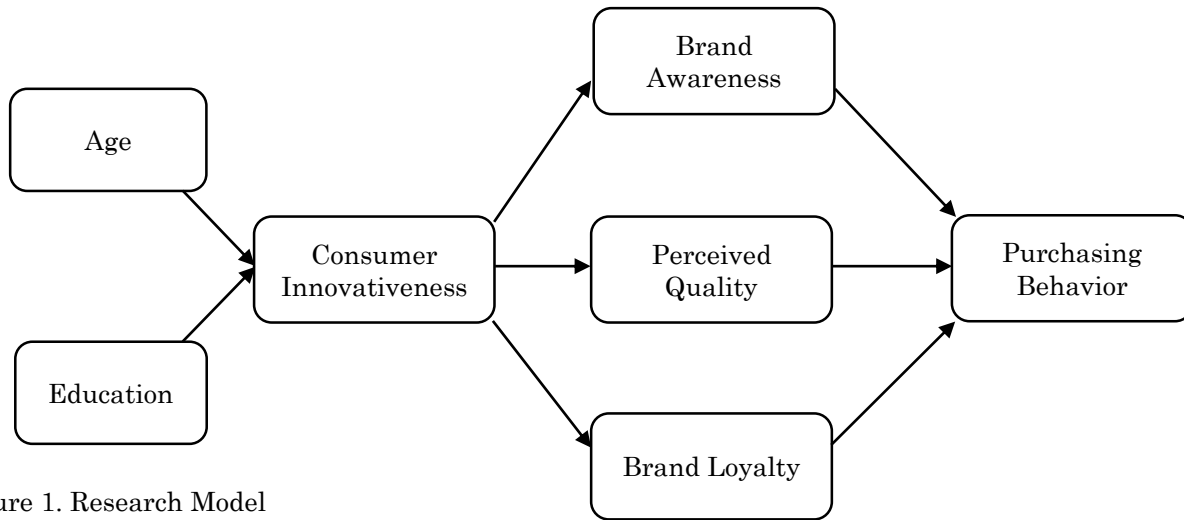


Figure 1. Research Model
Şekil 1. Araştırma Modeli

Data Collection and Survey Design:

The required data were collected through face-to-face surveys. Before the field study, ethics committee permission was obtained from the Kırşehir Ahi Evran University Social and Human Sciences Scientific Research and Publication Ethics Committee with the decision number 2025/01/36 dated 08.01.2025. The survey is divided into three sections. The first section collects information on participants' education, age, and the type and brand of agricultural equipment they own. The second section uses a five-point Likert scale with 14 statements to measure brand loyalty, perceived quality, brand awareness, and purchasing behavior, with responses ranging from 1 (strongly disagree) to 5 (strongly agree). The third section also utilizes a five-point Likert scale to evaluate consumer innovativeness through eight statements.

The statements used in the survey were adapted from previous scientific studies in the literature, ensuring their relevance to the research topic. The first scale employed is the brand loyalty scale, which consists of six statements adapted from Bloemer and Odekerken (2003). The brand awareness scale is measured using three statements from Higgins (2006), while the perceived quality scale includes three statements derived from the studies of Kim et al. (2003) and Mittal & Lassar (1998). The purchasing behavior scale comprises four statements adapted from Berens et al. (2005). Consumer innovativeness is measured using six statements adapted from the scale developed by Eryiğit and Kavak (2011) to suit the objectives of this study. The scales used in the study were taken from the literature and adapted to Turkish. The two-way translation method suggested by Brislin (1970) was applied in the adaptation process. First, the English scales were translated into Turkish, then an expert academic with a high command of another language was asked to check these translations and ensure linguistic equivalence by comparing them with the original text. In terms of cultural appropriateness, opinions were obtained from two separate academicians from the agricultural machinery and agricultural economics departments regarding the appropriateness of the scale expressions to the agricultural sector and context. Also, to ensure comprehensibility among the target population—farmers—a preliminary adaptation process was conducted. Once finalized, the questionnaire was carefully monitored during the initial stages of field application. As the items and questions were found to be well understood by the farmers, the survey was continued without modification. To ensure the validity and reliability of the scale adaptations, detailed information on Cronbach's alpha, confirmatory factor analysis, and construct validity is provided under the "Statistical Analysis and Results" section of this study.

