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Phenolic Profile and Antioxidant Capacity of *Helichrysum arenarium* Extracts: A Comprehensive LC-MS/MS and Antioxidant Analysis

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ABSTRACT

Helichrysum arenarium, commonly known as the immortal or everlasting flower, is a member of the Asteraceae family celebrated for its potential medicinal properties. This study aims to elucidate the phenolic profile and antioxidant properties of *H. arenarium* using advanced analytical techniques. Methanol extract of *H. arenarium* was analyzed using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) to identify and quantify various phenolic compounds. The phenolic profile revealed high concentrations of luteolin (744.57 mg 100^{-1} g^{-1}), quercetin (113.13 mg 100^{-1} g^{-1}), and naringenin (229.60 mg 100^{-1} g^{-1}), while other compounds were below the limit of quantification. The antioxidant capacity was evaluated using DPPH, ABTS, CUPRAC, and FRAP assays, showing moderate activity compared to standard antioxidants such as BHA, BHT, and Trolox. The methanol extract exhibited DPPH and ABTS radical scavenging activities of 19.14% and 26.91%, respectively, with FRAP and CUPRAC absorbance values of 0.441 and 0.653. These findings highlight the potential of H. arenarium as a source of natural antioxidants and pave the way for future research to optimize its therapeutic applications, especially in combating oxidative stress-related conditions.

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Helichrysum arenarium Ekstraktlarının Fenolik Profili ve Antioksidan Kapasitesi: Kapsamlı LC-MS/MS ve Antioksidan Analizi

ÖZET

Helichrysum arenarium, halk arasında ölümsüz veya sonsuz çiçek olarak bilinen, Asteraceae familyasına ait bir bitkidir ve potansiyel tıbbi özellikleriyle tanınmaktadır. Bu çalışmanın amacı, *H. arenarium*'un fenolik profilini ve antioksidan özelliklerini ileri düzey analitik tekniklerle açıklığa kavuşturmaktır. *H. arenarium*'un metanol ekstraktı, çeşitli fenolik bileşenleri tanımlamak ve miktarlarını belirlemek üzere Sıvı Kromatografi-Tandem Kütle Spektrometrisi (LC-MS/MS) kullanılarak analiz edilmiştir. Fenolik profil, luteolin (744.57 mg 100^{-1} g^{-1}), kuersetin (113.13 mg 100^{-1} g^{-1}) ve naringenin (229.60 mg 100^{-1} g^{-1}) gibi yüksek konsantrasyonlarda bileşenler içerirken, diğer bileşenler tespit sınırının altındadır. Antioksidan kapasite, DPPH, ABTS, CUPRAC ve FRAP testleri kullanılarak değerlendirilmiş ve standart antioksidanlar olan BHA, BHT ve Trolox ile karşılaştırıldığında orta derecede aktivite gözlenmiştir. Metanol ekstraktı, DPPH ve ABTS radikal temizleme aktiviteleri sırasıyla %19.14 ve %26.91, FRAP ve CUPRAC absorbans değerleri ise sırasıyla 0.441 ve 0.653 olarak belirlenmiştir. Bu bulgular, H. arenarium'un doğal antioksidan kaynağı olarak potansiyelini vurgulamakta ve özellikle oksidatif stresle ilgili koşullarla mücadelede terapötik uygulamalarını optimize etmek için gelecekteki araştırmaların yolunu açmaktadır.

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INTRODUCTION

Helichrysum arenarium, commonly known as the immortal or everlasting flower, is a species of flowering plant in the Asteraceae family, renowned for its diverse pharmacological properties and traditional medicinal uses. It has been traditionally utilized in various cultures for its medicinal properties, including its purported anti-inflammatory, antimicrobial, and antioxidant activities (Eroğlu et al., 2010; Umaz et al., 2023). Recent research has increasingly focused on the biochemical constituents of this plant, particularly its phenolic compounds, which are known for their significant antioxidant properties and potential health benefits. The presence and concentration of these phenolic compounds are critical in understanding the therapeutic efficacy of *H. arenarium*.

Phenolic compounds, a diverse group of secondary metabolites, play a crucial role in protecting plants against oxidative stress. They are also recognized for their ability to scavenge free radicals, thereby mitigating oxidative damage to cells and tissues. In the context of human health, phenolics have been linked to a reduced risk of chronic diseases associated with oxidative stress, such as cardiovascular diseases and cancer (Miller & Rice-Evans, 1997; Prior & Cao, 2000; Boudet, 2007; Halliwell & Gutteridge, 2007; Necip et al., 2021; Kaygısız et al., 2024; Uğur et al., 2024; Zengin et al., 2024). Understanding the profile of phenolic compounds in *H. arenarium* and their antioxidant efficacy is essential for harnessing its full therapeutic potential. In this context, Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) has emerged as a powerful analytical technique for the precise identification and quantification of phenolic compounds in plant extracts. LC-MS/MS provides high sensitivity and specificity, allowing for the detailed characterization of complex mixtures (Gao & Hu, 2015). This method is particularly valuable for profiling the diverse array of phenolic compounds present in *H. arenarium* and determining their relative concentrations.

To evaluate the antioxidant capacity of *H. arenarium*, several assays were employed, including ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), and CUPRAC (Cupric Reducing Antioxidant Capacity), tests. These assays are widely used to assess different mechanisms of antioxidant activity, providing a comprehensive overview of the plant's potential to neutralize oxidative species.

This study aims to elucidate the phenolic profile of *H. arenarium* and its antioxidant properties. This information will not only enhance understanding of the plant's medicinal value but also contribute to the development of natural antioxidants for therapeutic applications.

METHODS and MATERIALS

Chemicals

The compounds listed below were employed as standards in the LC-MS/MS analysis: acetohydroxamic acid (98%), vanillic acid (≥97%), catechin hydrate (≥ 99%), resveratrol (99%), thymoquinone (≥97%), caffeic acid (98%), gallic acid (98%), salicylic acid (99%), p-hydroxybenzoic acid (99%), phloridzin dihydrate (≥ 99%), oleuropein (≥ 80%), myricetin (≥96%), 2-hydroxy-1,4-naphthoquinone (97%), kaempferol, quercetin (98%) (≥97%), and alizarin (97%) from Sigma-Aldrich (Darmstadt, Germany); protocatechuic acid (97%), naringenin (≥95%), butein (≥98%), luteolin (≥98%), and silymarin (≥95%) from Merck (Darmstadt, Germany), ellagic acid (95%) and syringic acid (97%) from Fluka (Buchs, Switzerland); curcumin (≥99.5%) from Supelco (USA). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), trolox, trichloroacetic acid (TCA), and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were obtained from Sigma-Aldrich (Germany), and potassium persulfate ($K_2S_2O_8$), $CuCl_2$, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and potassium ferricyanide [$K_3Fe(CN)_6$] were obtained from Merck (Darmstadt, Germany).

Plant Samples

Helichrysum arenarium L. Moench plant was collected in May at Çelikhan town, Adiyaman, Turkey. The herb was washed with pure water and then dried in the shade at room temperature.

The Plant Sample Extraction

A 5 g portion of the powdered sample was subjected to extraction using 50 mL methanol as the extraction solvent, employing the maceration technique at ambient temperature for 24 hours. Following the extraction, the solution was filtered and evaporated to yield a dry extract. This extract was then reconstituted to a 1 mg mL^{-1} concentration and utilized for assays evaluating antioxidant capacity as well as for LC-MS/MS analysis.

LC-MS/MS Analyses

The analysis of 24 phytochemicals was conducted using a Shimadzu Nexera HPLC system coupled with a dual mass spectrometer (Kyoto, Japan). The liquid chromatograph was outfitted with LC-30AD binary pumps, a DGU-20A3R degasser, a SIL-30AC autosampler, and a CTO-10AS column oven. Separation of the analytes was achieved on an Inertsil ODS4 C18 reversed-phase analytical column (150 mm × 4.6 mm, 3 µm particle size). Gradient elution was performed at a flow rate of 0.5 mL min^{-1} and a column temperature of 40°C, with an injection volume of 4.0 µL. The mobile phase consisted of solvent A (water containing 5.0 mM ammonium formate and 0.1% formic acid) and solvent B (methanol containing 5.0 mM ammonium formate and 0.1% formic acid). The gradient elution program was as follows: 40-90% B from 0 to 20 minutes, 90-99% B from 20 to 23 minutes, 99-40% B from 23 to 24 minutes, and 4% B from 24 to 29 minutes. Mass spectrometric detection was carried out using a Shimadzu LCMS 8040 triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, operating in positive and negative ionization modes. Data acquisition and analysis were performed with Shimadzu LabSolutions software (Kyoto, Japan). Quantification of the analytes was achieved using multiple reaction monitoring (MRM) mode. Phenolic compounds were analyzed with two or three transitions per compound: the primary transition was used for quantification, while the additional transitions provided confirmation of the results.

Antioxidant Capacity

To determine the antioxidant capacity, the following tests were applied: DPPH free radical, ABTS cation radical scavenging activity, cupric reducing (CUPRAC), and ferric reducing (FRAP) methods (Miller, 1971; Elmastaş et al., 2006; Apak et al., 2004). UV/VIS Spectrophotometer (Shimadzu 2000S Model, Japan) was used for the detection of antioxidant capacity.

Statistical Analysis

The results were expressed as arithmetic mean ± standard error of the mean (sem); n = 3. Subsequently, the Tukey test was conducted to compare the antioxidant activity (DPPH, ABTS, FRAP, and CUPRAC) between the methanol extract, BHA, BHT, and Trolox. The results indicated significant differences across the treatments for all assays ($p < 0.001$).

Multiple linear regression analyses (Shimadzu LabSolutions software, Kyoto, Japan) were conducted to detect the concentrations of phenolic compounds identified in the LC-MS/MS data.

RESULTS and DISCUSSION

The LC-MS/MS system, noted for its high selectivity and sensitivity, was utilized to analyze phytochemicals present in *Helichrysum arenarium*. Key analytical parameters such as limits of detection (LOD), limits of quantification (LOQ), linear ranges, and coefficient of determination (R^2) for the studied analytes were determined, as summarized in Table 1.

Linear regression analyses were conducted to detect the concentrations of phenolic compounds identified in the LC-MS/MS data. Notably, all compounds exhibited a strong relationship ($R^2 > 0.99$), underscoring the high explanatory power of the regression models and the accuracy of the employed methodology. For instance, the linear regression equation derived for luteolin, y = 1389x - 40923, yielded an R^2 value of 0.9988. This correlation between luteolin concentration and peak area highlights the robustness of the analytical approach and the reliability of the quantification results.

For the phytochemical determination, a methanol extract of *H. arenarium* was utilized with the LC-MS/MS technique. Acetohydroxamic acid, catechin hydrate, syringic acid, fumaric acid, caffeic acid, phloridzin dihydrate, myricetin, quercetin, butein, naringenin, luteolin, and kaempferol from phenolic compounds were quantified in the methanol extract of *H. arenarium*. The concentration of luteolin was notably high at 744.57±4.21 mg 100^{-1} g^{-1}, while quercetin and naringenin concentrations were 113.13±1.22 mg 100^{-1} g^{-1} and 229.60±3.15 mg 100^{-1} g^{-1}, respectively. p-hydroxybenzoic acid, thymoquinone, curcumin, protocatechuic acid, salicylic acid, resveratrol, 2-hydroxy-1,4-naphthoquinone, ellagic acid, silymarin, and alizarin were under LOQ (Table 2).

The antioxidant capacity of the methanol extract of *H. arenarium* plant was assayed with the following tests: DPPH free radical scavenging, ABTS cation radical scavenging, cupric reducing (CUPRAC), and ferric reducing (FRAP). Results of antioxidant capacity are given in Table 3. DPPH and ABTS results were expressed as percentage radical scavenging activity, and CUPRAC and FRAP results were expressed as absorbance.

Table 1 Analytical parameters for LC-MS/MS analysis (Uğur & Güzel, 2023)
Çizelge 1. LC-MS/MS analizi için analitik parametreler

Compounds	Retention time (min)	Precursor ion (m z^{-1})	Product ion (m z^{-1})	Linear regression	LOD (µg L^{-1})	LOQ (µg L^{-1})	R^2	Linear range (µg L^{-1})
Acetohydroxamic acid	0.406	76.15	58	y = 216.91x + 6165.8	6.90	23.01	0.9989	20-750
Syringic acid	3.001	199.1	140.1	y = 112.03x + 1316.1	2.88	9.61	0.9994	10-500
Vanillic acid	2.762	168.95	65	y = 48.343x + 662.5	84.78	282.61	0.9993	250-1000
Resveratrol	3.606	229	135	y = 733.34x - 69955	41.83	139.43	0.999	250-1000
Thymoquinone	3.337	165	137	y = 349.23x - 2887.4	7.64	25.47	0.9971	20-500
Caffeic acid	2.836	179	135	y = 1227.2x - 5396.5	2.87	9.58	0.9948	10-100
Gallic acid	1.278	169.1	124.9	y = 305.07x - 1859.3	3.92	13.06	0.9981	10-100
Protocatechuic acid	3.556	181	108	y = 1382.2x - 4393.1	2.76	9.20	0.9967	10-500
p-hydroxybenzoic acid	3.555	137.2	93.1	y = 3831.2x - 94423	8.92	29.74	0.9996	40-500
Oleuropein	3.567	539.1	377	y = 324.26x - 5388.8	7.17	23.90	0.9997	40-750
Salicylic acid	3.558	137.2	93	y = 3838.2x - 149277	22.88	76.25	0.9977	75-1000
2-Hydroxy-1.4-naphthoquinone	3.664	173.1	145	y = 461.45x - 4553.8	2.07	6.91	0.9989	10-500
Phloridzin dihydrate	3.594	435.1	273.1	y = 120.23x - 9479.5	81.80	272.67	0.9989	250-1000
Ellagic acid	3.681	301.1	228.9	y = 18.841x + 911.46	23.74	79.14	0.9967	100-1000
Myricetin	3.644	317	179.1	y = 588.4x - 4990.6	4.34	14.45	0.9987	20-500
Butein	3.935	271	134.9	y = 62.943x - 2793	38.50	128.20	0.996	100-1000
Quercetin	3.891	301.1	150.9	y = 150.09x - 422.87	7.79	25.98	0.9997	20-500
Silymarin	3.996	481.1	453.1	y = 199.91x + 950.97	8.00	26.70	0.9997	40-750
Naringenin	3.952	271	150.9	y = 700.8x - 26469	68.40	228.10	0.9997	250-1000
Kaempferol	4.298	285	117	y = 62.513x - 821.08	3.90	13.00	0.9982	20-1000
Luteolin	4.069	285	150.9	y = 1389x - 40923	6.40	21.40	0.9988	40-1000
Catechin hydrate	2.532	291	139.1	y = 1717.9x - 563.99	2.05	6.84	0.9988	10-750
Alizarin	4.594	239	211	y = 26.512x - 1721	15.30	51.10	0.9991	60-2000
Curcumin	4.672	367.1	216.9	y = 1908.9x - 8252.1	12.80	42.70	0.9994	40-1000

The methanol extract of *H. arenarium* exhibited moderate antioxidant activity in comparison to synthetic antioxidants (BHA and Trolox). The DPPH radical scavenging activity of the extract was 19.14±2.13%, significantly lower than that of BHA (47.03±6.39%) and Trolox (57.81±4.09%). The ABTS assay revealed similar patterns, with the extract showing 26.91±2.60%, compared to BHA (93.65±4.71%) and Trolox (90.03±3.07%). Statistical analysis using the Tukey test indicated significant differences in antioxidant activity between the extract and the standards, $p < 0.001$. The test confirmed that the activity of the extract was significantly lower than both BHA and Trolox ($p < 0.001$, Table 3).

Table 2 Results of qualitative and quantitative determination of phytochemicals in *H. arenarium* extract by LC-MS/MS

Çizelge 2. H. arenarium ekstrakındaki fitokimyasalların LC-MS/MS ile kalitatif ve kantitatif tayin sonuçları

Compounds	Means ± sem (mg 100^{-1} g^{-1})	Compounds	Means ± sem (mg 100^{-1} g^{-1})
Acetohydroxamic acid	23.23±0.17	Kaempferol	202.63±7.90
Catechin hydrate	3.09±0.10	2-Hydroxy-1,4-naphthoquinone	< LOQ
Syringic acid	5.22±0.13	Curcumin	< LOQ
Fumaric acid	50.28±5.72	Ellagic acid	< LOQ
Caffeic acid	136.24±0.42	Thymoquinone	< LOQ
Phloridzin dihydrate	190.87±6.93	Protocatechuic acid	< LOQ
Myricetin	10.87±0.83	Salicylic acid	< LOQ
Quercetin	113.13±1.22	Silymarin	< LOQ
Butein	10.83±0.21	Resveratrol	< LOQ
Naringenin	229.60±3.15	*p*-hydroxybenzoic acid	< LOQ
Luteolin	744.57±4.21	Alizarin	< LOQ

Data represent arithmetic mean ± standard error of the mean (sem) of three independent samples.

Table 3 Antioxidant capacity of 0.2 mg/mL concentration of *H. arenarium* extracts, BHA, BHT, and trolox

Çizelge 3. 0.2 mg/mL H. Arenarium ekstraktı, BHA, BHT ve Trolox'a ait antioksidan kapasite sonuçları

	DPPH	ABTS	FRAP	CUPRAC
	(%)		(Absorbance)	
Methanol extract	19.14±2.13^{b}	26.91±2.60^{c}	0.441±0.020^{c}	0.653±0.011^{d}
BHA	47.03±6.39^{a}	93.65±4.71^{a}	0.669±0.034^{b}	1.995±0.010^{b}
BHT	21.71±4.21^{b}	58.21±2.66^{b}	1.232±0.034^{a}	2.278±0.021^{a}
TROLOX	57.81±4.09^{a}	90.03±3.07^{a}	1.265±0.028^{a}	1.812±0.032^{c}
p	*0.001*	*0.001*	*0.001*	*0.001*

Data represent average values ± standard deviation of three independent samples. Different letters $^{[a-d]}$ indicate significant differences according to a Tukey test. ($P < 0.001$).

The growing interest in natural products as therapeutic agents is evident in the increasing use of these substances to address various diseases. As a result, research into the effectiveness of plant-derived compounds has gained significant traction, highlighting their potential as novel treatment options. Among these compounds, phenolic substances are particularly noteworthy. These secondary metabolites, produced by plants, play crucial roles in their defense mechanisms, protecting them from herbivores, pests, pathogens, and a range of environmental stressors. Phenolic compounds are known for their diverse biological and pharmacological activities. They exhibit potent antioxidant properties, which help neutralize harmful free radicals in the body (Çuhacı et al., 2021). Additionally, they possess anti-inflammatory effects that can mitigate chronic inflammation, a key factor in many diseases. Their anti-tumor and anti-cancer properties are also of considerable interest, as they could potentially play a role in cancer prevention and treatment. Moreover, phenolic compounds have demonstrated antiviral and anti-allergic activities, further underscoring their potential therapeutic benefits (Sarker & Oba, 2020; Gülçin et al., 2002). Recent studies have highlighted the effectiveness of these phenolic compounds, particularly in the prevention and management of chronic conditions. They are increasingly recognized for their potential to address neurodegenerative diseases, such as Alzheimer's and Parkinson's, as well as metabolic disorders like diabetes (La Fata et al., 2014). Furthermore, their role in combating cardiovascular diseases and cancer underscores their importance in modern therapeutic strategies. As research continues, the full range of benefits offered by these plant-derived compounds is likely to become even more apparent.