Sampling Method:

The study was conducted in the central district of Kırşehir province during September and October of 2024. The survey was administered through face-to-face interviews conducted directly by the researcher in densely populated areas of the city. Prior to the implementation, participants were informed about the purpose and scope of the questionnaire, and their participation was based on voluntary consent. All interviews were conducted with agricultural producers, specifically those engaged in grain production, who are registered in the Farmer Registration System (ÇKS) and own at least one of the specified agricultural machines (plow, seeder, chisel plow, or disc harrow). To enhance the representativeness of the sample, the interview locations were diversified to include various neighborhoods and villages affiliated with the central district. The participants were selected using a simple random sampling method, which ensures that everyone in the population has an equal probability of being chosen, thereby minimizing selection bias. This approach not only reduces costs and time in field research but also enhances the validity of the findings. To determine the number of participants for the survey, a sample size calculation was conducted. The required sample size was determined using the formula for simple random sampling in finite populations. The sample size was determined using the formula proposed by Krejcie and Morgan (1970). The formula used is as follows:

$$n = \frac{N \cdot Z^2 \cdot p \cdot (1 - p)}{(N - 1) \cdot e^2 + Z^2 \cdot p \cdot (1 - p)}$$

n: Required sample size

N: Population size (in this case, appr. 21,500)

Z: Z-value corresponding to the desired confidence level (e.g., Z = 1.96 for a 95% confidence level)

p: Probability value or assumed proportion of success (commonly set at 0.5 or 50%)

e: Margin of error (also referred to as the allowable error or confidence interval; here, 0.05 or 5%)

The simple random sampling method was chosen to ensure that every farmer had an equal probability of being selected for the study. Where n is the required sample size, and N is the population size. According to the Ministry of Agriculture and Forestry, approximately 21,500 registered farmers are registered in the Farmer Registration System (ÇKS) in Kırşehir (Anonymus, 2022). Z is the Z-score corresponding to the desired confidence level (1.96 for 95%), p is the assumed proportion of the population possessing the characteristic of interest (set at 0.5 to maximize variability), and the acceptable margin of error (0.05).

The probability value $p=0.5$ was selected because it yields the maximum variance $p(1-p)$, which ensures the most conservative and robust sample size estimation. This approach is commonly adopted when there is no prior information about the population proportion. Based on this calculation, the minimum required sample size was determined to be 274 participants. To account for potential issues such as incomplete responses, an additional 10% of surveys were conducted, resulting in 306 valid responses for analysis. The distribution of participants by age and education is detailed in the analysis section of the study.

Statistical Analysis and Findings

For data analysis, the IBM SPSS Statistics (Version 27) software package was used, while SmartPLS 4.0 (Ringle et al., 2024) was employed for model testing. This study follows an explanatory research design, testing the formulated hypotheses. The research takes an inductive approach, starting with specific observations and drawing broader generalizations from them. The study specifically examines farmers in the Kırşehir region to draw general conclusions.

The demographic characteristics of the survey participants are shown in Table 1. Accordingly, the majority of the participants (60%) are between the ages of 30-49, which can be described as young-middle age. 122 participants (40%) are 50 years old and above. The distribution of the participants' educational status is close to each other. More than half (53%) are secondary school graduates, while almost half of the remaining participants (47%) are higher education graduates. It was determined that all participants in the survey have at least one piece of agricultural equipment.

Before moving on to a more detailed analysis of the data collected from the field, it was determined whether the data followed a normal distribution. For this, the skewness and kurtosis values were examined. A normal distribution is assumed when these values range between -1.5 and +1.5 (Tabachnick and Fidell, 2013). As a result of the analysis, the skewness and kurtosis values for the brand awareness scale were skewness: -0.628, kurtosis: 0.122; perceived quality: skewness: -0.485, kurtosis: 0.016; brand loyalty: skewness: -0.691, kurtosis: 0.393; purchase behavior: skewness: -0.245, kurtosis: -0.804; and consumer innovativeness: skewness: -0.479, kurtosis: 0.217. It was determined that the study data followed a normal distribution. Therefore, for the difference tests,

one-way analysis of variance (ANOVA) was chosen for the age variable, as it has three subgroups, and the independent samples t-test was used for the education variable, as it has two subgroups.