In the present study, different amounts of acetohydroxamic acid, catechin hydrate, syringic acid, fumaric acid, caffeic acid, phloridzin dihydrate, myricetin, quercetin, butein, naringenin, luteolin, and kaempferol in the methanol extract of *H. arenarium* were detected. In terms of phytochemical content, the high concentration of

luteolin aligns with previous studies (Sroka et al., 2004; Babota et al., 2018), which identified luteolin as a major phenolic compound in *H. arenarium*. And also, the results of luteolin, kaempferol, naringenin, caffeic acid, syringic acid, protocatechuic acid, and quercetin were comparable to those previously reported (Gradinaru et al., 2014; Grinev et al., 2016; Babota et al., 2018; Pljevljakušić et al., 2018). In one study conducted in Poland, Sroka et al. (2004) quantified caffeic acid, syringic acid, p-hydroxybenzoic acid, kaempferol, quercetin, and protocatechuic acid by HPLC in different solvent extracts of *H. arenarium*. On the contrary, p-hydroxybenzoic acid and protocatechuic acid were under LOQ in the present study. Babota et al. (2018), low amounts of luteolin and kaempferol in *H. arenarium* were found between 5.76-9.98 and 7.16-181.23 mg 100^{-1} g^{-1} using different extraction solvents, respectively. On the other hand, Jarzycka et al. (Jarzycka et al., 2013) found high amounts of naringenin (1740 mg 100^{-1} g^{-1}) by HPLC in *H. arenarium*. The differences between the phenolic compounds in *H. arenarium* plant depend on the soil, the processes after harvesting, the extraction process, and the analysis methods.

As shown in Table 3, the antioxidant capacity of *H. arenarium* extracts was compared with those of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trolox. *H. arenarium* extract showed lower activity than BHA, BHA, and trolox in all test results, too. The results of this study confirm the moderate antioxidant activity of *Helichrysum arenarium* methanol extracts, which were less potent compared to synthetic antioxidants such as BHA and Trolox. The significant difference in activity, as indicated by the Tukey test results ($p < 0.001$), suggests that while the extract has potential as a natural antioxidant, its efficacy is not as high as that of commercial alternatives. In previous studies, Babota et al. (2018) reported that the antioxidant capacity of *H. Arenarium* extract was 4.04 mg mL^{-1} (ABTS) and 4.91 mg mL^{-1} (DPPH). Umaz et al. (2023), the antioxidant activity results in two different populations of the *H. Arenarium*, found as 114.8-118.8 mg 100^{-1} g^{-1} and 0.642-0.766 absorbance according to DPPH and CUPRAC tests, respectively. The results obtained by Babota et al. (2018) using the DPPH and ABTS assays are significantly lower compared to the findings of the present study (Tablo 3). In addition, the DPPH assay results reported by Umaz et al. (2023) are also lower than those observed in the present study. On the other hand, the results from the CUPRAC assay conducted by Umaz et al. align closely with the results of the present study (Tablo 3). Antioxidant activity is primarily associated with phenolic compounds, which exhibit their effects through various mechanisms such as scavenging free radicals, providing reducing power, and chelating metal ions (Gülhan & Yangılar, 2022). The effectiveness of these compounds in combating oxidative stress is largely due to their phenolic hydroxyl groups. These hydroxyl groups are crucial, as they significantly enhance the compounds' ability to neutralize free radicals, thus contributing to their overall antioxidant potential (Işık, 2020; Güzel & Elmastaş, 2020).

The major phenolic compounds reinforce the potential of *H. arenarium* as a source of natural antioxidants. However, the moderate antioxidant activity observed may be due to factors such as extraction methods or environmental influences on the phytochemical content. Future research should focus on optimizing extraction methods to enhance the bioavailability of these phenolic compounds and further explore the therapeutic potential of *H. arenarium* extracts.

CONCLUSION

The analysis of *Helichrysum arenarium* using LC-MS/MS successfully identified and quantified several key phenolic compounds, including luteolin, quercetin, and naringenin, which are known for their antioxidant properties. Despite the moderate antioxidant activity of the methanol extract, as compared to synthetic antioxidants such as BHA, BHT, and Trolox, the presence of high levels of phenolic compounds suggests that *H. arenarium* has significant potential for further research and application as a natural antioxidant. The variations in antioxidant activity observed in different studies highlight the impact of extraction methods and plant sources on the efficacy of *H. arenarium*. Future studies should prioritize refining extraction processes and further elucidating the bioactive mechanisms of its phenolic components. Such efforts are critical to fully unlocking the therapeutic potential of *H. arenarium*, particularly in combating oxidative stress-related disorders and supporting the development of novel, natural antioxidant therapies.

Conflict of Interest

The authors declare they have no conflict of interest.

Contribution of the Authors as Summary

The authors declare the contribution of the authors is equal.

Ethics Statement

This study doesn't require an ethics committee decision.

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Comparison of Antioxidant and Antimicrobial Properties of Zinc oxide and Selenium oxide Nanoparticles using *Verbascum kotschyi* Boiss. & Hohen.

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ABSTRACT
Nanoparticle applications have been studied in many fields in recent years. Among these studies, the synthesis of nature-friendly and health-friendly nanoparticles through green synthesis attracts much attention. These investigations also highlight the significance of several plant species, many of whose worth and traits remain unknown. The goal of this work is to create zinc oxide and selenium nanoparticles from *Verbascum kotschyi* Boiss. & Hohen., a plant species that hasn't received much attention, and to ascertain the antioxidant and antibacterial qualities of these nanoparticles. To accomplish this, three distinct techniques (DPPH, CUPRAC, and FRAP) were used to assess the produced nanoparticles' *in vitro* antioxidant capabilities after SEM, EDX, and FTIR analyses. Furthermore, the disk diffusion technique was utilized to ascertain the antibacterial efficacy of these nanoparticles against both gram-positive and gram-negative bacteria and fungus. In conclusion, *V. kotschyi*-derived zinc oxide nanoparticles outperformed selenium nanoparticles in terms of antibacterial activity. But when it came to antioxidant activity, selenium nanoparticles outperformed zinc oxide nanoparticles. Thus, it was determined that the products created by nanoparticle synthesis from *Verbascum kotschyi* have properties that can be used in different fields.

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Verbascum kotschyi Boiss. & Hohen. Kullanılarak Sentezlenen Çinko Oksit ve Selenyum Oksit Nanopartiküllerinin Antioksidan ve Antimikrobiyal Özelliklerinin Karşılaştırılması

ÖZET
Son yıllarda nanopartiküllerin birbirinden farklı alanlarda kullanımı araştırılmaktadır. Bu çalışmalar içinde özellikle yeşil sentez ile doğa dostu ve sağlığa faydalı nanopartiküllerin sentezi oldukça ilgi çekmektedir. Aynı zamanda bu çalışmalar ile değeri ve pek çok özelliği bilinmeyen çok sayıda bitki türünün önemi de açığa çıkarılmaktadır. Bu nedenle bu bitki türlerinden biri olan ve üzerinde nanopartikül çalışmaları yapılmayan bir tür olan *Verbascum kotschyi*'den çinko oksit ve selenium nanopartiküller sentezleyip bu nanopartiküllerin antimikrobiyal ve antioksidan özelliklerini belirlemek amaçlanmıştır. *Verbascum kotschyi*'den sentezlenen nanopartiküllerin SEM, EDX, FTIR analizleri yapıldıktan sonra üç farklı yöntemle (DPPH, CUPRAC ve FRAP) *in vitro* antioksidan kapasiteleri belirlenmiştir. Ayrıca disk difüzyon yöntemi ile gram-pozitif, gram-negatif bakteriler ve mantar üzerine antimikrobiyal etkisi belirlenmiştir. Sonuç olarak *Verbascum kotschyi*'den sentezlenen çinko oksit nanaopartiküller selenyum nanopartiküllere kıyasla daha fazla antimikrobiyal özellik sergilemiştir. Ancak selenyum nanaopartiküller çinko oksit nanaopartiküllerden daha etkin antioksidan özellik sergilemiştir. Böylece *Verbascum kotschyi*'nin nanopartikül sentezi ile oluşturulabilecek ürünleri sayesinde farklı alanlarda kullanılabilecek özelliklere sahip olduğu tespit edildi.

Biyokimya

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Anahtar Kelimeler
Antimikrobiyal
Antioksidan
Yeşil sentez
Selenyum nanopartikül
Verbascum kotschyi

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INTRODUCTION

Because they differ from their bulk form in industrial domains by having specific electrical and optical properties, nanoparticles are useful across several domains, such as the agricultural, environmental, and biomedical (Nayak et al., 2021). In many areas, nanoparticles have been widely applied. Physical and chemical techniques are the primary means of identifying the nanoparticle synthesis processes; nevertheless, these techniques can be costly, hazardous, and adsorb on the nanoparticle surface (Mosalam & Marzouk, 2013; Kalishwaralal et al., 2016). Because of its advantages, including cost-effectiveness, ease of use and speed in manufacturing, friendliness with the environment, and other advantages, plant-mediated creation of nanoparticles has garnered an abundance of attention lately (Singh et al., 2014).

It is possible to create selenium nanoparticles using chemical, biological, and physical techniques. High temperatures, dangerous substances, and an acidic pH are required for chemical and physical procedures; these conditions are very hazardous and unfit for biological use (Iranifam et al., 2013). However, the biological production of SeNPs (Selenium Nanoparticles) is nontoxic, affordable, environmentally benign, and safe (Wadhwani et al., 2016). Furthermore, the organic materials that naturally coat the surface of biologically produced SeNPs make them more stable because they prevent nanoparticles from aggregating over time (Park et al., 2011).

The scientific community worldwide has been captivated by zinc oxide nanoparticles due to their exceptional catalytic activity, semiconducting nature, and ultraviolet filtration capabilities (Nagajyothi et al., 2013). Furthermore, reports indicate that these nanoparticles are biocompatible, non-toxic, and safe for biological systems (Anjum et al.,2021; Agarwal & Shanmugam, 2020; Bhuyan et al., 2015). Because zinc oxide nanoparticles can take in dangerous radiation like UV-A and UV-B, they are also used in sunscreen lotions and cosmetics (Ramesh et al., 2014). Zinc oxide is safe and can be utilized as a medicine, according to the US Food and Drug Administration (Lopez De Romaña et al., 2002). To eliminate infectious microorganisms, zinc oxide nanoparticles can be employed as an antibacterial substance. Shape, particle size, concentration, and duration of being in contact with the bacterial cell all affect how they inflict harm to the cell wall, seep inside, build up in the cell membrane, and eventually cause death by interfering with metabolic activities (Siddiqi et al., 2018).

Crucial trace elements in living things, zinc and selenium are crucial for immune system function, antioxidant defense, and antitumor activity in humans. Because biosynthesized zinc and selenium nanoparticles are more biodegradable, less toxic, and easily removed from the body, they are very advantageous (Zhuang et al., 2007; Schomburg, 2017).

Around 360 species of *Verbascum* L. (Scrophulariaceae) are known to exist in the world. There are roughly 249 species in Türkiye, among these, 191 are endemic (Huber-Morath, 1978; Georgiev et al., 2011; Güner et al., 2012). *Verbascum* species, also referred to as "sigirkuyrugu," have been utilized as a sedative, expectorant, mucolytic, anti-diarrheic, diuretic, and wound healer in traditional Turkish folk medicine (Baytop, 1999; Tuzlaci & Erol, 1999; Sezik et al., 2001).

The purpose of the selection of the *V. kotschyi* species in this study is to show the species' widespread spread in the Mardin province, the plant is being used for medical purposes by the public; and no previous study has been done (Mungan Kılıç & Kılıç, 2022; Eksik, 2020).

MATERIALS and METHODS

The aerial sections of *Verbascum kotschyi* were gathered from Mardin (Türkiye) - Mardin - Ortaköy highway, roadside, 37°17'07"N 40°46'32"E, 742 m altitude, on 11 May 2022. Flora of Türkiye (Huber-Morath, 1978) and Flora of Türkiye checklist (Güner et al., 2012) were used to identify plant species. An expert in botany verified the plants (Dr. Fatma Mungan Kılıç, Mardin Artuklu University), and one voucher specimen (Voucher No: M. Kılıç 264) was kept at Mardin Artuklu University in Türkiye.

60 g of *V. kotschyi* sample, which had been dried in the shade, was combined with 900 ml of distilled water and stirred with a magnetic stirrer for four hours at 60 °C. After passing through filter paper, the plant extraction was obtained. The extract was split into two halves. For 20 minutes at 60 °C, 250 ml of plant extract and 200 ml of 0.1 M $Zn(CH_3COO)_2 2H_2O$ were mixed in the first section. This solution appeared to be lightening in color. When the second portion of the extract (250 ml of plant extract) was mixed with 50 mM Na_2SeO_3 and heated to 60 °C for 20 minutes, a reddish color shift was noticed. The absorbance of both extracts was then recorded in the 200–800 nm

range using the UV-VIS spectrophotometer following a 24-hour incubation period at room temperature. Following a 30-minute centrifugation at 5000 rpm for both solutions, the precipitates were incubated for three hours at 100 °C in etuve.

By measuring the DPPH radical reduction potential of zinc nanoparticles (VkZnO) and selenium oxide nanoparticles (VkSe) produced from *V. kotschyi* using 60 µM DPPH radical solution, an antioxidant capacity analysis test was carried out. Trolox and the sample (VkZnO, VkSe) produced at several doses (10, 20, 30 µg ml^{-1}) were measured for their absorbance at 517 nm to determine their DPPH reduction capacities (Makhlouf-Gafsi et al., 2018).

The FRAP technique was employed to analyze the antioxidant capacity of VkZnO and VkSe solutions at varying concentrations. The solutions were mixed with 20 mM $FeCl_3$ solution and FRAP reagent (10 volumes of 0.3 M acetate buffer (pH:3.6), 1 volume of 10 mM TPTZ solution, and 1 volume of 20 mM $FeCl_3.6H_2O$ solution), and their total absorbance at 593 nm was noted (Gülçin, 2012).

The CUPRAC technique was used to perform antioxidant testing. VkZnO, VkSe, and Trolox solutions were made at varied concentrations. 0.01 M $CuCl_2$, $7.5x10^{-3}$ M neocuprin solution, and 1 M ammonium acetate buffer (pH: 6.5) were included, and their absorbances were measured at 450 nm (Apak et al., 2007).

The molecular structure of the *V. kotschyi*-derived nanoparticles was determined by SEM, EDX, and FTIR analyses. Zinc oxide nanoparticles (VkZnO) at a concentration of 100 mg ml^{-1} and selenium oxide nanoparticles (VkSe) at a concentration of 60 mg ml^{-1}, which were produced from *V. kotschyi*, were used for antimicrobial activity testing. Water acted as the negative control in these tests, while the antibiotic rifampin (5 µg) acted as the positive control. Using the disk diffusion technique, antimicrobial activity tests were conducted on gram (-) bacteria *(P. aeruginosa, E. coli, K. pneumoniae)*, gram (+) bacterium *(S. aureus)*, and fungus *(C. albicans)* (Wayne, 1997).

Statistical Analysis

In this investigation, every experiment was carried out in triplicate. The findings are presented as the mean value ± standard deviation. To ascertain if the antioxidant and antimicrobial properties of VkZnO and VkSe varied substantially from one another, data were analyzed using a one-way ANOVA for multiple comparisons of means. The threshold for statistical significance was $p<0.050$. Statistical analyses were performed separately for each method in antioxidant analyses. This statistical study revealed that VkZnO had antibacterial activity. VkSe and VkZnO showed antioxidant capacities, especially VkSe, in CUPRAC, FRAP, and DPPH methods. $p<0.050$ was found in the antibacterial and antioxidant tests with nanoparticles. The intergroup significance Fp: 0.027 in the FRAP method, significance Cp: 0.030 in the CUPRAC method, and intergroup significance Dp: 0.026 in the DPPH method were found. Significance p:0.000 was found in antimicrobial analyses.

RESULTS and DISCUSSION

UV-VIS, SEM-EDX, and FTIR analyses were carried out to elucidate whether the nanoparticles synthesized from *Verbascum kotschyi* were synthesized chemically and the molecular structure of the synthesized nanoparticles. Figure 1 displays the UV-VIS data of zinc oxide and selenium oxide nanoparticles that were made from *V. kotschyi*.

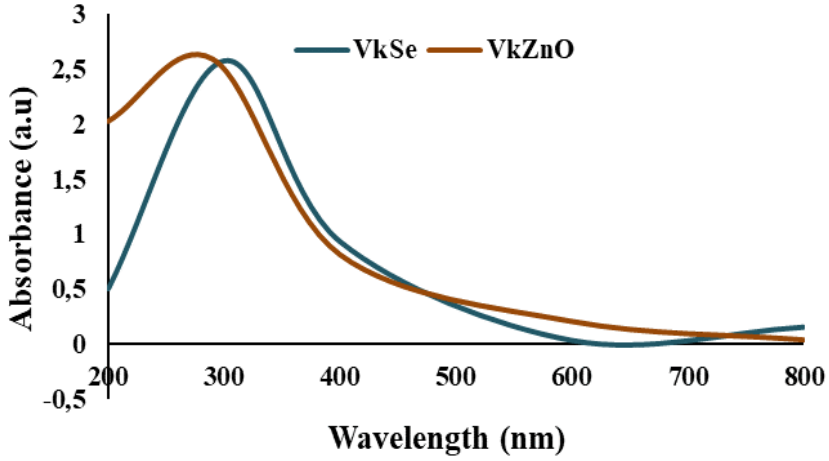


Figure 1. UV-VIS spectrophotometer results of VkZnO and VkSe nanoparticles
Şekil 1. VkZnO ve VkSe nanoparçacıklarının UV-VIS spektrofotometre sonuçları

The highest absorbance of VkZnO was measured at 290 nm. The synthesis of the zinc oxide nanoparticle is shown by this peak. SPR (surface plasmon resonance) happens when light interacts with the movable surface electrons of VkZnO nanoparticles (Akintelu & Folorunso, 2020; Al-Dhabi & Arasu, 2018). The synthesis of zinc oxide nanoparticles was demonstrated by the appearance of the peak at 295 nm wavelength, which is where the SPR of VkZnO nanoparticles is produced (Rajakumar et al., 2018; Akintelu & Folorunso, 2020).

The surface plasmon vibration peak at 300 nm in the UV-VIS spectrum seen in Figure 1 verified the production of VkSe nanoparticles (Yang et al., 2008; R. R. Mishra et al., 2011; Mishra et al., 2013; Srivastava & Mukhopadhyay, 2015). SEM images of zinc oxide nanoparticles produced from *V. kotschyi* are displayed in Figure 2.

VkZnO was visible in SEM pictures as stones and pieces of broken rock. It failed to take on a regular, symmetrical form. Figure 3 shows VkSe nanoparticle SEM images.