Table 1. Demographic Characteristics of Participant's
Çizelge 1. Katılımcıların Demografik Özellikleri

		Frequency	Percent
Age	18-29	71	23.2
	30-49	113	36.9
	50 and above	122	39.9
	Total	306	100.0
Education	Secondary Education	162	52.9
	Higher Education	144	47.1
	Total	306	100.0

To test the hypotheses of the study, first, the difference between the age and education levels of the participants and their consumer innovativeness was tested. To examine this, a one-way analysis of variance (ANOVA) was conducted to determine whether participants' acceptance of innovations differed by age. The results revealed a significant difference between age groups, $F(2, 303, N=306) = 6.25, p = .002$. This indicates that technology acceptance levels vary significantly based on participants' age.

The analysis results have revealed a significant difference between the groups. To identify the groups, a post-hoc test was performed. Since the result of the Levene test was significant ($p = .001$), the homogeneity of variances between the groups could not be ensured. Since equal variance could not be achieved, a post-hoc test was performed to see the difference between the groups, and the Games-Howell test, which gave the strongest result, was selected. The test results show that there is a significant ($p < .001$) difference in the acceptance of innovations between participants in the 18-29 age group and those in the 30-49 age group. Similarly, a significant difference was found in the acceptance of innovations between the 30-49 age group and participants aged 50 and over. Participants aged 30-49 tend to research, accept, and adopt innovations more than those in other age groups. The 18-29 age group shows lower interest in accepting innovations, and middle-aged people are more accepting of innovations than other age groups.

To determine the relationship between participants' educational status and their acceptance of innovations, an independent samples t-test was applied. According to the test results, it was determined that the homogeneity of variances between the groups was not ensured, as per the Levene test ($p < .001$). The two-tailed significance value of the table was $p = .028$. Therefore, a significant difference in the acceptance of innovations based on educational status was identified. According to the analysis, individuals with higher education (mean: 3.8550) are more innovative or accept innovations more than those with secondary education (mean: 3.7346). The Cohen's d effect size test result showed a medium effect size of .475 and a medium level difference between the two groups. A significant effect of the education variable is seen, $t(300, N=306) = 6.74, p = .002$, people with higher education are more accepting of innovations than those with secondary education.

As a result of the conducted difference tests, hypotheses 1 and 2 were accepted.

Before testing the model constructed in the theoretical framework, the reliability and factor structures of the survey statements were tested with exploratory factor analysis. The Cronbach's alpha coefficient method was used for reliability. A Cronbach's alpha reliability coefficient value of 0.80 or above indicates the reliability of the scales (Altuntas et al., 2015). Robinson et al. (1991) state that in exploratory research, the reliability coefficient can drop to 0.60.

Exploratory factor analysis was conducted to identify the factor structure of the scales measuring brand awareness, perceived quality, brand loyalty, purchase behavior, and consumer innovativeness. First, the Kaiser-Meyer sample adequacy test result was calculated. According to the result, a value of 0.791 was found, indicating that the sample is adequate for factor analysis. The Bartlett's test of sphericity also resulted in $p < .001$. As a result of the factor analysis, a 3-factor structure was revealed that explained more than half of the total variance (54.853%) of the scales with brand equity elements. The identified factor structure shows that the measurement was conducted in line with the research's objectives.

In the purchase behavior scale, two statements were included, and the reliability of the scale, calculated as a single dimension, was found to be 0.825.

The consumer innovativeness scale consisted of 8 statements; however, during the analysis, two statements that were deemed irrelevant in the correlation matrix were removed. The statement "I don't spend much time checking new agricultural machines" was removed from the scale, and after reanalysis, the statement "When I buy a new

agricultural machine, I decide on my own” was also removed. As a result, a single-factor structure with a high reliability coefficient of 0.827 was found.

The reliability coefficients and factor structures of the scales are presented as a whole in Table 2 below.

Table 2. Reliability Coefficients and Factor Structures of the Scales
Çizelge 2. Ölçeklerin Güvenirlik Katsayıları ve Faktör Yapıları

	Factor Loading	Variance Explained
Brand Loyalty (Cronbach's Alfa:0,853; CR:0,894; AVE: 0,791)		21,468
When I want to change my agricultural equipment, I will still buy “A” brand.	0,898	
Even if other brands make different promises, I will always prefer the “A” brand that I am currently using.	0,812	
“A” brand agricultural equipment is always my first choice.	0,793	
When I want to buy a new model of the “A” brand that I am using, if that product is not available, I wait for it to arrive.	0,787	
I recommend the “A” brand agricultural equipment I use to my circle.	0,745	
I love the “A” brand farming equipment I use	0,712	
Brand Awareness (Cronbach's Alfa:0,829; CR:0,917; AVE: 0,813)		17,132
I recognize "A" brand agricultural equipment when I see it.	0,862	
I have no difficulty remembering the symbol and logo of "A" brand agricultural equipment.	0,847	
I can immediately distinguish "A" brand agricultural equipment from others.	0,810	
Perceived Quality (Cronbach's Alfa:0,866; CR:0,907; AVE:0,764)		16,253
The "A" brand agricultural equipment I use provides me with high quality.	0,914	
The "A" brand agricultural equipment is durable.	0,853	
The after-sales service of the "A" brand agricultural equipment I prefer is unmatched.	0,821	
Purchasing Behavior (Cronbach's Alfa:0,825; CR: 0,916; AVE: 0,729)		13,777
When I buy agricultural equipment, the "A" brand comes first.	0,874	
I can pay a higher price for the "A" brand agricultural equipment I use.	0,776	
Consumer innovativeness (Cronbach's Alpha:0.827; CR: 0,887; AVE:0,733)		16,240
When I am going to buy a new agricultural equipment, I prefer to consult my farmer friends who have experience with that brand.	0,851	
I am constantly in search of new equipment.	0,822	
I prefer to be in places where information is given about new agricultural equipment.	0,784	
I follow websites that introduce new agricultural equipment.	0,731	
I trust the information of my experienced friends before deciding to buy new agricultural equipment.	0,705	
I do research on new models of agricultural equipment.	0,70	

As presented in detail in Table 2 above, all the scales used in the study exhibit high reliability coefficients. Although the purchasing behavior scale includes a limited number of items, which is generally considered a psychometric risk, the results of this study indicate that this limitation does not pose a significant threat to scale validity. The high values of Cronbach’s alpha ($\alpha = 0.825$), composite reliability ($CR > 0.90$), and average variance extracted ($AVE > 0.70$) demonstrate that the scale maintains sufficient reliability and validity despite the low number of items. According to the results of the confirmatory factor analysis, the factor loadings (λ) of the items exceed 0.700 at a significance level of $p < .001$. The composite reliability ($CR > 0.70$) values of the scales range between 0.887 and 0.917, indicating highly satisfactory levels. Similarly, as seen in Table 2, the average variance extracted ($AVE > 0.50$) scores fall between 0.733 and 0.813, well above the accepted threshold.

After the high reliability levels of the scale expressions were demonstrated, the variance inflation factor (VIF) values were checked for each scale expression. Because the multicollinearity value is expected to be low. Otherwise, the high level of relationship between the independent variables puts the reliability of the model at risk. As a result of the checks, if the VIF value is < 5 , there is no problem in the linear connection (Craney & Surles, 2002). The VIF values calculated for the variables in the examination varied between 1.478 and 4.324, and it was determined that the value less than 5 for each expression was within acceptable limits.

The Fornell-Larcker criterion was checked to evaluate the discriminant validity of the model before the path analysis. This criterion is an important indicator in terms of showing that the latent variables in the model are separated from each other and measure different structures. To assess discriminant validity, the square root of the average variance extracted for each factor was compared with the correlations between factors, and the results are presented in Table 3. The findings indicate that the square root of the average variance extracted is higher than the corresponding correlation coefficients between factors, confirming that the measurement model possesses discriminant validity.

Table 3. Correlation Coefficient Matrix and Square Roots of Average Variance Extracted Scores
Çizelge 3. Korelasyon Katsayı Matrisi ve Ortalama Varyansın Karekökleri Çıkarılan Puanlar

	BL	BA	PQ	PB	CI
BL	0,889				
BA	0,434	0,901			
PQ	0,461	0,472	0,874		
PB	0,345	0,376	0,381	0,853	
CI	0,546	0,568	0,623	0,487	0,856

BL: Brand loyalty, BA: Brand awareness, PQ: Perceived quality, PB: Purchasing Behavior,
CI: Consumer innovativeness

After determining the reliability, factor structures, composite and discriminant validity of the scales, path analysis was conducted to examine the relationship between consumer innovativeness and the brand equity elements, namely perceived quality, brand awareness, and brand loyalty, as well as to determine the impact of these elements on purchasing behavior. Path analysis was performed using the partial least squares (PLS) structural equation method via the SmartPls 3.0 program. The analysis was conducted by examining the R² value for the explanatory power of the structural model. The model, factor loadings, path coefficients, and the R² values of the latent variables are shown in Figure 2 below.