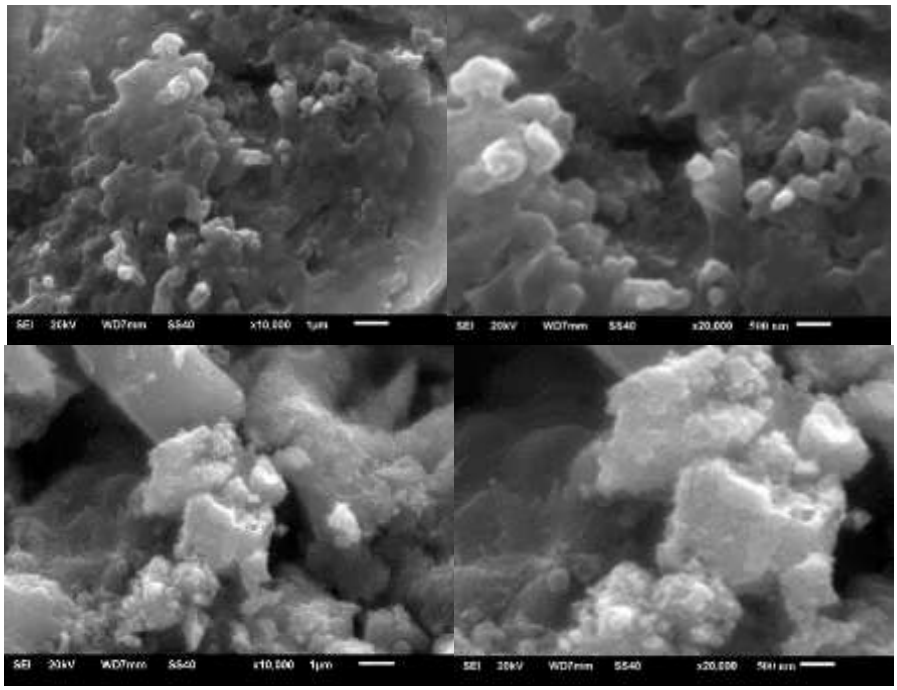


Figure 2. VkZnO nanoparticles SEM images
Şekil 2. VkZnO nanoparçacıklarının SEM görüntüleri

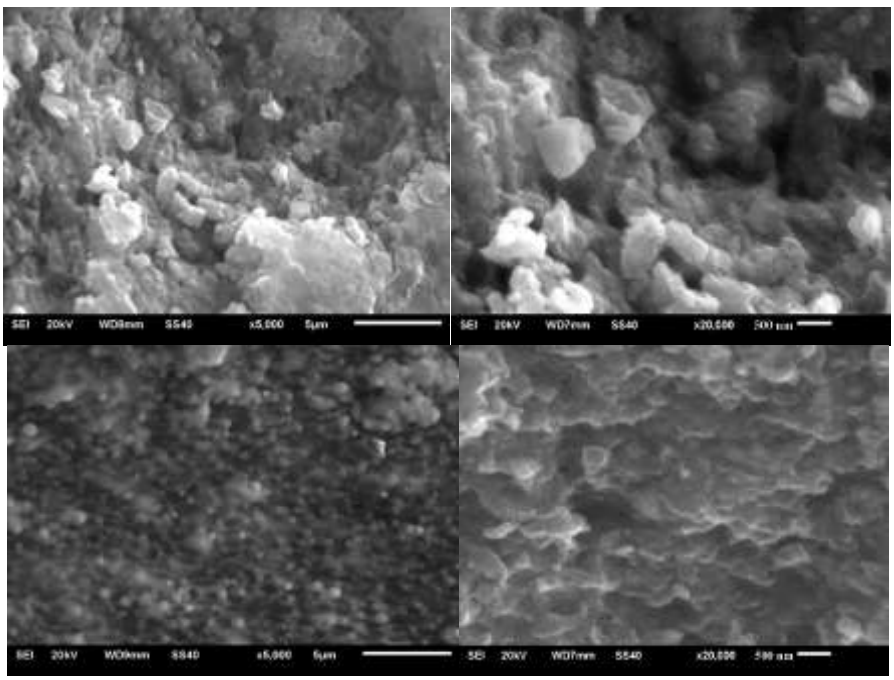
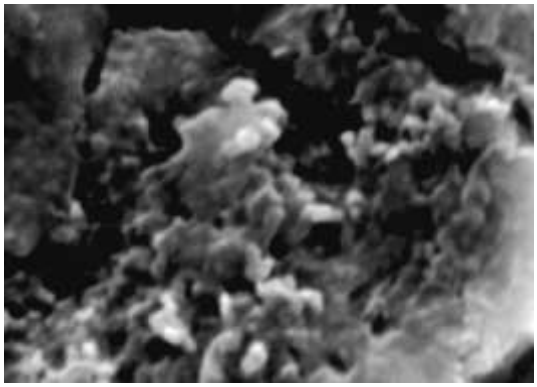
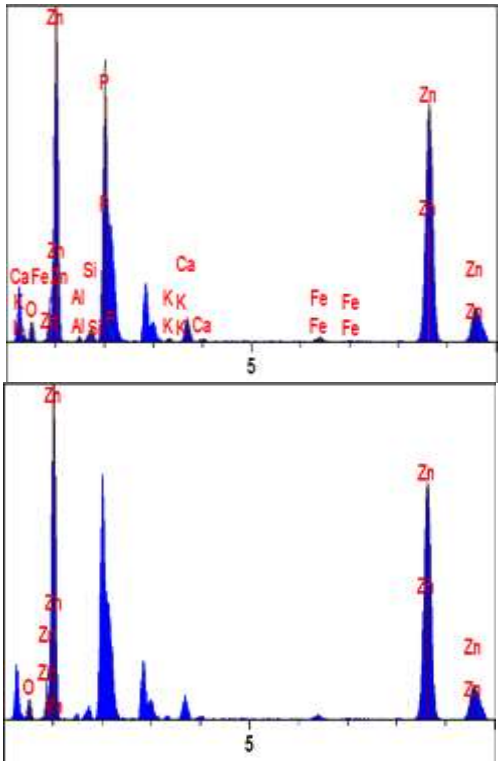


Figure 3. VkSe nanoparticles SEM images
Şekil 3. VkSe nanoparçacıklarının SEM görüntüleri

In the SEM images, VkSe produced tiny spherical spots and patterns that resembled reliefs on karst cave ceilings. Figure 4 displays the VkZnO nanoparticle EDX (Energy Dispersive X-Ray Spectroscopy) data.



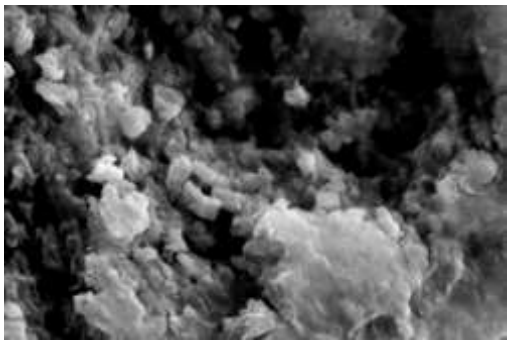


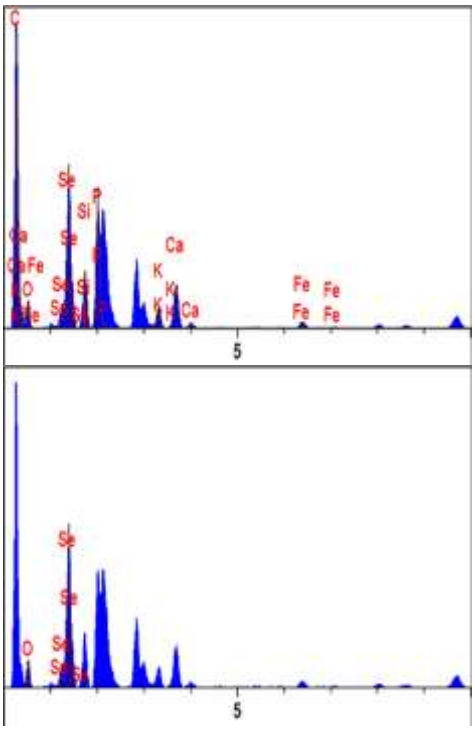
Elt.	Line	Intensity (c s^{-1})	Error 2-sig	Conc	Units
O	Ka	48.71	2.176	3.091	wt.%
Al	Ka	16.11	3.204	0.483	wt.%
Si	Ka	43.04	3.655	1.006	wt.%
P	Ka	887.12	8.433	18.451	wt.%
K	Ka	13.53	3.438	0.228	wt.%
Ca	Ka	91.11	3.889	1.494	wt.%
Fe	Ka	25.45	2.912	0.571	wt.%
	Ka	1,317.08	9.681	74.675	wt.%
			Total	100.000	wt.%

Figure 4. EDX spectroscopy results of VkZnO nanoparticles
Şekil 4. VkZnO nanoparçacıklarının EDX spektroskopisi sonuçları

VkZnO nanoparticles' elemental composition was ascertained by EDX analysis. In addition to Zn and O, the data show that VkZnO also contains elements like P and Ca.

Figure 5 shows the VkSe nanoparticles EDX data.





Elt.	Line	Intensity (c s^{-1})	Error 2-sig	Conc	Units
C	Ka	1,002.42	8.442	68.494	wt.%
O	Ka	84.65	2.883	9.540	wt.%
Si	Ka	223.96	5.510	2.412	wt.%
P	Ka	568.21	7.261	6.111	wt.%
K	Ka	100.17	4.214	1.102	wt.%
Ca	Ka	218.75	4.841	2.492	wt.%
Fe	Ka	43.30	2.873	0.954	wt.%
Se	Ka	65.69	2.691	8.895	wt.%
			Total	100.000	wt.%

Figure 5. EDX spectroscopy results of VkSe nanoparticles
Şekil 5. VkSe nanoparçacıklarının EDX spektroskopisi sonuçları

Selenium oxide nanoparticles were produced from *V. kotschyi*, as demonstrated by the results of the EDX study. Figure 6 shows the VkZnO nanoparticles' FTIR (Fourier Transform Infrared Spectroscopy) findings.

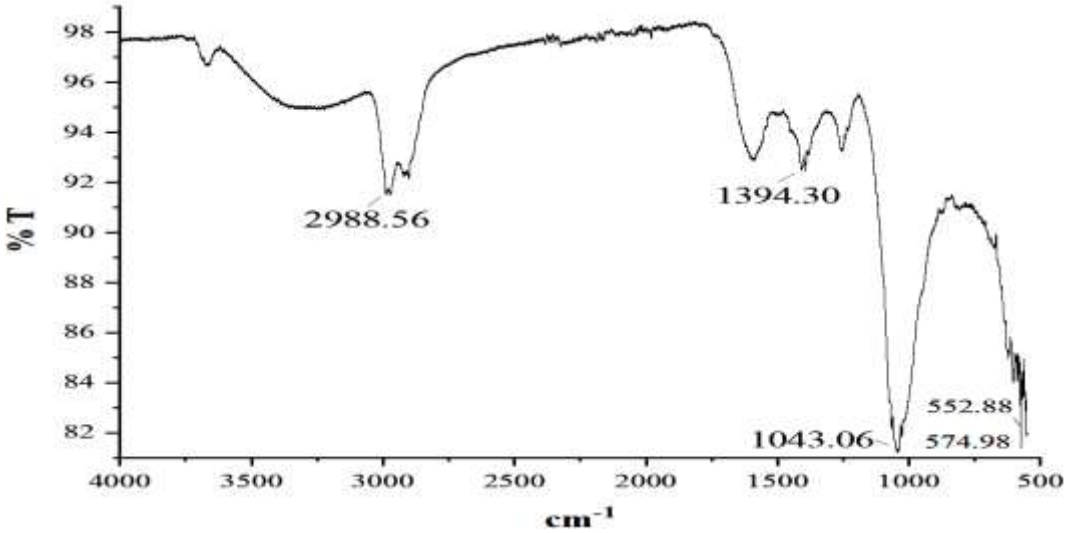


Figure 6. FTIR analysis results of VkZnO nanoparticles
Şekil 6. VkZnO nanoparçacıklarının FTIR analiz sonuçları

The intergroup vibration peaks in the 2988, 1394, 1043, and 574 cm^{-1} bands were produced by VkZnO nanoparticles. A variety of bioactive compounds, including amines, carboxylic acids, phenols, and alcohols, may help to stabilize zinc oxide nanoparticles (Fakhari et al., 2019). The aromatic C=C bond is shown by the stretching of 1394 cm^{-1}, the C-O stretching is demonstrated by the observed peak of 1043 cm^{-1}, and the Zn-O stretching is indicated by the bands of 574 cm^{-1} (Dole et al., 2011; Sangeetha et al., 2011; Kumar et al., 2014; Fakhari et al., 2019).

Figure 7 displays the VkSe nanoparticles FTIR analysis findings.

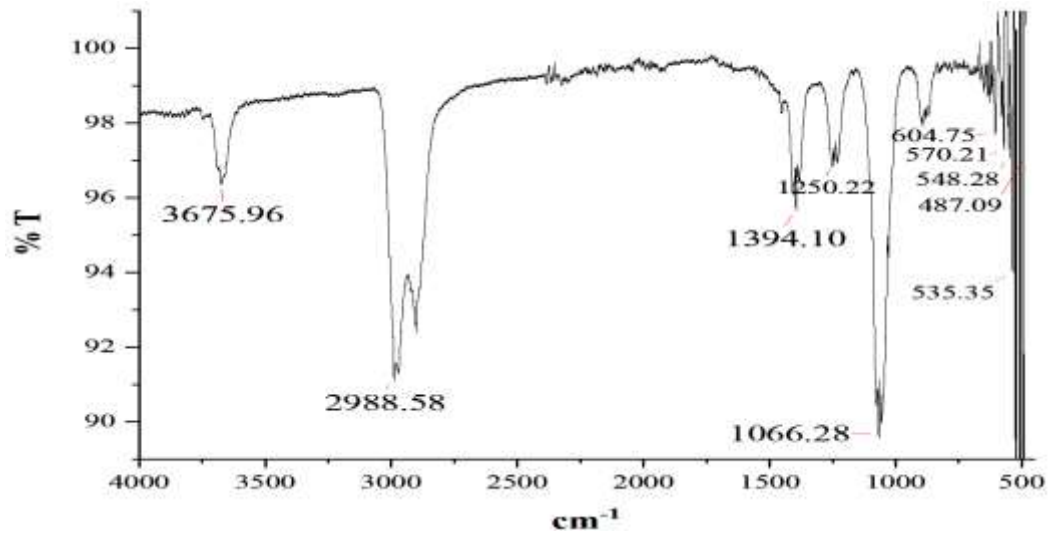


Figure 7. FTIR analysis results of VkSe
Şekil 7. VkSe'nin FTIR analiz sonuçları

The molecular bonds of VkSe nanoparticles gave vibration peaks in the 3675, 2988, 1394, 1250, 1066, 604, 570, 548, 535, and 487 cm^{-1} bands. The FTIR spectrum's stretching vibration bands, which range from 604 to 487 cm, could show how Se-NPs attach to (OH) groups as Se-O (Salem et al., 2021). FTIR analysis results of VkSe and VkZnO are presented in Table 1.

Table 2 displays the findings of the antioxidant investigation performed with VkZnO and VkSe nanoparticles.

Table 1. FTIR analysis results of VkSe and VkZnO
Çizelge 1. VkSe veVkZnO'nun FTIR analiz sonuçları

Samples	Intergroup vibration peaks	Molecular group	References
VkZnO, VkSe	2988 cm^{-1}	Methyl, methylene, and methoxy groups	(Kora & Rastogi, 2016; Kumar et al., 2014)
VkZnO, VkSe	1394 cm^{-1}	Aromatic C=C bond	(Dole et al., 2011; Kumar et al., 2014; Pillai et al., 2020)
VkZnO	1043 cm^{-1}	C-O	(Sangeetha et al., 2011; Kumar et al., 2014)
VkZnO	574 cm^{-1}	Zn-O stretching	(Kumar et al., 2014; Fakhari et al., 2019).
VkSe	3675 cm^{-1}	O-H	(Pillai et al., 2020)
VkSe	1066 cm^{-1}	C-O vibration	(Kumar et al., 2014)
VkSe	604 - 487 cm^{-1}	Se-O	(Salem et al., 2021)

Table 2. Antioxidant capacity test results of VkZnO and VkSe nanoparticles
Çizelge 2. VkZnO ve VkSe nanoparçacıklarının antioksidan kapasitesi test sonuçları

Samples	DPPH		CUPRAC		FRAP	
	IC_{50} (µg ml^{-1})	Trolox equivalent (µg ml^{-1})	($A_{0,5}$)	Trolox equivalent (µg ml^{-1})	($A_{0,5}$)	Trolox equivalent (µg ml^{-1})
VkZnO	37.63 ±0.02	0.643	195.8±0.020	0.0958	166.55±0.01	0.0811
VkSe	16.71±0.01	1.448	100.88±0.05	0.186	83.77±0.03	0.161
Trolox	24.21±0.05	-	18.75±0.5	-	13.5±0.05	-

$A_{0,5}$: Concentration corresponding to 0.5 absorbance, IC_{50}: Concentration that inhibits 50% of the radical.

In the DPPH method, IC_{50} values were calculated with the help of the graph and equation created with the % inhibition values corresponding to different concentration values of nanoparticles and are presented in Table 2. In CUPRAC and FRAP methods, the increase in absorbance is directly proportional to the amount of antioxidants. In these methods, concentrations($A_{0.5}$) corresponding to 0.5 absorbances were calculated for both nanoparticles and Trolox with the help of the linear graph created using the absorbance values corresponding to the concentration values. IC_{50} and $A_{0.5}$ values of both nanoparticles and standard (Trolox) were calculated (Ercan et al., 2024). Thus, they were compared in terms of antioxidant capacity. Additionally, in Table 2, the antioxidant capacities of both nanoparticles in the three methods are given as Torolox equivalents. The antioxidant properties of both nanoparticles were less active compared to Trolox. According to the DPPH, CUPRAC, and FRAP techniques, VkSe nanoparticles showed greater antioxidant capabilities than VkZnO nanoparticles.

Table 3 presents the antimicrobial analysis findings for VkZnO and VkSe nanoparticles.

Table 3. Inhibition zone diameters (mm) for VkZnO and VkSe nanoparticles in antimicrobial tests
Çizelge 3. Antimikrobiyal testlerde VkZnO ve VkSe nanoparçacıklarının inhibisyon bölgesi çapları (mm)

Samples	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	*S. aureus*	*C. albicans*
VkZnO	8.0±0.0	10.0±1.40	12.0±0.05	11.3±1.15	-
VkSe	-	7.0±0.0	-	-	-
Rifampin	16.0±3.6	12.3±0.6	11.3±1.15	20.0±0.0	12.6±1.50

When it came to antibacterial characteristics, VkZnO nanoparticles outperformed VkSe nanoparticles. In comparison to zinc nanoparticles, selenium nanoparticles had a very slight inhibitory impact on *K. pneumoniae*. However, VkZnO showed an inhibitory effect on *P. aeruginosa, K. pneumoniae, E. coli,* and *S. aureus* bacteria. VkZnO exhibited an inhibitory effect of 50% on *P. aeruginosa*, 81.3% on *K. pneumoniae*, 106.2% on *E. coli*, and 56.5% on *S. aureus* compared to the rifampin antibiotic. Zinc oxide nanoparticles react in both acidic and alkaline environments and release free Zn^{2+} ions as a result of their amphoteric nature. When these free Zn^{2+} ions interact with biomolecules like proteins and carbohydrates, they may disrupt the essential processes of bacteria (Siddiqi et al., 2018). Zinc oxide nanoparticles may have an advantage over selenium nanoparticles in antibacterial activity testing due to this characteristic. Antioxidant characteristics were demonstrated by both *V. kotschyi* nanoparticles. One of the generated nanoparticles, VkSe, had a higher antioxidant activity.

There are few studies on *V. kotschyi* in the literature. If we talk about some studies conducted with Verbascum

species; Silver nanoparticles were synthesized with *Verbascum thapsus* and their photocatalytic activities were examined (Elemike et al., 2016). The antimicrobial activity of *Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss species was examined and it was reported that *Verbascum L.* species demonstrated antibacterial efficacy against gram (+) bacteria, and yeasts, but did not show activity against gram (-) bacteria (Dülger et al., 2002). According to different research, *V. sinuatum*'s beneficial bioactive components have anti-inflammatory, anti-cancer, cardiovascular, antibacterial, antidiabetic, and neuroprotective properties (Donn et al., 2023). Because of its antioxidant and antibacterial properties, *Verbascum pseudoholotrichum* has also been proposed as a food, chemically based, and pharmacology agent (Yabalak et al., 2022). *Verbascum thapsus* was used to create gold nanoparticles, which were then shown to have antiproliferative properties in a different study (Soto et al., 2022). *Verbascum thapsus* was used to create zero-valent iron nanoparticles once more, and the activity of Cr (VI) reduction was measured (Saleh et al., 2021).

V. sinaiticum showed antibacterial, antifungal, and antioxidant properties and this plant was highly suitable for the synthesis of Zinc-ferric bimetallic nanoparticles (Dinakarkumar et al., 2024). ZnONPs can be used in many biomedical applications such as anticancer, wound healing, drug delivery, antibacterial and diabetes treatment, anti-inflammation, and biological imaging (Emsen et al., 2023; Jiang et al., 2018; Mishra et al., 2017; Xiong, 2013; Zhang & Xiong, 2015). SeNPs are nanomaterials that attract attention due to their various therapeutic benefits such as anticancer, antioxidant, anti-inflammatory, and anti-diabetic effects (Khurana et al., 2019). *V. kotschyi* has not been the subject of any nanoparticle research reported yet. Unknown plants must be discovered and various species with antibacterial and antioxidant capabilities must be introduced via the manufacturing of nanoparticles. This study will introduce *V. kotschyi* and close what is lacking in the literature concerning *V. kotschyi*.

CONCLUSIONS and SUGGESTIONS

Because of their antioxidant and antibacterial qualities, *Verbascum kotschyi*-derived zinc and selenium oxide nanoparticles are good candidates for application in a range of product development fields, encompassing food preservatives, pharmaceuticals, and cosmetics. The durable and advantageous properties of *V. kotschyi* nanoparticles, a little-known species, may make them useful in product development.

Contribution Rate Statement Summary of Researchers

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

Disclosure Statement

The authors report no conflicts of interest.

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Some Physiological Effects of Bisphenol A on *Lemna gibba* L., A Free-Floating Aquatic Macrophyte

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ABSTRACT
The present study was carried out to evaluate the effect of bisphenol A (BPA) on *Lemna gibba*, a free-floating aquatic macrophyte, in a climate cabinet under controlled conditions. *L. gibba* was collected from natural water sources in Gaziantep (Türkiye) and acclimatized for two weeks in containers containing 10% nutrient solution. Macrophytes were treated with 1.5, 17.2, and 50 mg/L BPA for 96 hours. Chlorophyll a, chlorophyll b, carotenoid, protein, and total soluble carbohydrate contents were declined following BPA application. Contrary to this, an elevation in the contents of NP-SH, H_2O_2, and malondialdehyde were detected. In conclusion, correlation analyses showed that the changes may be related to BPA-induced oxidative stress.

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Bisfenol A'nın Serbest Yüzücü Makrofitlerden *Lemna gibba* L. Üzerindeki Bazı Fizyolojik Etkileri

ÖZET
Bu çalışma, kontrollü koşullar altında bir iklimlendirme dolabında BPA'nın *Lemna gibba* üzerindeki etkisini belirlemek amacıyla yapıldı. Makrofitler Gaziantep'teki (Türkiye) doğal su kaynaklarından toplandı ve %10 besin çözeltisi içeren kaplarda iki hafta boyunca aklimatize edildi. Makrofitler 96 saat boyunca 1.5, 17.2 ve 50 mg/L BPA ile muamele edildi. BPA'nın klorofil a, klorofil b, karotenoid, protein ve toplam çözünür karbonhidrat içeriğinde azalmaya neden olduğu belirlendi. Bunların aksine, protein olmayan sülfidril gruplar (NP-SH), H_2O_2 ve malondialdehit (MDA) içeriklerinde artışlar tespit edildi. Sonuç olarak, korelasyon analizleri bu değişikliklerin BPA kaynaklı oksidatif stresle ilişkili olabileceğini göstermektedir.

Bitki Fizyolojisi

Araştırma Makalesi

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Anahtar Kelimeler
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Lemna gibba,
Fizyolojik etkiler,
Oksidatif stres

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INTRODUCTION
Industrially important monomer, bisphenol A (BPA: 2,2-Bis (4- hidroksifenil) propane; Fig. 1) is a synthetic chemical used extensively to synthesize epoxy resins, polymer materials, and polycarbonate plastics. For example, BPA is found in baby bottles, water bottles, dental fillings, thermal paper, toys, medical devices, etc. Therefore, since it has widespread use in many products, it causes a wide distribution of BPA in the environment, thus it appears as a potential pollutant (Manzoor et al., 2022).

The duckweed contains the smallest flowering plants. Therefore, it can be considered as a model plant. Due to their wide tolerance range, they have a high potential to be used in bioremediation studies. Due to their high protein content, they can be used as feed for fish, other animals, and even humans. Additionally, they may be important to use as a biofuel due to their high starch content (Coskun et al., 2018). *L. gibba* can continue to grow in eutrophied waters with low oxygen and excess carbon dioxide, low light, and very salty waters. On the other hand, it is considered as a potential indicator for the aquatic ecosystems (Thingujam et al., 2024).
Pollution of the environment due to anthropogenic activities causes negative effects on living things. The deterioration of environmental health caused by rapid industrialization, urbanization, and increasing population pressure brings along many environmental problems. In addition, although studies on the structure, amount,

physicochemical behavior, and effects of pollutants have increased greatly in recent years, more research is still needed on the effects of these pollutants on living things. Therefore, this study was carried out to determine the effects of BPA, which is an important environmental pollutant, on some physiological properties of *L. gibba*, a free-floating macrophyte.

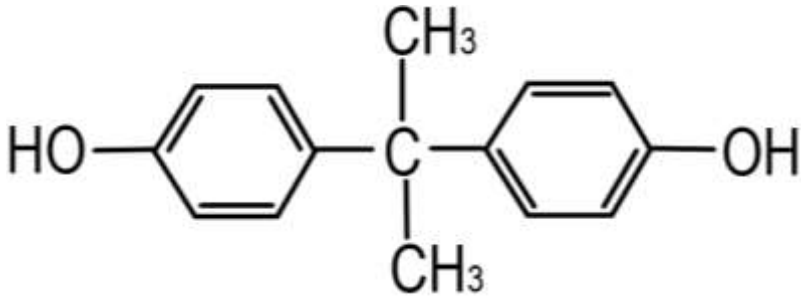

Fig. 1. Structure of BPA
Şekil 1. BPA'nın yapısı

MATERIALS and METHODS

Plant material and BPA treatment

L. gibba was collected from water bodies in the province of Gaziantep (Türkiye) and acclimated to 10% nutrient solution in a climate chamber (Snijders Scientific, Netherlands) in determined conditions (light/dark regimes of 16/8 h, light level 120 µE.m^{-2}.s^{-1}, temperature 23±1 °C) for two weeks before BPA treatments. Three different BPA concentrations were applied in the study. The BPA concentration of 1.5 mg/L was chosen because it is the upper safe limit for drinking water according to the US EPA (Geens et al., 2011). The concentration of 17.2 mg/L is the hazardous landfill leachate concentration (Yamamoto et al., 2001). The concentration of 50 mg/L was preferred because it causes possible contamination by BPA and this concentration is often used to indicate the toxicity of BPA in plants (Dogan et al., 2010; Cinar & Dogan, 2020). Thus, healthy macrophytes were treated with concentrations of 1.5, 17.2, and 50 mg/L of BPA with 10% nutrient solution (Öztürk et al. 2002) in triplicate in 100 ml glass vessels. A 10% nutrient solution (without BPA) was used as a control. The macrophytes were harvested after 96 hours. Ultrapure water was used in all applications and analyses. The reagents used in the study were of analytical grade.

Physiological analysis

Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid contents were determined spectrophotometrically according to Lichtenthaler & Wellburn (1985). The anthrone method was used to measure total soluble carbohydrate content (Plummer, 1978) using glucose as standard. The method introduced by Lowry et al. was used to determine the protein content using bovine serum albumin as standard (Lowry et al., 1951). Non-protein sulfhydryl groups (NP-SH) were determined according to Ellman's method (Ellman, 1959). Malondialdehyde (MDA) level was determined by Zhou (2001). Hydrogen peroxide was determined according to Sergiev et al. (1997).

Data Analysis

Statistical analyses were performed using the SPSS software program. The least significant difference (LSD) test was used to compare the data. Pearson correlation was applied to evaluate the relationship among physiological changes.

RESULTS and DISCUSSION

Plants need the synthesis and accumulation of organic substances for growth and development, which in turn depends on their photosynthesis. Chlorophyll molecules bound to the proteins of photosynthetic membranes harvest sunlight (Von Wettstein et al., 1995) Carotenoids are photosynthetic pigments that play an important role in physiological processes such as light harvesting and energy transfer, photoprotection, and stabilization of light-harvesting pigment-protein complexes (Sutherland et al., 2022) Therefore, since the chlorophyll content is directly related to photosynthesis, respiration, and energy (Qiu et al., 2013) the effect of BPA applications on the photosynthetic pigment content of *L. gibba* was determined (Fig. 2). Chlorophyll content was dose-dependent manner decreased at 1.5, 17.2 and 50 mg/L BPA concentrations by 3.1% (p=0.631), 7.5% (p=0.228) and 43.7% (p=00006), respectively, when compared to the control. Similarly, chlorophyll a and carotenoid contents at 50 mg/L BPA decreased by up to 34.3% (p=0.02) and 40.5% (p=0.01), respectively, when compared to the control. Photosynthesis is strongly affected by stress factors, including BPA (Qiu et al., 2013). Previous studies have reported that BPA causes oxidative stress by triggering the formation of reactive oxygen species (ROS) in plants (Dogan et al., 2020; Qiu et al., 2013; Dogan et al., 2012). There was a positive relationship between H_2O_2 content and photosynthetic pigments (r=0.703 and p=0.011 for Chl a; r=0.552 and p=0.063 for Chl b; and r=0.629 and

p=0.028 for carotenoids). Besides, regression analysis showed a positive correlation between MDA content and photosynthetic pigments (r=0.708 at p=0.010 for Chl a, r=0.563 at p=0.057 for Chl b, and r=0.632 at p=0.027 for carotenoids). According to the findings, BPA reduced the photosynthetic pigment content in *L. gibba* cells, which may be due to the oxidative stress of BPA. Furthermore, the decrease in BPA-treated *L. gibba* may be due to peroxidation of chloroplast membrane lipids (Cinar & Dogan, 2020; Qiu et al., 2013).

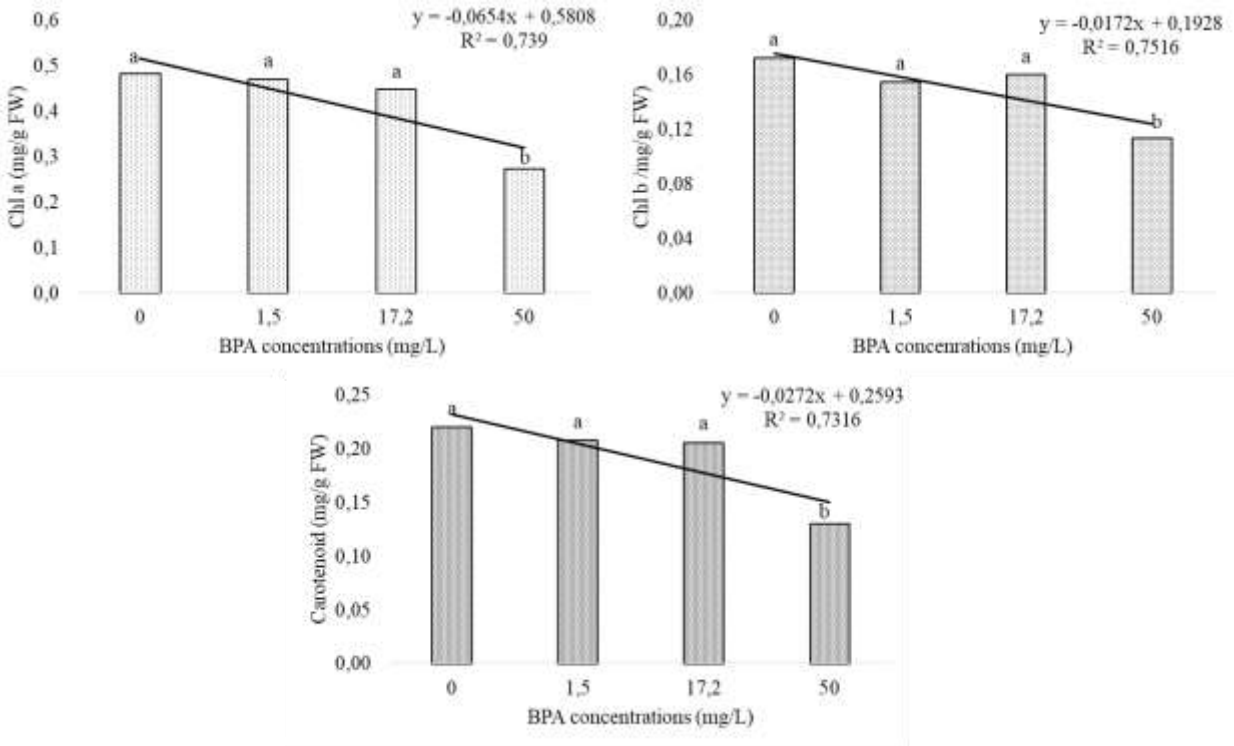

Fig. 2. Photosynthetic pigment contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test (p<0.05).

Şekil 2. BPA uygulamaları sonrası L. gibba'nın fotosentetik pigment içerikleri. Farklı harfler LSD testine göre istatistiksel önemi göstermektedir (p<0.05).

Protein content and their statistical evaluations of *L. gibba* tissues are presented in Fig. 3. Protein content was decreased at 1.5, 17.2, and 50 mg/L BPA as 3.1% (p=0.811), 29.0% (p=0.047) and 58.3% (p=0.02), respectively, when compared to the control. Correlation analyses showed that there was a positive relationship between H_2O_2 content and protein content (r=0.600; p=0.039). The findings may indicate that BPA is mediated by oxidative stress resulting in ROS production in *L. gibba* cells, thus may be a reason for the decreased protein content (Dogan et al., 2012; Cinar & Dogan, 2020; Halliwell, 1987).

Total soluble carbohydrate contents at 1.5, 17.2, and 50 mg/L BPA were found to be decreased by 26.9% (p=0.023), 35.8%(p=0.006), and 36.9% (p=0.005), respectively, when compared to the control (Fig. 3). The positive correlation was found between the H_2O_2 and total carbohydrate contents suggesting BPA elicited oxidative stress (r=0.015; p=0.964). Chronic exposure to BPA has been reported to cause oxidative stress and impair carbohydrate metabolism through the downregulation of carbohydrate metabolizing enzymes (Ul Haq et al., 2020).

There is evidence that non-protein thiols contribute to plant stress tolerance. In the majority of aerobic cells, the main non-protein thiol is glutathione (GSH), which is an important antioxidant, playing a central role in ROS scavenging in the GSH-ascorbate cycle (Noctor et al., 2012). In *L. gibba*, NP-SH content increased up to 79.9% by BPA (p=0.00007) (Fig. 4). Positive correlation was determined between NP-SH and H_2O_2 contents (r= 0.543; p=0.088). This may indicate that increased GSH content under stressful conditions is related to the tolerance of *L. gibba* to BPA stress.

As previously mentioned, BPA causes oxidative stress by inducing ROS. For this purpose, H_2O_2 and MDA contents were determined to evaluate whether BPA caused oxidative stress in *L. gibba* cells. H_2O_2 content increased by 17.1% (p=0.058) and 20.1% (p=0.031) in 1.5 and 17.2 mg/L BPA, respectively, compared to control, but decreased by 32.4% (p=0.003) in 50 mg/L BPA (Fig. 4). MDA is an important biomarker of oxidative stress (Dogan et al. 2010; Akbulut et al., 2020). Similar findings were also found in MDA content (Fig. 4). Correlation analysis revealed a significant and positive relationship between H_2O_2 and MDA (r=0.994; p<0.00001), confirming the status of oxidative stress.

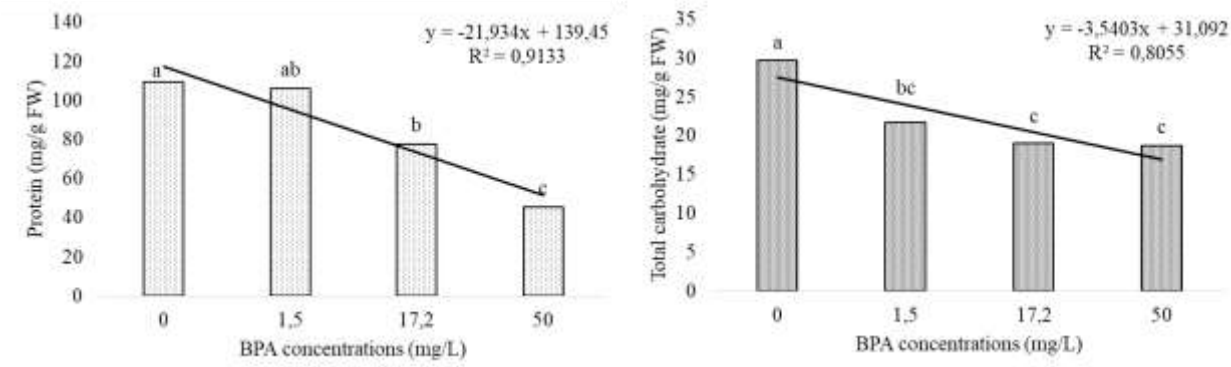

Fig. 3. Protein and total carbohydrate contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test ($p<0.05$).

Şekil 3. BPA uygulamalarından sonra L. gibba'nın protein ve toplam karbonhidrat içerikleri. Farklı harfler LSD testine göre istatistiksel önemi göstermektedir ($p<0.05$).