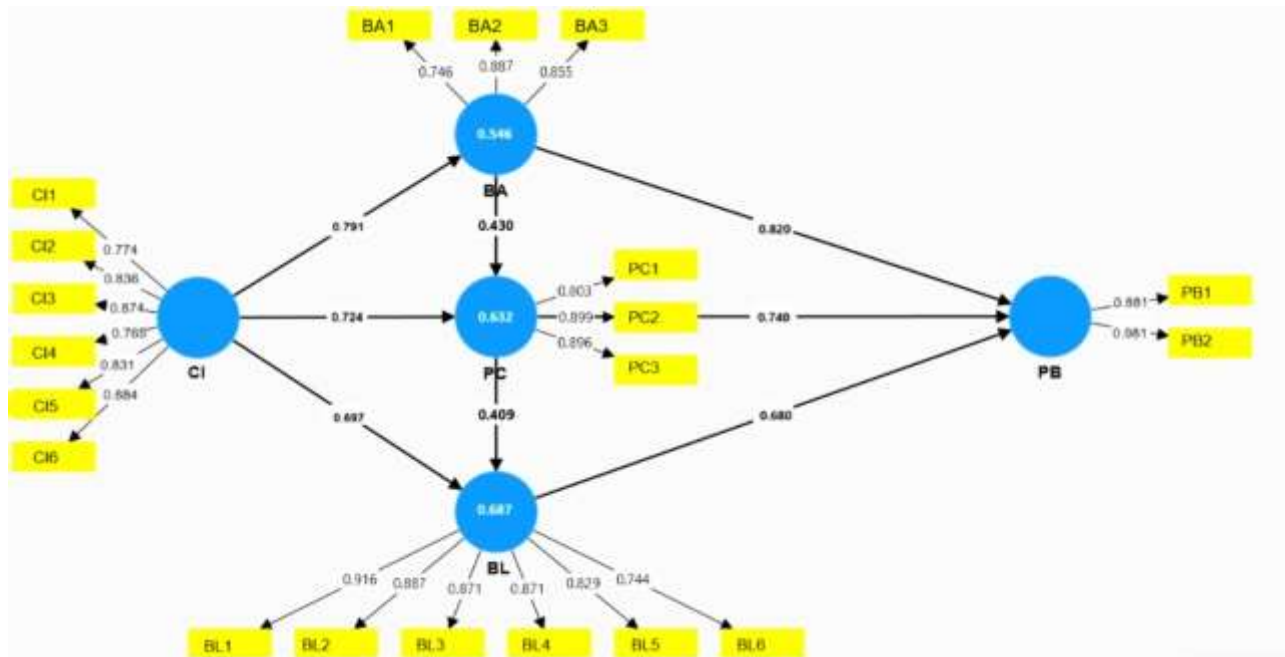


Figure 2. Structural Model Path Analysis
Şekil 2. Yapısal Model Yol Analizi

In addition to evaluating the explanatory power of the model, model fit was assessed using the Standardized Root Mean Square Residual (SRMR) value. The SRMR of the model was found to be 0.052, which is below the threshold value of 0.08, indicating an acceptable model fit (Hu & Bentler, 1999). In Pls-sem, SRMR alone is considered a sufficient fit index (Henseler et al, 2015), but here the normal fit index (NIF) value of the model is also given. The normal fit index (NIF) value for the model's goodness of fit was calculated as 0.923. A value of NIF above 0.90 is considered an acceptable fit (Bentler & Bonett, 1980). PLS is an analytical method that does not require assumptions regarding distribution and sample size, unlike covariance-based structural models (Altıntaş, 2017). To assess the data consistency of each scale, the rho_A coefficient was calculated. Accordingly, the values were

determined as follows: perceived quality (0.884), brand loyalty (0.929), brand awareness (0.832), purchasing behavior (0.940), and consumer innovativeness (0.759).

The significance of the model was tested at a 0.05 error probability level ($p < .001$). The table value of 1.96 was taken as the critical value, and the t-values were calculated using the bootstrapping method. The test type was set as two-tailed, and the subsample size was determined as 1000. The path coefficients of the model, t-values, and significance levels are presented in Table 4 below.