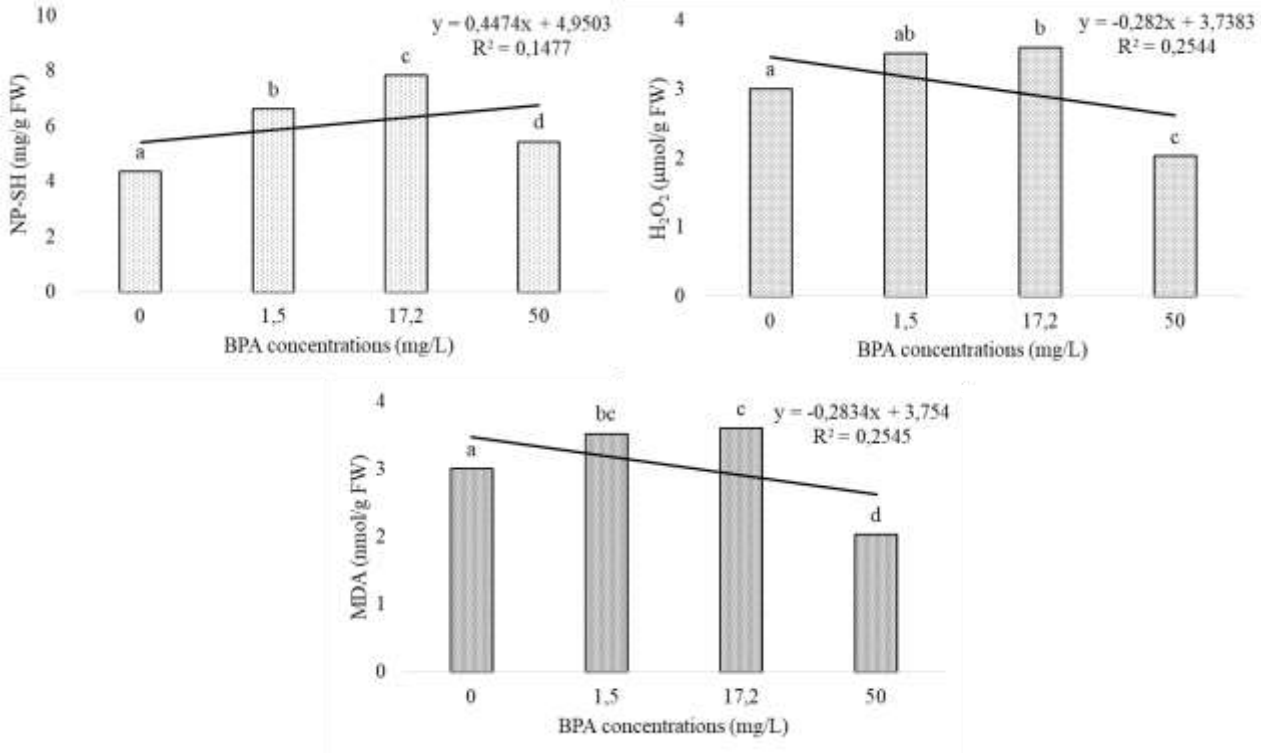

Fig. 4. NP-SH, H_2O_2, and MDA contents of *L. gibba* after BPA applications. Different letters indicate statistical significance according to the LSD test ($p<0.05$).

Şekil 4. BPA uygulamaları sonrası L. gibba'nın NP-SH, H2O2 ve MDA içerikleri. LSD testine göre farklı harfler istatistiksel anlamlılığı göstermektedir ($p<0,05$).

CONCLUSION

This study investigated the effects of BPA on some physiological processes in *L. gibba* to clarify the effect of BPA on aquatic plants and to provide a reference for the assessment of the ecological risk of BPA in the environment. It was determined that BPA caused a decrease in photosynthetic pigments, protein, and total soluble carbohydrate contents. On the other hand, increased NP-SH contents may be related to their tolerance to BPA-induced stress. It was demonstrated by the increase in the content of H_2O_2, a type of ROS, that BPA triggered oxidative stress in *L. gibba* cells. In addition, an increase in MDA, which is both an indicator of oxidative stress and a marker of lipid peroxidation, clearly demonstrated this situation as well.

Contribution of Authors

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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Effects of Zinc Oxide Nanoparticle on Antioxidant System in Bean Leaves

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ABSTRACT
Nanotechnology can be most simply defined as technology at the nanoscale. Heavy metal stress often induces reactive oxygen species (ROS) and causes oxidative stress. Antioxidant enzymes, metabolites, flavonoids, carotenoids, polyols, cytosolic ascorbate, and peroxiredoxin play roles in ROS scavenging. Certain antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) defend against metal toxicity. In this study, the effects of zinc nanoparticles on certain biochemical parameters in the leaves of bean (Phaseolus vulgaris L.) were examined. For this purpose, ZnO nanoparticle concentrations of 0.1 mM, 0.01 mM, and 0.001 mM were applied. At the end of 120 hours, malondialdehyde, proline, glutathione, total soluble protein, and the activities of superoxide dismutase and catalase enzymes were determined. As a result, all findings from this study revealed that ZnO nanoparticle applications activated antioxidant defense mechanisms in the leaves of Phaseolus vulgaris L. It was determined that the mentioned ZnO nanoparticle exhibited more pronounced effects, especially at lower doses. Nano-sized metals were found to exert toxic effects on the leaves of *Phaseolus vulgaris* L."

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Research Article

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Nanoparticle
Zinc oxide

Çinko Oksit Nanopartikülünün Fasulye Bitkisi Yapraklarinda Antioksidan Sistem Üzerine Etkileri

ÖZET
Nanoteknolojinin en basit tanımı, nanoskalada teknoloji olarak ifade edilebilir. Ağır metal stresi genellikle reaktif oksijen türlerini (ROS) indükler ve oksidatif stres oluşturur. Antioksidan enzimler, metabolitler, flavonoidler, karotenoidler, polioller, sitozolik askorbat ve peroksiredoksin gibi maddeler ROS temizlenmesinde rol oynar. Katalaz (CAT), Askorbat peroksidaz (APX), Süperoksit dismutaz (SOD) ve Glutatyon redüktaz (GR) gibi bazı antioksidan enzimler metal toksisitesine karşı savunma yapar. Bu çalışmada, çinko nanopartikülünün fasulye (*Phaseolus vulgaris* L.) yapraklarındaki bazı biyokimyasal parametreler üzerindeki etkileri incelendi. Bu amaçla 0.1 mM, 0.01 mM ve 0.001 mM ZnO nanopartikül konsantrasyonları uygulandı. 120 saat sonunda malondialdehit, prolin, glutatyon, toplam çözünür protein ve süperoksit dismutaz ve katalaz enzim aktiviteleri belirlendi. Sonuç olarak, bu çalışmadan elde edilen tüm sonuçlar ZnO Nanopartikül uygulamalarının *Phaseolus vulgaris* L. yapraklarında antioksidan savunmayı aktive ettiğini ortaya koydu. Bahsi geçen ZnO nanoparçacığın, özellikle düşük doza bağlı olarak daha ciddi etkiler gösterdiği belirlendi. Nano boyuttaki metaller, *Phaseolus vulgaris* L. yapraklarında toksik bir etki oluşturdu.

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INTRODUCTION

The simplest definition of nanotechnology is nanoscale technology (Ramsden, 2016). Metallic nanoparticles increase seed germination, shoot and root growth, biomass production, and physiological activity (El-Gazzar et al., 2020). Plants produce reactive oxygen species in response to stress from various effects. Abiotic stresses such as UV rays, heavy metals, salinity, drought, herbicides, high temperature, and pollution are among these effects (Mehla et al., 2017). Oxidative stress occurs when the balance between antioxidants and reactive oxygen species (ROS) is disrupted. Nanotoxicity is also a factor that can cause oxidative stress (Cekic et al., 2017). Nanomaterials can induce excessive ROS production as a regulatory mechanism to protect stressed plant cells from further oxidative damage (Venkatachalam et al., 2017). Concentrations of nanoparticles (NPs) above the optimal ranges of metals such as Zn, Cu, Ag, Ce, and Ti produce stress and/or toxicity by generating ROS and disrupting cellular metabolism (Mehla et al., 2017). ZnO NPs affect the normal functionality of cells by causing changes in the structures of molecules such as proteins and DNA. Genotoxicity studies have shown that ZnO NPs affect plant DNA (Reddy et al., 2018). It has been stated that the application of ZnO and CuO NPs can lead to the oxidation and denaturation of proteins and the alteration of their structure and functions (Hidour et al., 2022). By controlling glucose metabolism and promoting the activity of antioxidant enzymes, ZnO nanoparticles improve the stress resistance of plants (Liu et al., 2022). Stress-responsive genes and transcription factors, which adapt to different stress situations, are distributed differently in response to stress signals, also causing the formation of ROS. Nanomaterials can boost photosynthesis, encourage plant development, and increase biomass and protein levels (Fazelian et al., 2020). Nevertheless, they have the potential to alter crop plant morphology and physiological processes when administered in high quantities. For example, the application of large concentrations of NPs in the root zone inhibits root growth, modifies the uptake of water and nutrients, and decreases leaf development and biomass output (Usman et al., 2020). Zinc oxide nanoparticles (ZnO NPs) have been shown to improve plant metabolism by increasing plant defense and stress tolerance (Hidour et al., 2022). However, some harmful effects of NPs on chlorophyll have resulted in oxidative stress, which slows down plant growth by affecting photosynthesis (Li et al., 2016). Nanotechnology is fundamentally a mindset—a way of thinking about the world based on precise perceptions at the atomic level. Zinc is an essential micronutrient for plants and plays a vital role in metabolic activities, including the synthesis and breakdown of macromolecules necessary for growth. It is crucial for chlorophyll synthesis and plays an important role in regulating plant growth hormones such as Indole-3-acetic acid (IAA) (Reddy et al., 2018). Many previous studies have reported the harmful effects of ZnO NPs on different plant species. For example, ZnO NPs have been shown to inhibit the growth of wheat and soybean plants (Salehi et al., 2021). In studies by Reddy-Pullagurala et al., ZnO NPs at 100 mg/L gradually delayed the germination period of Macrotyloma uniflorum. Under similar cultivation conditions, 1600 mg/L of ZnO NPs reduced germination by 40% in Medicago sativa and 20% in Solanum lycopersicum (Reddy et al., 2018). There are studies (Liu et al., 2022) indicating that ZnO NPs have increased the growth rate, enhanced biomass, and improved root growth of cluster bean (*Cyamopsis tetragonoloba* L.) and pearl millet (Pennisetum americanum) by promoting the increase of certain enzymes. Similar results have been observed in tomatoes (*Solanum lycopersicum*) and cabbage (*Brassica oleracea* L.), as well as in plants such as lettuce (*Lactuca sativa* L.). The main outcomes include positive changes in internal components such as chlorophyll, carbohydrates, and antioxidant enzymes (Liu et al., 2022). This study investigated the effects of ZnO nanoparticles at different concentrations on the antioxidant defense system of bean plant leaves at the 120th hour. Previous studies have highlighted both the positive and negative effects of ZnO nanoparticles (NPs) on plant growth; however, this study specifically investigates the impact of ZnO NPs on the antioxidant defense system of the bean plant.

MATERIAL and METHOD

Experimental Material

In our study, oilseed beans (*Phaseolus vulgaris* L.) seeds were used and obtained commercially. Germination tests were conducted, and plant cultivation and application procedures were carried out. Samples were frozen in liquid nitrogen and stored in a deep freezer at -40°C.

Germination of Plants and Formation of Experimental Groups

Following surface sterilization, seeds were soaked in water for 24 hours using an aquarium pump. Then, the planting process was applied to pots. The seeds were grown under controlled conditions at 25±2°C with 60-65% humidity. Plants irrigated with Hoagland culture solution (Hoagland and Arnon, 1938) were treated with ZnO nanoparticles prepared at concentrations of 0.1 mM, 0.01 mM, and 0.001 mM, added to the irrigation water after 12 weeks. The pH of the prepared nutrient solution was adjusted to be in the range of 5.6–5.8. As a result of the literature review, it was decided to use the current doses. Perlite was used as the cultivation medium. To observe the long-term effects, samples were collected at the 120th hour and analyzed. The experimental materials were grown in pots with a diameter of 21 cm and depth of 18 cm. The study was to include 12 pots for each concentration

and time, and one seed per pot. Due to the possibility of insufficient germination, 6 pots were prepared as replacements for each application and time. After applications, analyzes were performed with 6 randomly selected plants from concentration and time and 6 randomly collected leaves from each of these plants. Subsequently, samples were taken at 120 hours and transferred to the freezer. The results were analyzed with three replications.

Sampling of Plant Samples

Random samples were taken from plants in sufficient quantities, covered with aluminum foil, and rapidly frozen in liquid nitrogen. They were stored for analyses to be conducted at a temperature of -40°C.

Antioxidant Enzyme Analyses

The Superoxide dismutase (SOD) enzyme activity was performed with reference to the method specified by Sairam et al. (2002). The SOD activity was measured by recording a decrease in optical density of nitroblue tetrazolium (NBT). 3 ml of assay mixture consisted of 13 mM methionine, 25 mM nitroblue tetrazolium chloride, 0.1mM EDTA, 50mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.1 ml enzyme. Reaction was initiated with the addition of riboflavin and kept under two 15W fluorescent lamps for 15 minutes. Reaction was stopped by closing the light and using assay mixture without enzyme, giving the maximum coloration, as control. Complete assay mixture, which was not illuminated, was used as blank. SOD activity of one unit was defined as the amount of enzyme needed to cause 50% inhibition of NBT at 560 nm and was expressed in Unit/g.
Catalase activity (CAT) determination was carried out according to Aebi (1984). To measure CAT activity, the extract was combined with phosphate buffer and hydrogen peroxide (H_2O_2), and the variation in absorbance was recorded at a wavelength of 240 nm.

Determination of Malondialdehyde Content

HPLC apparatus was used for the determination of malondialdehyde content (Karatas et al. 2002). After the tissues were homogenized with Tris buffer, 1 mL of the resulting supernatant was taken and 10% perchloric acid ($HClO_4$) was added. The mixture was centrifuged at 5000 rpm for 5 minutes. The supernatant was then analyzed using an HPLC device through vials. In the HPLC system, a mobile phase consisting of a mixture of 30 mmol KH_2PO_4 and methanol (%82.5-%17.5 with H_3PO_4 at pH = 4.0) was used, along with an ODS-3 HPLC column (150 mm x 4.6 mm, 5 µm). The mobile phase flow rate was set to 1 mL/min, and the wavelength of the PDA detector was set to 244 nm. The results were calculated using the calibration curve obtained from standard mixtures, and the analysis was performed with Class VP 6.26 software (Shimadzu, Kyoto, Japan).

Prolin

The proline content was determined based on its reaction with ninhydrin which forms a colored complex. After adding 2-propanol, the absorbance of the sample solution and a reference solution at 510 nm using a spectrophotometer was determined. Results were expressed in proline milligrams per kilogram of honey (Codex Alimentarius Commission 2001).

Total Soluble Protein

Total protein contents of yeast cells were determined as Lowry's method (Lowry et al. 1951).

Measurement of GSH Content with HPLC

The GSH content was determined using an HPLC apparatus (Klejdus et al. 2004; Yilmaz et al. 2009). 1 mL of homogenate was taken, 1 mL of 10% TCA was added and it was deproteinized. After centrifugation at 6000 rpm, 1 mL was taken into autosampler vials. In quantitative measurements, analysis was performed using Shimadzu brand fully automatic HPLC device at 214 nm, and LC-10 ADVP UV-visible pump, SPD-M10AVP, PDA detector, CTO-10ASVP column oven, SIL-10ADVP autosampler, DGU-14A degasser unit and Class VP 6.26 operating program (Shimadzu, Kyota Japan)

Statistical Analyses

All experimental data were replicated three times under the application conditions. The experimental groups were subjected to a comparative analysis of variance with their respective control groups. The statistical analyses of the data were performed using SPSS 25.0 software. Analysis of variance (ANOVA) and the least significant difference (LSD) test were used to compare the groups with the control group. The data are presented as mean ± SD (standard deviation) and statistical significance was determined at $p<0.05$. Additionally, regression curves were statistically determined.

RESULTS and DISCUSSION

Prolin Content

The table provides the analysis of proline content in samples taken from *P. vulgaris* L. leaves at 120-hour intervals with NP applications. In the samples taken at 120 hours, a noticeable increase was recorded compared to the

control. The application at 0.01 mM concentration at 120 hours showed a high increase compared to the control but measured lower than other concentrations. The values at all of concentrations ($p<0.05$) are statistically significant. There was a significant increase in proline values at 120 hours compared to the control. In conclusion, ZnO NP significantly increased proline values at 120 hours.

Table 1. Effects of application group on Proline content in leaves of *P. vulgaris* L. plant
Çizelge 1. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Prolin miktarına etkileri

Application groups (120th hour)	Proline content (mg/g)
Control	**52,92±1.68**
0.1 mM ZnO	70.97±1.2*
0.01 mM ZnO	82.00±1.1*
0.001 mM ZnO	83.90±1.2*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Proline plays a significant role in the physiological characteristics and growth of plants, assisting them in overcoming various stresses (Hayat et al., 2012). The accumulation of proline is a common response to various stresses (Verbruggen and Hermans, 2008) and is also an important indicator of lipid peroxidation (Dai et al., 2019). It is evident that as the concentration of stress decreases, the amount of proline increases in this study. This indicates that the plant is under stress. Additionally, it can be concluded that nanomaterials facilitate greater absorption, leading to an increase in proline content through accumulation.

It should be noted that the study reported by Amooaghaie et al. (2016), suggesting that the application of nanoparticles at low concentrations is suitable for plant growth, contradicts our findings. In their study, high proline content was determined at low concentrations, indicating that the plant was under stress when evaluated in conjunction with the antioxidant system. Due to differences in the material used, concentration, and method, results may vary.

In the case of Zn nanoparticles applied to Faisal and Shiralee varieties of canola at different concentrations (5 mg/L, 15 mg/L, and 25 mg/L), it was reported that they caused an increase in proline content. For mustard plants (*B. juncea*) treated with ZnO NPs, a gradual increase in proline content up to a concentration of 1000 mg/L in leaves and a decrease at 1500 mg/L were observed (Rao & Shekhawat, 2013). These studies align with our results, where an overall increase in proline content was determined.

Our research indicates that, at the 120th hour of application, the higher proline content compared to the control suggests that the applied nanoparticle molecules create stress conditions in the plant, leading to an increase in proline content as a part of the defense mechanism.

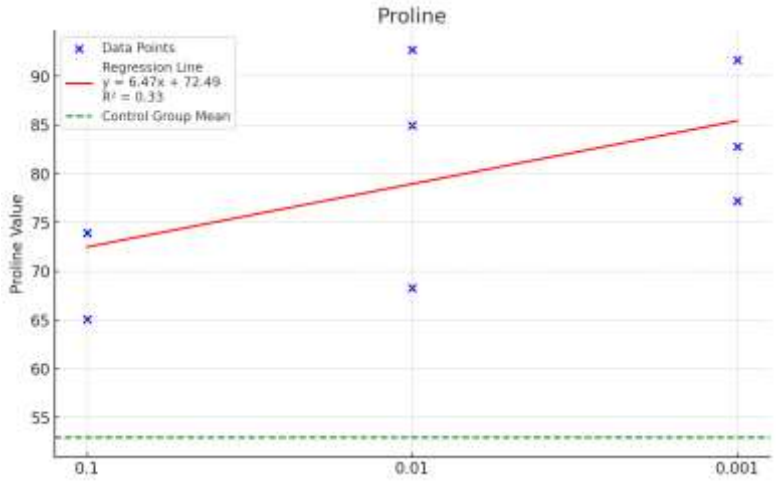

Figure 1. Regression curve of Proline content of application concentrations at 120th hour
Şekil 1. *Uygulama konsantrasyonlarının 120. saatteki Prolin içeriğine ait regresyon eğrisi*

Malondialdehyde Content

The table presents the effects of application groups on Malondialdehyde (MDA) content in leaves of *Phaseolus vulgaris* L. plants, as observed in Table 2. At 120 hours of application, it was observed that ZnO applications at 120 hours caused the increased MDA content increased as the concentrations decreased and all ZnO aplications higher than control.

Table 2. Effects of application group on Malondialdehyde (MDA) content in leaves of *Phaseolus vulgaris* L. plant
Çizelge 2. P.vulgaris L. bitkisi yapraklarında uygulama gruplarının Malondialdehit (MDA) içeriğine etkileri

Application groups (120th hour)	MDA content (**nmol/g**)
Control	88±31,6
0.1 mM ZnO	147.92±28.71*
0.01 mM ZnO	157.82±10.56*
0.001 mM ZnO	179.66±23.31*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Hydroperoxides accumulating in the environment disrupt membrane integrity. Large classes of biomolecules are affected by free radicals and their derivatives, with lipids being the most sensitive among them (Gulcin et al., 2004). Biological membranes are composed of a combination of polyunsaturated fatty acids, amphipathic lipids, and membrane proteins. Lipid peroxidation (LPO) is a process that begins with the oxidation of polyunsaturated fatty acids by radicals and extends in the form of autocatalytic chain reactions, culminating in the conversion of lipid peroxides into aldehyde derivatives, hydrocarbon radicals, and some volatile products (Unal, 1999; Kirecci, 2018). The membrane damage caused by lipid peroxidation (LPO) is irreversible.
At 120 hours, it was observed that as the concentration decreased in the ZnO application groups, the MDA content increased. This indicates that over time, superoxide radicals increase the MDA content. In a study conducted by Li et al. (2012) on tomato seedlings, it was noted that ZnO NPs applied to tomato seedlings (at concentrations of 10 and 50 mg/L) significantly increased the MDA content in the roots due to the increased concentration of H_2O_2. In the same study (Li et al., 2012), despite the increase in H_2O_2 content in wheat leaves, the unchanged MDA content and SOD activity were associated with POD, CAT, GR, and APX activities, as well as osmotic regulation. In cotton plants, it has been noted that exposure to high concentrations of nanoparticles leads to a decrease in the activities of SOD, CAT, and POX enzymes, resulting in lower lipid peroxidation (low MDA) (Venkatachalam et al., 2016). Faizan et al. (2021) reported a significant decrease in MDA by 31%, H_2O_2 by 28%, and O_2 by 31% concentrations in tomatoes upon the application of ZnO NPs. Separate applications of Zn and ZnO to tomato and wheat plants showed a significant increase in MDA content and H_2O_2 accumulation in tomato plants, but in wheat plants, although the H_2O_2 content slightly increased, the MDA content remained unchanged. However, 200 mg Zn/L and ZnO resulted in a significant increase in MDA content in wheat. Sunflower plants have been shown to exhibit effects related to water scarcity, such as electrolyte leakage, increased lipid peroxidation, H_2O_2 production, and proline accumulation, causing damage to the membrane (Ramadan et al., 2022). In our study, it was concluded that after a 120-hour nanoparticle application period, there was an increase in MDA content, and this increase was particularly higher at low concentrations. Although zinc is a molecule required for plants, in nano size and at low concentrations, it has caused increases in MDA content due to absorption.

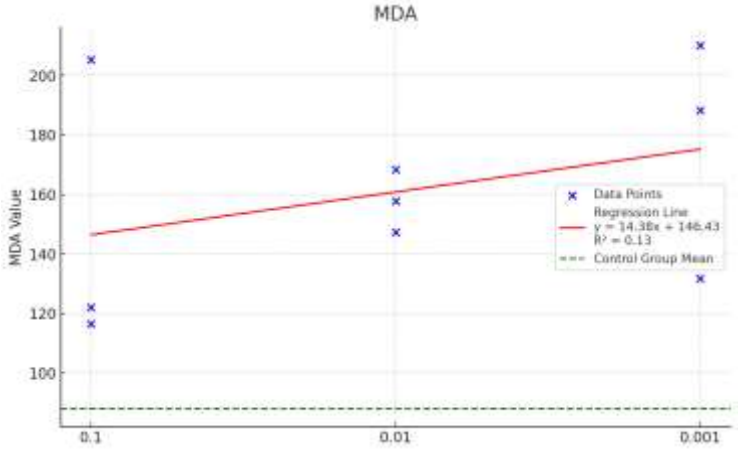

Figure 2. Regression curve of MDA content of application concentrations at 120th hour
Şekil 2. *Uygulama konsantrasyonlarının 120. saatteki MDA içeriğine ait regresyon eğrisi*

Glutathione Content

The table examines the effects of application groups on Glutathione (GSH) content in leaves of *Phaseolus vulgaris* L. plants, as observed in Table 3.Upon examining Table 3 regarding the effects on GSH content in the leaves of *Phaseolus vulgaris* L. plants, the lowest GSH content was obtained at 120 hours with the application of 0.001 mM ZnO nanoparticles (56.75±13.14 nmol/g), and this decrease was statistically significant ($p<0.05$).

Table 3. Effects of application groups on Glutathione (GSH) content in leaves of *P. vulgaris* L. plant
Çizelge 3. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Glutatyon (GSH) miktarına etkileri

Application groups (120th hour)	GSH content (**nmol/g**)
Control	**335,91±33.6**
0.1 mM ZnO	119.92±33.6*
0.01 mM ZnO	77.92±7.58*
0.001 mM ZnO	56.75±13.14*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Scientific observations on many plants have shown that glutathione is a significant player in determining their relative tolerance. Glutathione plays a role in detoxifying reactive oxygen species (ROS) through the ascorbate-glutathione cycle. Accumulated metal ions are detoxified by phytochelatins synthesized from glutathione in plants exposed to heavy metals (Yadav, 2010). Oxidative stress occurs when the balance between ROS and antioxidant defenses is disrupted (Tarrahi et al., 2018). Glutathione is a tripeptide found in various cellular organelles (Millar et al., 2003). Glutathione is a non-enzymatic antioxidant. ZnO NPs can induce oxidative stress by disrupting cellular metabolism or consuming cellular enzymatic and non-enzymatic antioxidants, thereby causing damage to cellular lipids, proteins, and nuclear DNA (Alkaladi, 2019).

At 0.001 mM concentration, ZnO was measured at the lowest level of 56.75 nmol/g after 120 hours. A generally decreasing level of GSH indicates an increase in oxidative stress. According to Alkaladi (2019), sub-lethal doses of ZnO NPs in Nile tilapia plants inhibit the activities of glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione S-transferase (GST), alongside gene expression. Researchers reporting a decrease in GSH (glutathione) levels also noted an increase in lipid peroxidation (LPO) levels, indicating a significant increase in oxidative stress due to these results.

A study reports that Cu NP applied to tomato plants under salt stress resulted in a 13% increase in GSH levels compared to the control group. The study indicates that applying only salt resulted in an 81% increase in GSH, while Cu NP treatment led to a significantly high GSH content of 337%. According to the results, it was concluded that Cu NP has a positive effect on the GSH content of the plant (Pérez-Labrada et al., 2019). In the current study, however, the opposite result was obtained. It was observed that the application of ZnO NP led to a decrease in GSH content. This result may be due to differences in the experimental material used. Additionally, Cu NP and ZnO NP have different properties. Therefore, obtaining different results in living tissues, particularly in plants, can be inevitable. Overall, the impact of nanoparticles is influenced by various factors, including dose, treatment duration, application method, type of nanoparticle, and the plant species (Santás-Miguel et al., 2023).

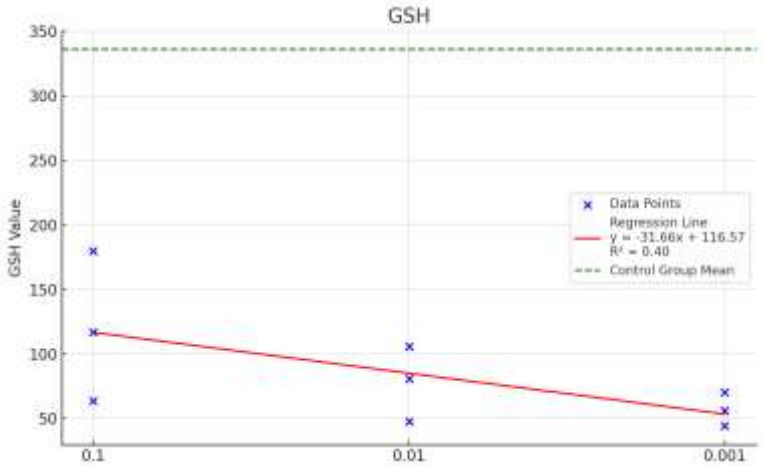

Figure 3. Regression curve of GSH content of application concentrations at 120th hour
Şekil 3. *Uygulama konsantrasyonlarının 120. saatteki GSH içeriğine ait regresyon eğrisi*

Total Soluble Protein Content

Changes in total soluble protein content are seen in Table 4. When examining the effects on the total soluble protein content in the leaves of *Phaseolus vulgaris* L. plants, it is observed that at 120 hours, as the concentrations of ZnO decrease, the soluble protein content also decreases.

A study reported an increase in protein levels in tomato, cauliflower, and cabbage plants as a result of the optimal use of ZnO NPs (Singh et al., 2013). Mehrian et al. (2015) investigated the effects of silver NPs on tomato plants. According to this study, significant decreases in protein content were observed as the concentration of Ag NPs

increased. In a study conducted with kidney beans, changes in Total Soluble Protein (TSP) content due to CeO2 NP applications were examined. The results indicated that protein content was not affected by the treatments conducted for 7 days, but significantly high protein contents were determined in the roots. The study also reported decreases in protein content in the leaves (Majumdar et al., 2014).

Table 4. Effects of application groups on Total Soluble Protein content in leaves of *P.vulgaris* L. plants
Çizelge 4. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Toplam Çözünebilir Protein miktarına etkileri

Application groups (120th hour)	Total Soluble protein Content (**mg/g**)
Control	1.61±0.19
0.1 mM ZnO	1.33±0.03*
0.01 mM ZnO	1.16±0.01*
0.001 mM ZnO	1.28±0.04*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: p<0.05

In a different study, the effects of ZnO NPs were investigated in *Leucaena leucocephala* plants under stress conditions induced by Pb and Cd applications. According to the research findings, the total soluble protein content did not increase in samples treated with ZnO NPs alone (Venkatachalam et al., 2017). A study reported that salinity stress and the application of different concentrations of Zn NPs did not significantly affect the total soluble protein values in spinach plants (Zafar et al., 2022). Additionally, a study conducted with corn plants suggests that the negative effects caused by arsenic can be mitigated with ZnO NPs (Khan et al., 2022).
Similarly, in our study, decreasing protein contents were observed in leaf tissues, which is generally consistent with the literature. Proteomic analyses are considered necessary for a better understanding of the mechanism.

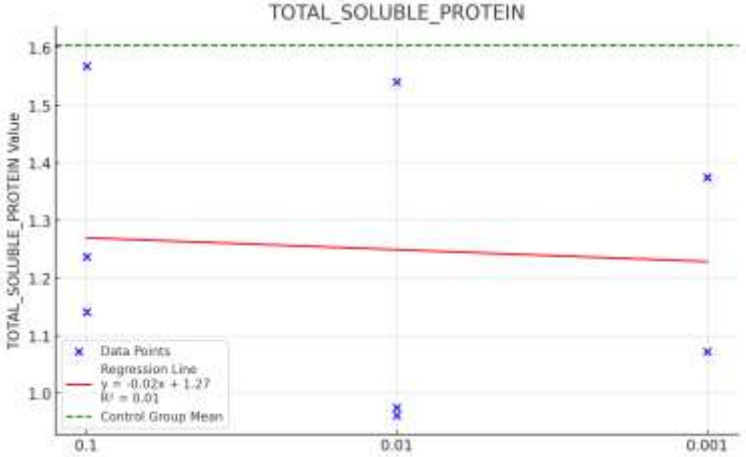

Figure 4. Regression curve of Total Soluble Protein content of application concentrations at 120th hour
Şekil 4. *Uygulama konsantrasyonlarının 120. saatteki Toplam Çözünebilir Protein içeriğine ait regresyon eğrisi*

Superoxide dismutase and Catalase enzyme activities

The superoxide dismutase (SOD) enzyme activity in leaves of *Phaseolus vulgaris* L. plants are presented in Table 5. The results obtained indicate that, in all concentrations of ZnO application, lower SOD enzyme activities were determined compared to the control. The effects of application group on catalase (CAT) enzyme activity in the leaves of *Phaseolus vulgaris* L. plants were examined based on the results in Table 5. It is observed that the effects of ZnO NP increase in all concentrations compared to the control. It is observed that CAT activity increases with decreasing concentrations of ZnO NP.

Table 5. The effects of application groups on Superoxide dismutase (SOD) and Catalase enzyme activities in leaves of *P. vulgaris* L. Plant
Çizelge 5. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Süperoksit dizmutaz (SOD) ve Katalaz (CAT) enzim aktivitelerine etkileri

Application groups (120th hour)	SOD Enzyme Activities (unit/g)	CAT Enzyme Activities (unit/g)
Control	**7.82±0.37**	**66.65±0.67**
0.1 mM ZnO	7.24±0.12*	121.09±4.44*
0.01 mM ZnO	7.20±0.05*	132.31±5.61*
0.001 mM ZnO	7.18±0.2*	206.64±8.45*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: p<0.05.

In this current study, in the ZnO NP application group, the SOD activity is lower compared to the control group. The lower SOD (Superoxide dismutase) activity at high concentrations may disrupt the antioxidant response involved in clearing reactive oxygen species from leaves, similar to the findings of Salehi et al. (2021). In another study with corn plants, high concentrations of ZnO NPs reduced SOD activity (Srivastav et al., 2021). On the other hand, zinc nanomaterials have been shown to increase SOD and POX enzyme activities, while also demonstrating a protective role against oxidative damage by reducing CAT activity (Venkatachalam et al., 2017).
In the results of this study, in all applications of ZnO NP, an increase in Catalase (CAT) activity compared to the control was observed. In the ZnO NP applied group, as the concentration decreased, CAT activity in all groups was significantly higher compared to the control.
In maize plants, SOD activity increased with the application of ZnO NPs at a concentration of 50 mg/L, but a decrease was observed with increasing concentrations (Srivastav et al., 2021). In light of the data, it can be said that superoxide radicals are dismutated by the SOD enzyme and then broken down and removed from the environment by CAT activity. Additionally, the high CAT activity can be explained by the excessive formation of H_2O_2 in the environment. Higher SOD activities have been identified in ZnO applications, possibly due to the Zn-SOD isoenzyme structure. When all the results and the literature are considered together, it can be seen that there is consistency in the relationships between the enzyme activities obtained and NP applications.

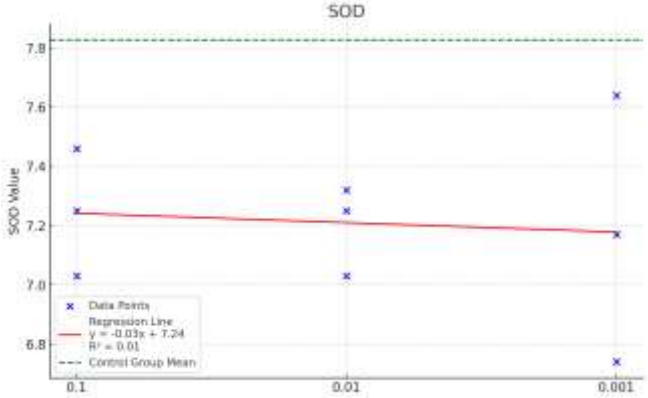

Figure 5. Regression curve of SOD and CAT enzyme activities of application concentrations at 120th hour
Şekil 5. *Uygulama konsantrasyonlarının 120. saatteki SOD enzim aktivitesine ait regresyon eğrisi*

Figure 6. Regression curve of CAT enzyme activity of application concentrations at 120th hour
Şekil 6. *Uygulama konsantrasyonlarının 120. saatteki CAT enzim aktivitesine ait regresyon eğrisi*

CONCLUSION

As a result, all findings from the present study revealed that ZnO NP application activates the antioxidant defense system in *Phaseolus vulgaris* L. leaves. It was determined that the metal nanoparticles in question caused negative effects, especially following exposure to low doses and for a duration of 120 hours. This situation may be attributed to their significantly small size. Nano-sized metals caused a toxic effect on the leaves of *Phaseolus vulgaris* L. Biological systems make it challenging to clearly elucidate the antioxidant mechanism. Obtaining consistent results in living cells is not possible due to factors such as the organism's genetics, developmental stage, applied molecules, and dosage, which play a primary role. However, according to the results of this study, nano-sized metals have induced a toxic effect on the leaves of *Phaseolus vulgaris* L. It should be noted that the duration of exposure to NPs also plays a role in the formation of this toxic effect. Numerous studies have reported that nanoparticles help cope with stress conditions in plants (You and Chan, 2015; González-García et al., 2021; Mushtaq et al., 2020). Specifically, it has been noted that nanoparticle applications lead to an increase in

antioxidant enzyme activities. The results suggest that nanoparticles act as agents that promote antioxidant defense. In the present study, similar results were obtained, and it was concluded that, in addition to stressing the plant, the rising antioxidant defense markers stimulate the plant's defense system. The increase in antioxidant activities will be an important defense response for the plant to overcome various stresses. Metal oxide nanoparticles are used in many fields. However, there is a potential danger that these substances can lead to serious adverse effects. The results obtained will contribute to the literature, emphasizing the need for more detailed research in this area. The results of this study emphasize the necessity of careful consideration in the use of nanomaterials. Particularly, in the face of global environmental disasters such as impending scarcity and drought, it is essential to establish sustainable systems and develop technologies.

Contribution Rate Statement Summary of Researchers

The authors declare no conflict of interest.

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Analysis of the Variations Within *Quercus ilex* L. and the Evaluation of Morphological Types Based on Chloroplast and Nuclear DNA Sequences

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ABSTRACT

Quercus ilex, evaluated within evergreen oaks, has a wide geographic distribution in the Mediterranean basin. Hybridization and gene flow are effective and frequently observed mechanisms in *Q. ilex*. Additionally, weak reproductive barriers between closely related taxa in zones of geographical contact further increase genetic diversity and subsequent taxonomic problems. Two morphological types, known as rotundifolia and ilex, are defined based on the variations between *Q. ilex* populations appearing as a result of all these factors. However, it is still controversial whether morphological types: ilex and rotundifolia are subspecies of *Q. ilex* or two separate species. In this study, short DNA sequences that consist of matK gene-partial trnK gene intron of chloroplast DNA and ITS1-5.8S rRNA gene-ITS2 of nuclear DNA were used to overcome such difficulties and to reveal the variations between *Q. ilex* populations. All *Q. ilex* populations based on both barcoding regions were determined and examined using the Molecular Evolutionary Genetics Analysis (MEGA 11). The analysis such as base substitutions, variable and parsim-info sites, transitional and transversional base substitution ranges (%), and nucleotide frequencies (%) was performed and transitional substitutions according to the transversional substitutions for both barcoding regions were observed in the high-value. Furthermore, the sequences belonging to nuclear DNA in comparison to other barcoding regions exhibited higher variable and parsim-info sites. Finally, Maximum Parsimony (MP) dendrograms for both barcoding regions were drawn to evaluate the populations belonging to *Q. ilex* in terms of their variations, phylogenetic-evolutionary relationships, and taxonomic status. Although both barcoding regions support the separation of *Q. ilex* populations based on different morphological types, matK gene-partial trnK gene intron sequences exhibited clearer and more informative results than ITS1-5.8S rRNA gene-ITS2 sequences.