Table 4. Path Coefficients Significance Test Results (Bootstrapping)
Çizelge 4. Yol Katsayıları Önem Testi Sonuçları (Bootstrapping)

	Path Coefficient	t-value	p
Consumer innovativeness > Brand loyalty	0.697	3.385	.001
Consumer innovativeness > Perceived quality	0.724	5.594	.001
Consumer innovativeness > Brand awareness	0.791	6.516	.001
Brand awareness > Purchasing behavior	0.820	14.589	.001
Perceived quality > Purchase behavior	0.740	11.694	.001
Brand loyalty > Purchase behavior	0.680	5.875	.001
Brand awareness > Perceived quality	0.430	4.396	.001
Perceived quality > Brand loyalty	0.409	4.105	.001

The model has provided satisfactory results, indicating that consumer innovativeness influences brand loyalty, perceived quality, and brand awareness, and that these elements translate into purchasing behavior. According to the path analysis results, as seen in Table 4, consumer innovativeness has a strong impact on brand loyalty (0.697), perceived quality (0.724), and brand awareness (0.791). The effects of brand awareness, perceived quality, and brand loyalty on purchasing behavior were calculated as 0.820, 0.740, and 0.680, respectively, highlighting them as three key factors in understanding purchasing behavior.

Although not initially within the study's scope, the model also revealed that brand awareness affects perceived quality and that perceived quality contributes to brand loyalty.

CONCLUSION and DISCUSSION

The level of agricultural mechanization in Türkiye holds critical importance for sustainable production and increased productivity. This study provides data that reveals farmers' attitudes toward technology and their brand preferences, thereby offering insights into the development of public policies aimed at enhancing the diffusion of technology. Furthermore, it presents guiding results for domestic manufacturers operating in the agricultural machinery sector in terms of target market segmentation, product positioning, and brand management. This study aims to contribute to a better understanding of consumer behavior in Türkiye's agricultural machinery sector by examining the relationships between consumer innovativeness and key brand equity components—brand awareness, perceived quality, and brand loyalty. The results indicate that consumer innovativeness directly enhances brand awareness, perceived quality, and brand loyalty, while also indirectly influencing purchasing behavior through these factors. In this context, the study highlights that introducing innovative products not only meets evolving consumer demands but also reinforces brand loyalty and competitive advantage. Consumer innovativeness thus serves as a strategic guide for firms in product development and plays a critical role in shaping brand perception. Moreover, the study emphasizes how brands can transform their engagement with target audiences, particularly in an era where digital platforms and social media empower consumers to rapidly access information about brands and products.

Consumer innovativeness is a critical variable that reflects individuals' attitudes toward new products and technologies, their willingness to learn, and their trial behaviors. In this context, in a sector such as agricultural machinery, which is open to technological developments, innovative consumers are not only the first users of new products but also pioneers of industry transformation. The findings of the study indicate that this consumer group forms strong connections with the key elements that constitute brand value. Innovative individuals make more informed decisions, closely follow brand attributes, and demonstrate greater loyalty to the brands they are satisfied with. This study, conducted with farmers operating in the Kırşehir region, reveals that participants aged 30-49 are more innovative compared to other age groups. Similarly, higher education graduates exhibit more innovative behaviors than those with a secondary education. These findings are consistent with Rogers's (1983) *Diffusion of Innovations Theory*, which asserts that individuals with higher education, better access to information, and broader social networks are more likely to adopt innovations early. The greater innovativeness observed in the 30–49 age group may stem from their more active involvement in production processes and a higher tendency to engage with technology. Conversely, the lower innovativeness of the 18–29 age group may be due to limited experience in

agricultural decision-making and restricted access to financial resources. These demographic differences offer valuable guidance for companies aiming to segment their target market. Marketing strategies tailored to younger and less innovative segments, especially those that raise awareness and simplify the use of new technologies, may help boost adoption rates in the long term. The model developed through confirmatory and exploratory analyses shows that consumer innovativeness has strong and significant effects on brand awareness, perceived quality, and brand loyalty. Additionally, it was found that all three of these factors significantly influence purchase behavior. The model also revealed that brand awareness affects perceived quality, and perceived quality in turn influences brand loyalty. These results indicate that consumer innovativeness plays a central role not only as an individual characteristic but also in shaping brand perception and guiding purchase behaviors. For brands operating in the agricultural machinery sector, this underscores the importance of understanding innovative consumer profiles and developing marketing strategies tailored to these profiles.

The findings reached in this study largely align with the existing literature. Consistent with Im et al. (2003) and Steenkamp and Gielens (2003), our findings affirm the positive influence of consumer innovativeness on brand equity. Even in a sector with more rational and high-cost products like agricultural machinery, the increase in brand awareness due to innovation reveals that consumers show more attention and interest towards innovative products. Additionally, the effects on perceived quality and brand loyalty indicate that innovative individuals structure their experiences with brands in a more meaningful way. This suggests that, particularly for innovative consumers, the relationship between product quality and trust in the brand becomes more critical.

Another important finding of the study is that the components of brand value directly influence purchasing behavior. This result supports Aaker's (1991) brand value model and reveals that factors such as brand awareness, perceived quality, and brand loyalty are not only crucial in brand building but also in actual purchase decisions. Furthermore, the impact of brand awareness on perceived quality and perceived quality on brand loyalty shows that brand value has a hierarchical and interactive structure within itself. This result is also consistent with Keller's (1993) brand knowledge model.

The detection of significant differences in consumer innovativeness based on age and education level is consistent with Rogers's (1983) Diffusion of Innovations Theory. In particular, younger and more educated individuals tend to adopt innovations more quickly, contributing to the identification of open technology user profiles in the agricultural sector. In this context, it can be said that companies operating in the agricultural machinery sector need to develop more effective segmented strategies by considering these demographic characteristics in their marketing and communication strategies.

Koçkaya (2022), in a pioneering study in the domestic literature on the branding processes of agricultural equipment, identified brand as a significant factor in purchasing decisions and emphasized the importance of innovation not only in product development but also in the branding process. The results obtained in this study reveal important areas regarding decision-making processes in the agricultural equipment sector by revealing how innovative products affect consumer perception and brand loyalty, and show how consumer innovation, when combined with brand loyalty, shapes purchasing decisions.

Based on the findings of this study, several recommendations are presented for companies operating in the agricultural machinery sector, policymakers, marketing experts, and researchers. Firstly, as indicated by the statistical analysis results, brand awareness (0.82) emerges as the most influential variable on brand value, followed by perceived quality (0.74) and brand loyalty (0.68). This outcome highlights the critical role of awareness creation as a marketing strategy. Therefore, companies should prioritize strategies aimed at increasing brand awareness. In product categories that require high investments, such as agricultural machinery, brand recognition strengthens consumer trust and directly impacts purchasing decisions. In this context, industry trade fairs, digital platforms, and local agricultural consultancy channels should be used effectively. At the same time, communication strategies for perceived quality should be strengthened, with clear emphasis on product durability, service networks, and technical support advantages.

It is essential to emphasize that age and education level are crucial factors in target audience segmentation. Individuals aged 30-49 are more willing and inclined to adopt agricultural innovations. Educational initiatives and promotional activities aimed at this age group will facilitate faster acceptance of innovative products. Incentives such as additional service discounts for innovative farmers (age group 30-49) can increase the pace of adoption. Technological innovations in products should be presented in a simple and comprehensible manner, and creative marketing strategies, such as virtual reality applications, social media campaigns, and influencer partnerships, should be adopted, particularly targeting the younger and more educated farmer segment, as they are effective in creating brand awareness.

The impact of education level on innovation adoption should also not be overlooked. Highly educated individuals are more likely to adopt innovative agricultural equipment and demonstrate a more analytical approach in the

process. Therefore, companies should offer technical content and educational programs tailored to educated consumers. Furthermore, collaborations with early adopters in this group will help create effective marketing strategies for other segments.

Brand awareness stands out as one of the most powerful factors in creating brand value. Therefore, companies should prioritize advertising and marketing strategies to promote their products. Digital platforms and agricultural trade fairs can help companies establish effective communication. Farm equipment companies can increase brand awareness by hosting 'How It Works' videos, farmer testimonials, and live Q&A sessions on social media like Instagram and YouTube. However, strategies regarding distribution channels, such as establishing closer relations with farmers and local dealer networks, organizing demo tours for innovative products, and allowing farmers to try out the machines and see the technical features on site, will have an impact on increasing customer confidence. Pricing strategies such as flexible payment plans that fit farmers' budgets, opportunities to benefit from local incentives, and financing options offered through the banking system will also have an impact on increasing brand awareness.