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Quercus ilex L. İçerisindeki Varyasyonlarin Analizi ve Kloroplast ve Nükleer DNA Sekanslari Temelinde Morfolojik Tiplerin Değerlendirmesi

ÖZET

Herdem yeşil meşeler içerisinde değerlendirilen *Quercus ilex*, Akdeniz temelinde geniş coğrafik dağılıma sahiptir. Hibridizasyon ve gen akışı *Q. ilex*'de etkili ve sıklıkla gözlenen mekanizmalardır. Ayrıca, coğrafik olarak temaslı bölgelerde, yakın ilişkili taksonlar arasında zayıf üreme bariyerleri, *Q. ilex* içerisindeki genetik çeşitliliği ve sonrasında taksonomik problemleri arttıran diğer bir önemli durumdur. Rotundifolia ve ilex olarak bilinen iki morfolojik tip, tüm bu faktörlerin sonucu olarak ortaya çıkan, *Q. ilex* populasyonları arasındaki varyasyonlar temelinde tanımlanır. Ancak, morfolojik tipler: ilex ve rotundifolia nın *Q. ilex*'in alttürlerimi yoksa iki ayrı türmü olup olmadığı hala tartışmalı durumdur. Bu çalışmada, kloroplast DNA'ya ait matK geni-kısmi trnK gen intronu ve nükleer DNA'ya ait ITS1-5.8S rRNA geni-ITS2 den oluşan kısa DNA

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Kloroplast DNA
Nükleer DNA
MEGA 11

sekansları, bu tarz zorlukların üstesinden gelmek ve *Q. ilex* populasyonları arasındaki varyasyonları ortaya çıkarmak için kullanıldı. *Q. ilex*'e ait tüm populasyonlar heriki barkodlama bölgesi temelinde belirlendi ve Molecular Evolutionary Genetics Analysis (MEGA 11) kullanarak incelendi. Baz değişimleri, varyasyonlu ve parsim info bölgeler, transisyonel ve transversiyonel baz değişim oranları (%) ve nükleotid frekansları (%) gibi analizler gerçekleştirildi ve her iki barkodlama bölgesi için transisyonel baz değişimlerinin transversiyonel değişimlere göre daha yüksek değerde olduğu gözlemlendi. Ayrıca, nükleer DNA'ya ait sekanslar diğer barkodlama bölgesi ile karşılaştırmada daha yüksek varyasyonlu ve parsim info bölgeler sergiledi. Son olarak her iki barkodlama bölgesi için Maximum Parsimony (MP) dendrogramlar, varyasyonlar, filogenetik-evrimsel ilişkiler ve taksonomik statüler açısından *Q. ilex*'e ait populasyonları değerlendirmek için çizildi. Her iki barkodlama bölgesi, *Q. ilex* populasyonlarının farklı morfolojik tipler temelinde ayrımını desteklemesine rağmen, özellikle matK geni-kısmi trnK gen intron sekansları, ITS1-5.8S rRNA geni-ITS2 sekanslarından daha açık ve bilgilendirici sonuçlar sergiledi.

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INTRODUCTION

Quercus ilex L., commonly known as holm oak, is an evergreen tree or shrub a natural distribution across the central and western Mediterranean basin, Aegean Islands, Balkan regions, North Africa, western parts of Türkiye and the limited coastal areas of Black Sea in Türkiye (Barbero et al., 1992; de Rigo & Caudullo, 2016; Suicmez & Avci, 2023).

Q. ilex, a dominant species in the Mediterranean forests, faces threats from various factors such as vertebrate and invertebrate species that rely on this tree for sustenance and habitat, differentiation in the geographical distribution under ecological and climatic changes, destruction for its high economic value and inadequate protection strategies (Yılmaz, 2018; Hernandez-Agüero et al., 2022; Rey et al., 2023; Suicmez & Avci, 2023). Additionally, the aging tree populations with poor regeneration capacity also impact species diversity and distribution (Rey et al., 2023).

Q. ilex, evaluated within evergreen oaks, has two main morphological types known as rotundifolia and ilex (Saenz de Rivas, 1967, 1970; Peguero-Pina et al., 2014; Bensaci et al., 2021). The rorundifolia type has small and round thick leaves, while the ilex type features elongated and large pointed leaves (Tutın et al., 1964; Peguero-Pina et al., 2014). Furthermore, three different morphotypes - ilex, rotundifolia, and intermediate - within holm oak were defined by Michaud et al. (1995) and Lumaret et al. (2002). The distribution areas for the individuals with intermediate morphotypes exhibiting characteristics between ilex and rotundifolia were identified in coastal areas of eastern and northern Spain and south France (Languedoc and Roussillon). Rotundifolia morphotype is characterized by dry distribution areas of the Mediterranean climate such as North Africa and the interior region of Spain (Tutın et al., 1964; Lumaret et al., 2002; Vázquez Pardo et al., 2002; Peguero-Pina et al., 2014). Ilex morphotypes are distributed from Greece to the French Riviera along France's Atlantic coast (Lumaret et al., 2002; Peguero-Pina et al., 2014). In addition to the distribution areas stated for morphotypes, *Q. ilex* has natural populations in Türkiye. Yılmaz et al. (2013) evaluated the *Q. ilex* in five separate populations in their study based on the molecular diversity of evergreen oaks in Türkiye. Similarly, the relationships of *Q. ilex* populations based on their morphological variabilities were examined by Yılmaz et al. (2017). Comprehensive studies, including *Q. ilex* populations in Türkiye, are necessary to better understand the species' genetic diversity.

The classification of morphological types (ilex and rotundifolia) within *Q. ilex* as either subspecies (*Q. ilex* subsp. *ilex* and *Q. ilex* subsp. *rotundifolia*) or separate species remains controversial (Tutın et al., 1964; Saenz de Rivas, 1967; Amaral-Franco, 1990; Govaerts and Frodin, 1998; Vázquez Pardo et al., 2002; Soto et al., 2007; de Rigo & Caudullo, 2016; Sousa et al., 2021).

Furthermore, the taxonomic status of these morphotypes has not been completely clear and resolved yet. Today,

the separation of ilex and rotundifolia types within *Q. ilex* is based on their morphological characters and geographic distribution.

Hybridization and gene flow are effective mechanisms frequently observed in the genus *Quercus*. They are important processes in genetic diversity, evolution, and speciation of the genus. Hybridization, commonly observed between taxa with weak reproductive barriers in zones of geographical contact, complicates taxonomy (Bacilieri et al., 1996; Borazan & Babaç; 2003). Similar hybridization behaviors in *Q. ilex* were reported by Schnitzler et al. (2004) and Lopez de Heredia et al. (2018). *Quercus* x *turneri* 'Pseudoturneri' as a hybrid resulting from a crossing of *Q. ilex* L. and *Q. robur* L. is stated by Schnitzler et al. (2004). Hybridization between *Q. suber* L. and *Q. ilex* L. in the zones where they form mixed stands has been known and reported for a long time (Lopez de Heredia et al., 2018). Two evergreen oaks: *Q. ilex* and *Q. coccifera* are closely related taxa that have extensive distribution areas in the Mediterranean region and co-occurred in mixed stands where hybridization may take place (de Casas et al. 2007; Ortego & Bonal, 2010). Consequently, introgression as a result of hybridization between *Q. ilex* and *Q. coccifera* in the distribution areas overlapping frequently appeared (Jimenez et al., 2004; Lopez de Heredia et al., 2007; Ortego & Bonal, 2010).

These factors have an important effect on the variation in morphological characters and distribution of *Q. ilex*. Variations within oak species complicate species identification due to fuzzy species boundaries. (Bacilieri et al., 1996; Borazan & Babaç; 2003; Petit et al., 2003; Yılmaz, 2018). All these factors increase the taxonomic problems in *Q. ilex* which has extensive distribution areas and makes problematic the taxon. To overcome such difficulties and to collect the taxa in the correct systematic categories, short DNA sequences that contain enough information to identify the species and reveal the phylogenetic relationships between taxa are frequently used as a molecular approach. In this study, matK gene-partial trnK gene intron sequence data of chloroplast DNA and ITS1-5.8S rRNA gene-ITS2 sequence data of nuclear DNA were acquired from the National Center for Biotechnology Information (NCBI) and later it was aimed to: i) evaluate the ability of chloroplast and nuclear DNA barcoding regions to reveal phylogenetic relationships among *Q. ilex* populations from different localities, ii) determine the variations between *Q. ilex* populations, iii) create a phylogenetic tree and make suggestions about the taxonomic status of *Q. ilex* populations according to the results provided from the phylogenetic tree, and iv) present more informative and comprehensive results about the taxonomic and phylogenetic relations of morphological types within *Q. ilex*.

MATERIALS and METHODS

All sequence data for *Q. ilex* populations, covering both barcoding regions (matK gene-partial trnK gene intron of chloroplast DNA and ITS1-5.8S rRNA gene-ITS2 sequence of nuclear DNA) from past to present, were obtained from the NCBI database. Sequences of the 18S rRNA gene- ITS1- 5,8S rRNA gene- ITS2- 28S rRNA gene, in addition to ITS1-5.8S rRNA gene-ITS2, were collected and then the sequences containing ITS1- 5,8S rRNA gene-ITS2 were extracted from these regions. Finally, sequence data of all extracted regions for *Q. ilex* populations were combined to provide more effective and comprehensive results about the variations between *Q. ilex* populations and the taxonomic status of morphological types within *Q. ilex*.

A total of 37 *Q. ilex* populations were analyzed for compatibility of sequence information based on matK gene-partial trnK gene intron. Additionally, 20 populations of *Q. ilex* for the region containing ITS1 and ITS2 sequence data were detected and examined in this study. GenBank codes for both barcoding regions were presented in the Appendix. The multiple sequence alignments for *Q. ilex* populations were separately performed for both DNA sequences using the Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura et al. 2021). The probabilities of substitution from one base to another base were determined and subsequently, variable and parsim-info sites which are important indicators in phylogenetic relationships were computed for both barcoding regions belonging to nuclear and chloroplast DNA. Transitional and transversional base substitution ranges (%) were computed for examined DNA sequences. Finally, nucleotide frequencies of matK gene-partial trnK gene intron and ITS1-5.8S rRNA gene-ITS2 sequences were determined and presented as G+C % and A+T/U %.

Dendrograms showing bootstrap values on their branches and inferred the evolutionary history were created using the Maximum Parsimony (MP) method. These MP dendrograms for both barcoding regions were used to evaluate the phylogenetic relationships between *Q. ilex* populations, determine the variations among populations from different geographic regions, and provide more informative results about morphological types within *Q. ilex*.

RESULTS and DISCUSSION

The sequence data for ITS1-5.8S rRNA gene-ITS2 of nuclear DNA and, matK gene-partial trnK gene intron of chloroplast DNA were acquired from the NCBI for all *Q. ilex* populations. The alignment lengths for a total of 37 *Q. ilex* populations were determined as 695 bp based on the sequence information of matK gene-partial trnK gene

intron. The variable and parsimony informative sites expressing the nucleotide substitutions are critical indicators for determining the variations and relationships among morphological types in *Q. ilex* populations from different habitats. In this study, based on the matK gene-partial trnK gene intron, the variable, and parsimony informative sites were observed in 9 and 8 nucleotides, respectively. The probabilities of substitutions between bases for matK gene-partial trnK gene intron were determined and shown in Table 1.

Table 1. The probabilities of substitution (r) from one base (row) to another base (column) for matK gene-partial trnK gene intron (Transitional substitutions are shown in bold).

Çizelge 1. matK geni-kısmi trnK gen intronu için bir bazdan diğerine değişim olasılıkları (Transisyonel baz değişimleri koyu renkli gösterilir)

	A	**T**	**C**	**G**
A	-	*6.9*	3.06	**0.58**
T	6.71	-	**17.89**	3.32
C	6.71	**40.37**	-	3.32
G	**1.17**	6.9	3.06	-

The highest substitutions were observed as 40.37% from C to T and then 17.89% from T to C. Moreover, transitional and transversional base substitutions were computed from Table 1 as 60.01% and 39.99%, respectively. This indicates that transitional substitutions are higher than the transversional substitutions for matK gene-partial trnK gene intron sequences belonging to *Q. ilex* populations.

The alignment lengths for a total of 20 *Q. ilex* populations based on the ITS1-5.8S rRNA gene-ITS2 sequences were determined to be 604 bp. The variable and parsimony informative sites were observed in 58 and 32 nucleotides, respectively. The probabilities of substitutions between bases for ITS1-5.8S rRNA gene-ITS2 sequences were determined and the highest substitutions detected as 33.02% from T to C and then 20.24% from A to G. (Table 2). Moreover, transitional and transversional base substitutions were computed as 83.81% and 16.19%, respectively. This indicates that transitional substitutions are significantly higher than the transversional substitutions for the region containing ITS1-5.8S rRNA gene-ITS2 sequences.

Table 2. The probabilities of substitution (r) from one base (row) to another base (column) for ITS1-5.8S rRNA gene-ITS2 sequences (Transitional substitutions are shown in bold).

Çizelge 2. ITS1-5.8S rRNA geni-ITS2 sekansları *için bir bazdan diğerine değişim olasılıkları (Transisyonel baz değişimleri koyu renkli gösterilir)*

	A	**T**	**C**	**G**
A	-	*1.45*	2.69	**20.24**
T	1.52	-	**33.02**	2.43
C	1.52	**17.85**	-	2.43
G	**12.7**	1.45	2.69	-

Transition/transversion ratios for purines (k_1) and pyrimidines (k_2) were determined and compared for both barcoding regions. It was observed that the transition/transversion ratio of pyrimidines (5.84) was higher than that of purines for matK gene-partial trnK gene intron sequences. The transition/transversion ratio for purines (k_1), pyrimidines (k_2), and overall were determined as 8.34, 12.27, and 4.81, respectively for ITS1-5.8S rRNA gene-ITS2 sequences. In other words, pyrimidines in the aspect of the transition/transversion ratio show a higher value than purines in the comparison, similar to the results provided from matK gene-partial trnK gene intron (Table 3).

It was determined that DNA sequences analyzed for *Q. ilex* populations consist primarily of A and T/U bases (68.09%) for the region that contains matK gene-partial trnK gene intron sequences. Conversely, it was observed that the percentage of G and C bases was higher (63.22%) than A+T/U bases (36.78%) for ITS1-5.8S rRNA gene-ITS2 sequences (Table 3).

Furthermore, table 4 shows the variable nucleotides for both barcoding regions were formed to understand the phylogenetic and evolutionary relationships between *Q. ilex* populations.

Finally, Maximum Parsimony (MP) dendrograms for both barcoding regions were drawn to evaluate the *Q. ilex* populations in terms of their variations, phylogenetic-evolutionary relationships, and taxonomic status (Figure 1, 2).

The examined *Q. ilex* populations show a wide geographic distribution in the Mediterranean basin. In other words, *Q. ilex* populations analyzed in this study consist of samples from three continents. MP dendrogram based on matK gene-partial trnK gene intron sequences separated the *Q. ilex* populations into three main groups (Figure 1).

The populations resolved in Group I showed two separate clusters: a and b. Cluster a consists of the populations from Croatia, Italy, and France, while cluster b consists of the samples from Albania, Croatia, island populations (Sardinia and Zafferana Etna) of Italy with Apulia, France, and Malta (another island population) that has the very close locality to Zafferana Etna. Group II consists of the samples collected in Morocco, three populations of Algeria, five populations of Spain, and three population of France and Greece.

Table 3. The information of the *Q. ilex* populations examined is based on matK gene-partial trnK gene intron and ITS1-5.8S rRNA gene-ITS2 sequences.

Çizelge 3. matK geni-kısmi trnK gen intronu and ITS1-5.8S rRNA geni-ITS2 sekansları temelinde incelenen *Q. ilex* populasyonlarının bilgileri.

DNA regions	Pop. (Number)	Alignment length (bp)	Variable site	Parsim-info site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion Purine (k_1)	Pyrimid. (k_2)	Overall (R)	Nucleotide frek. (%) A+T/U G+C
matK gene-trnK intron	37	*695*	9	8	60.01	39.99	0.17	5.84	1.27	68.09/31.91
ITS1-5.8S-ITS2	20	604	58	32	83.81	16.19	8.34	12.27	4.81	36.78/63.22

Table 4. *Q. ilex* populations and variable sites belonging to a) matK gene-partial trnK gene intron sequences b) ITS1-5.8S rRNA gene-ITS2 sequences (The numbers show variable nucleotides).