To foster brand loyalty, post-sale service quality should be improved, loyalty programs should be developed, and feedback mechanisms based on user experiences should be established, thereby building long-term relationships with customers. Considering that previous positive experiences strongly influence brand loyalty, ensuring the sustainability of these experiences is essential.

Consumer innovativeness strengthens purchasing behavior through its impact on brand value. Companies should increase research and development investments and introduce innovative products to the market by improving existing products. Considering geographical differences, such as disc harrows or models suitable for soil structure, will contribute to creating a difference in regional markets. Also, implementing product development programs based on consumer feedback will enhance customer satisfaction and contribute to the innovation process. By collecting machine usage data with IoT sensors, interactions such as providing farmers with customized maintenance reminders or productivity analytics can strengthen brand loyalty.

Lastly, to accelerate the adoption of innovative products, practical initiatives such as training programs and field trials are recommended. Additionally, offering strong after-sales support will improve brand perception and increase customer satisfaction. In a digitalized world, companies should promote their innovative products through personalized marketing strategies on digital platforms.

The study's limitations were outlined in the methodology section. However, it is important to note that this research was conducted with farmers in Kırşehir and centered on specific product groups. The sample size was determined as 306 individuals with a 5% confidence level, and participants were selected using a random method. Age and education were chosen as demographic variables that could be more easily generalized. Moreover, the statistical methods used and the explanatory power of the model positively contribute to the generalizability of the study's results. Nevertheless, broader studies including different demographics, geographical areas, and product categories may produce different outcomes. The use of face-to-face surveys may have introduced social desirability bias. Future research using alternative measurement tools might yield varied results. Furthermore, as the study was conducted in 2024, consumer behavior may have evolved over time. Macroeconomic and technological factors that influence innovation adoption, such as rising prices or shifts in consumer priorities, were not extensively addressed. Similarly, government incentives for agricultural equipment purchases were not incorporated into the research model. The R^2 values from the model also suggest that additional variables may influence consumer behavior.

For future research, several directions can be recommended. The fact that this study is limited to the province of Kırşehir limits the ability of the results to represent all agricultural producers in Turkey. Since the socioeconomic structure of the region, types of agricultural activities, and machinery usage habits may differ, direct generalization of the findings to other regions should be considered carefully. In future studies, it will be possible to reach more holistic results by including different geographical regions. Studies conducted in different regions and with diverse farming communities and cultures may offer new perspectives. Replicating the findings in wider samples would enhance the generalizability of the results. Longitudinal studies could be used to observe changes over time in the relationship between consumer innovativeness and brand equity. In addition to survey methods, experimental designs may help assess the effects of specific marketing strategies or communication techniques. Future studies may incorporate socioeconomic variables, such as farm size, income, and access to credit, into the model as control variables. Furthermore, exploring the mediating role of perceived quality or the moderating effects of demographic factors like education level could lead to a more comprehensive understanding of the model. The research focused only on soil processing machines. However, it is evaluated that other agricultural machines such as tractors, harvesters, spraying, and irrigation systems that appeal to different areas of use may also create different perceptions in terms of consumer innovation. Therefore, including these product groups in the model in

future research will allow for a more comprehensive evaluation. Although the study structures the components of brand equity based on Aaker's classic model, the findings show that the effects of brand awareness and perceived quality on purchase behavior align with contemporary studies (e.g., Keller & Lehmann, 2003). Additionally, contemporary literature highlights new elements such as digital touchpoints (Christodoulides & de Chernatony, 2010) and consumer productivity. However, due to the unique conditions of the agricultural sector, this research does not cover new-generation brand equity factors such as social media influence and user reviews. This also reveals the application limitations of the study within the sector context. In sectors dominated by rational decisions, like agricultural equipment, the impact of these contemporary factors may be limited; nonetheless, with future digitalization, these variables could also be integrated into the model. Finally, incorporating psychological traits such as risk-taking and willingness to learn, as well as economic variables like income and investment orientation, would enrich the theoretical and practical contributions of future studies in this area.

Contribution Rate Statement Summary of Researchers

Literature review, ethics committee process, writing stage, translation, survey design, statistical analysis, language expression checks, publication process were carried out by the corresponding author. The second author carried out only the collection of data from the field.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

Ethics Statement

Ethics Committee Approval for this study was obtained from the Kırşehir Ahi Evran University Social and Human Sciences Scientific Research and Publication Ethics Committee with the decision dated 08.01.2025 and numbered 2025/01/36.

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