Çizelge 4. Q. ilex populasyonları ve varyasyonlu nükleotid bölgeleri a) matK geni-kısmi trnK gen intron sekansları b) ITS1-5.8S rRNA geni-ITS2 sekansları (Numaralar varyasyonlu nükleotidleri gösterir).

a)

	1 3 4 4 4 4 4 5 5
	4 1 0 2 3 3 5 6 8
	0 3 2 6 1 9 5 0 9
Quercus ilex (Italy-1)	- T A G T C C G A
Quercus ilex (Türkiye-1)	C T T G A T T G A
Quercus ilex (Italy-2)	- T A G T C C G A
Quercus ilex (Türkiye-2)	C T T G A T T G A
Quercus ilex (Greece-1)	C T T G A T T A A
Quercus ilex (Spain-1)	T C T T C C C G A
Quercus ilex (Algeria)	T C T T C C C G A
Quercus ilex (Albania)	- T A G C C C G A
Quercus ilex (Greece-2)	T T T T C C C G A
Quercus ilex (Croatia-1)	- T A G T C C G A
Quercus ilex (Italy-3)	- T A G C C C G A
Quercus ilex (Spain-2)	T C T T C C C G A
Quercus ilex (Malta)	- T A G C C C G A
Quercus ilex (Türkiye-3)	C T T G A T T G A
Quercus ilex (France-1)	- T A G C C C G A
Quercus ilex (France-2)	- C T G C C C G A
Quercus ilex (Algeria/Souk)	- C T T C C C G A
Quercus ilex (Algeria/Mascara)	- C T T C C C G A
Quercus ilex (Spain/Coll de Corniols)	- C T T C C C G A
Quercus ilex (Spain/Mallorca)	- C T T C C C G A
Quercus ilex (Italy/Zafferana Etna)	- T A G C C C G A
Quercus ilex (Greece/Skyathos)	- T T G A T T G A
Quercus ilex (Greece/Ikaria)	- T T G A T T G A
Quercus ilex (Greece/Drymaia)	- T T G A T T G A
Quercus ilex (Greece/Crete)	- T T G A T T A A
Quercus ilex (France/Lacanau)	- C T T C C C G A
Quercus ilex (France/Rennes-le-Chateaux)	- C T T C C C G A
Quercus ilex (France/Nice)	- T A G C C C G A
Quercus ilex (France/Olmeto)	- T A G T C C G A
Quercus ilex (Croatia/Split)	- T A G T C C G A
Quercus ilex (Croatia-2)	- T A G C C C G A
Quercus ilex (Spain-3)	T C T T C C C G A
Quercus ilex (Italy-Apulia)	- T A G C C C G A
Quercus ilex (Italy-Sardinia)	- T A G C C C G A
Quercus ilex (Italy-Latium)	- T A G T C C G A
Quercus ilex (Morocco/Tangers)	T C T T C C C G A
Quercus ilex (Israel/Mt. Tabor)	C T T G C C C G C

b)

	1 1 1 1 1 1 1 1 1 1 1 1 1
	1 2 2 2 4 5 6 6 7 7 7 8 8 8 9 9 0 1 1 1 3 3 3 3 4 5 6 6 6
	8 7 8 9 9 2 3 8 3 5 8 1 4 8 0 9 1 3 4 9 0 2 4 7 0 1 2 3 8
Quercus ilex (Italy/Basilicata)	A A C A G A T A C C C C C A T C C T T C G T G C G C A C G
Quercus ilex (Türkiye/Northern Türkiye)	G G . G . G . G . T . . . G C . T C G . C C . T . T . G .
Quercus ilex (France/Provence)	G G . G . G . G . . . T A G C . . C G . C C . . . . . G .
Quercus ilex (Morocco-1)	G G T G . G C G . . . T A G C . . C G . C C A . . . . G .
Quercus ilex (Morocco-2)	G G . G . G . G T . . T A G C T . C G . C C . . . . . G T
Quercus ilex (Spain/Arboretum El Bosque)	G G . G A G . G T . G T A G C . . C G . T C . . . T . G .
Quercus ilex (Spain/Andalucia)	G G . G . G . G T . . T A G C T . C G . C C A . . . . G T
Quercus ilex (Greece/Mainland Greece)	G G . G . G . G T . G T A G C . . C G T T C . . . T G G .
Quercus ilex (Spain/Huesca)	G G . G A G . G T . G T A G C . . C G . T C . . . T . G .
Quercus ilex (Spain/Sierra de Tolono)	G G . G . G . G T . . T A G C T . C G . C C . . . . . G T
Quercus ilex (Greece/Corfu)	G G . G . G . G T . G T A G C . T C G . C C . T . T . G .
Quercus ilex (Italy/Lecce)	G G . G . G . G T . G T A G C . . C G . T C . . A T . G .
Quercus ilex (Spain/Binifaldo)	G G . G . G . G T . . T A G C . . C G . C C . . . . . G .
Quercus ilex (Spain/Algarrobet)	G G . G . G . G T . . T A G C . . C G . C C . . . . . G .
Quercus ilex (Spain/Constantina)	G G . G A G . G T . . T A G C . . C G . T C . . . T . G .
Quercus ilex (France/Brignoles)	G G . G . G . G T . . T A G C . . C G . C C . . . . . G .
Quercus ilex (Spain/Pinet)	G G . G . G . G T . . T A G C T . C G . C C . . . . . G T
Quercus ilex (France/Corse Island)	G G . G . G . G T . . T A G C . . C G . C C . . . T . G .
Quercus ilex (Italy/Abruzzo)	G G . G . G . G T . G T A G C . . C G . T C . . . T . G .
Quercus ilex (Italy/Latium)	G G . G . G . G T . G T A G C . . C G . T C . . . T . G .
	1 1 1 1 1 2 2 3 3 3 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5
	7 7 8 9 9 7 8 2 4 9 1 2 3 4 7 8 8 9 1 3 3 4 5 5 6 7 7 8 9
	1 9 0 5 6 6 3 0 8 5 0 0 8 6 9 6 9 9 2 0 1 1 1 3 4 4 5 9 6
Quercus ilex (Italy/Basilicata)	A C G C T A G G G C T T G G C T C A A G T C T C G C T T G
Quercus ilex (Türkiye/Northern Türkiye)	G . . . C G . A . . C C . . . C . G . . C . A . . . C C .
Quercus ilex (France/Provence)	G . . . C G . . . . . C . . . C . G G . C . . . . . C C .
Quercus ilex (Morocco-1)	G T . . C G . . . . . C . . . C . G G . C . . . . . C C .
Quercus ilex (Morocco-2)	G . . . C G . . . . . C A . . C . G . . C A . T . . C C .
Quercus ilex (Spain/Arboretum El Bosque)	. . A . . . . . . T . . . A T C . G . . C . . . .
Quercus ilex (Spain/Andalucia)	G . . . C G A . . . . C A . . C . G . . C A . T . . C C .
Quercus ilex (Greece/Mainland Greece)	. . . . C . . . C . G . . C . . . .
Quercus ilex (Spain/Huesca)	. . A . . . . . . T . . . A T C . G . . C . . . .
Quercus ilex (Spain/Sierra de Tolono)	G . . . C G . . . . . C . . . C . G . . C . . T . . C C .
Quercus ilex (Greece/Corfu)	. . . T C G . . . . . C . . . C . G . . C . . . . . C C C
Quercus ilex (Italy/Lecce)	. . . . C . . . . C . . . .
Quercus ilex (Spain/Binifaldo)	G . . . C G . . . . . C . . . C . G . A C . C . . . C C .
Quercus ilex (Spain/Algarrobet)	G . . T C G . . . . . C . . . C . G . . C . . . . G C C C
Quercus ilex (Spain/Constantina)	. . A . . G . . . T . . . A T C . G . . C . . . .
Quercus ilex (France/Brignoles)	G . . . C G . . . . . C . . . C . G . . C . C . . . C C .
Quercus ilex (Spain/Pinet)	G . . . C G . . . . . C A . . C . G . . C A . T . . C C .
Quercus ilex (France/Corse Island)	G . . . C G . . . . . C . . . C . G . . C A . T A . C C .
Quercus ilex (Italy/Abruzzo)	. . . . C G . . A . . C . . . C G . . . C . . . . . C C .
Quercus ilex (Italy/Latium)	. . . . C G . . A . . C . . . C . G G . C . . . . G C C .

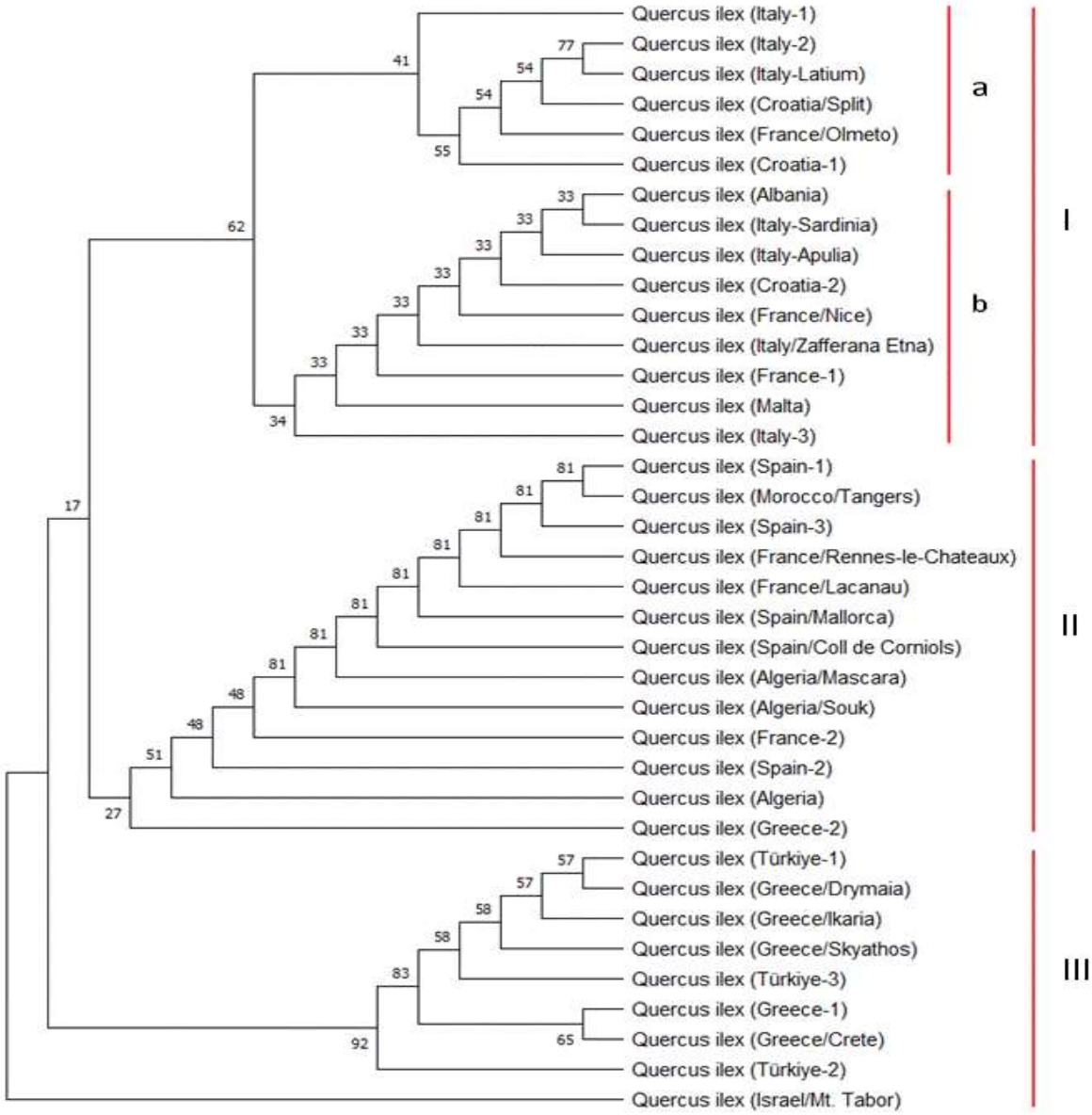

Figure1. Maximum Parsimony tree provided from matK gene-partial trnK gene intron sequences of *Q. ilex* populations.

Şekil 1. Q. ilex populasyonlarının matK geni-kısmi trnK gene intron *sekanslarından elde edilen MP ağacı.*

In summary, populations from dry distribution areas of the Mediterranean climate such as North Africa, alongside samples from Spain, were clustered together, forming a separate group in dendrogram. Additionally, the populations from Albania to France generate Group I, distinct from Spanish and African populations (Morocco and Algeria). However, French populations were observed in both Group I and II, and they exhibited the highest variations compared to the other populations.

Hybridization and introgression are commonly observed mechanisms in the genus *Quercus,* especially in overlapping zones due to weak reproductive barriers (Kremer et al., 2002; Borazan & Babaç; 2003; Petit et al., 2003). Furthermore, these mechanisms give rise to morphological variations and later make it hard to identify the taxa because of insufficient diagnostic morphological characters. Similar behaviors for *Q. ilex* have been observed and reported by many researchers (Jimenez et al., 2004; Schnitzler et al., 2004; de Casas et al. 2007; Lopez de Heredia et al., 2007; Ortego & Bonal, 2010; Lopez de Heredia et al., 2018). In this concept, the variations that were related to hybridization and introgression, in addition to the variations caused by different climatic and ecological factors in the populations showing wide geographical distribution are observed and different morphological types (rotundifolia and ilex) are defined within the *Q. ilex*. The distribution areas of rotundifolia morphotype are stated by dry climatic Mediterranean areas of North Africa and Spain (Tutın et al., 1964; Vázquez Pardo et al., 2002; Peguero-Pina et al., 2014), while regions between Greece and French Riviera are evaluated as distribution areas

for ilex morphotype (Lumaret et al., 2002; Peguero-Pina et al., 2014). Some regions, such as southern France and, eastern and northern Spain, contain samples with intermediate morphotypes between rotundifolia and ilex (Michaud et al., 1995; Lumaret et al., 2002). However, the distribution areas and the taxonomic status of morphological types are not still completely resolved, due to the evaluation of the variations within the *Q. ilex* in terms of only their distribution areas and morphological characters. This study provides important data to evaluate the variations among *Q. ilex* populations from different geographic regions based on nucleotide sequences that consist of both nuclear and chloroplast DNA. Moreover, thus it is aimed to provide more informative results about morphological types. MP tree based on matK gene-partial trnK gene intron sequences separated the *Q. ilex* populations into Group I (Albania, Croatia, France, and Malta) and Group II (North Africa and Spain). This result supports the separation of *Q. ilex* populations based on morphological types. Furthermore, the distribution areas of the rotundifolia and ilex morphotypes, as stated by many researchers, align with the study results. The samples with intermediate morphotypes that were defined by Michaud et al. (1995) and Lumaret et al. (2002) are characterized by the distribution areas such as south France and coastal areas of eastern and northern Spain. Similarly, *Q. ilex* populations in France were observed in both Group I and II (Figure 1). A total of eight populations from Türkiye and Greece formed a distinct group with the outmost species in the phylogenetic tree and they were clustered together in Group III exhibiting higher variations in comparison to the other populations. The single population from Israel merged from outermost to the clade consisting of Türkiye and Greece populations, showing the highest variation in the phylogenetic tree.

MP dendrogram based on ITS1-5.8S rRNA gene-ITS2 sequences separated the *Q. ilex* populations into two main groups (Figure 2).

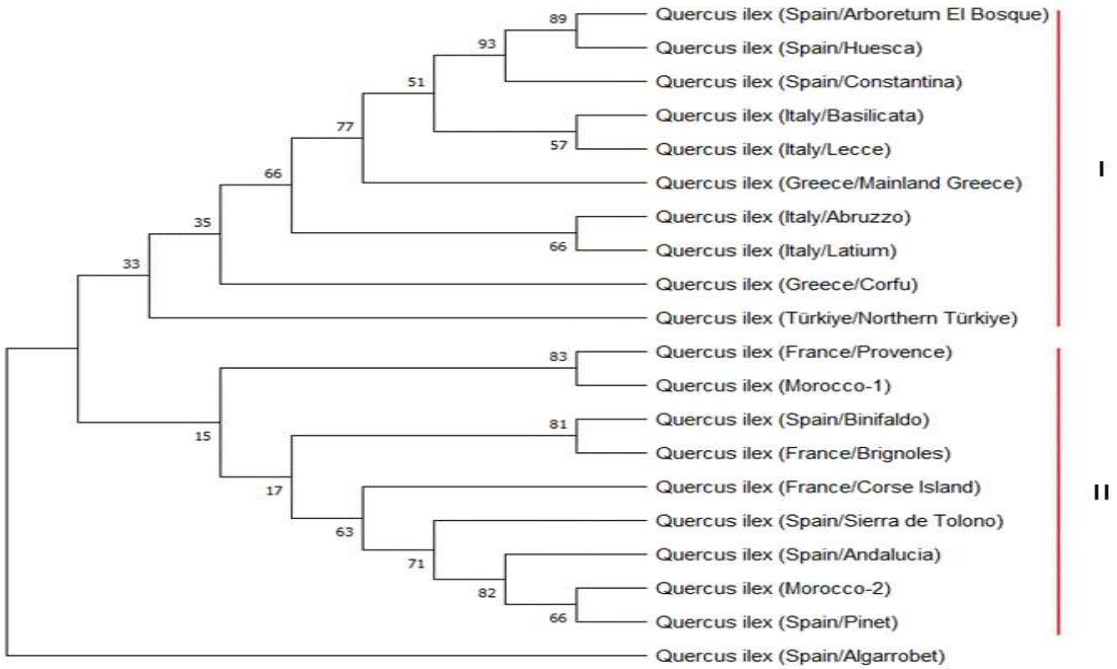

Figure2. Maximum Parsimony tree provided from ITS1-5.8S rRNA gene-ITS2 sequences of *Q. ilex* populations.
Şekil 2. Q. ilex populasyonlarının ITS1-5.8S rRNA geni-ITS2 *sekanslarından elde edilen MP ağacı.*

The samples resolved in Group I consist of ten populations from Spain, Italy, Greece, and Northern Türkiye. The Spanish populations in Group I are from the interior regions of Spain. Also, it can be stated that the populations were clustered in a phylogenetic tree according to the distribution areas.

Group II consists of the samples collected from two populations in Morocco, three populations in France, and five populations in Spain. Provence and Brignoles are two populations that have close distribution areas on the Mediterranean coast of South France. Corse Island is another population from France that lies southeast of the French mainland on the Mediterranean Sea. These France populations were clustered together with Moroccan populations characterized by other dry distribution areas of the Mediterranean climate (Figure II). Similarly, the populations that have distribution areas on the Mediterranean coast of Spain such as Andalucía, Pinet, and Algarrobet (island population) were clustered in Group II.

The MP tree provided from ITS1-5.8S rRNA gene-ITS2 sequences separated the *Q. ilex* populations characterized

by dry Mediterranean climate into Group II. In this concept, the separation of *Q. ilex* populations aligns with the distribution areas of the rotundifolia morphotypes noted by many researchers (Tutın et al., 1964; Lumaret et al., 2002; Vázquez Pardo et al., 2002; Peguero-Pina et al., 2014. However, further studies with more samples from the wide distribution areas in the Mediterranean region are necessary for support.

Both barcoding regions of the chloroplast and nuclear DNA support the separation of *Q. ilex* populations based on different morphological types. Especially, matK gene-partial trnK gene intron sequences in the aspect of the ability to reveal the variations and phylogenetic relationships between *Q. ilex* populations exhibited more clear and informative results than ITS1-5.8S rRNA gene-ITS2 sequences. Additionally, matK gene-partial trnK gene intron sequences have lower sequence variations among populations examined than the region containing ITS sequences.

Therefore, matK gene-partial trnK gene intron sequences are strongly recommended for further studies to reveal variations within *Q. ilex* more clearly and in detail, including samples from all distribution areas. It should also be noted that there are many problems related to the data in NCBI, such as missing habitats and country information in the database. The taxon such as *Q. ilex* which has a wide distribution area is under the influence of different ecological and climatic conditions causing variations. In other words, habitat information of the samples collected from different geographical regions is very important to evaluate variations within the taxon. Deficiencies in this sense complicate interpretation and lead to mistakes in evaluating results. Furthermore, Türkiye is another important region for *Q. ilex* populations with distribution areas that consist of northwest parts and the limited coastal areas of the Black Sea (Yılmaz et al., 2013; Yılmaz et al., 2017). The molecular diversity of *Q. ilex* and their phylogenetic relationships with evergreen oaks in Türkiye were evaluated by Yılmaz et al. (2013). Nevertheless, there is not still enough information about the genetic diversity of *Q. ilex* based on the population genetics. This makes it necessary to conduct studies including the *Q. ilex* populations collected from all distribution areas to obtain more comprehensive and effective results. Finally, DNA sequences preferred in the evaluation of phylogenetic relationships and the determination of genetic diversity have a highly important role in the success of the study, due to variability in species identification and separation ability of the same barcoding region in different plant groups. In other words, it is very important to determine the barcoding regions giving the most accurate and consistent results for the plant group examined. In this concept, although both barcoding regions that consist of matK gene-partial trnK gene intron and ITS1-5.8S rRNA gene-ITS2 sequences provide significant information regarding variations between *Q. ilex* populations, DNA sequences belonging to matK gene-partial trnK gene intron is particularly recommended for their ability to reveal the diversity between populations more clearly, detailed, and meaningfully.

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Author's Contributions

The authors contributed equally.

Statement of Conflict of Interest

The author has declared no conflict of interest.

Appendix

FM244453, FM244439, FM244427, FM244422, FM244411, FM244365, FM244363, FM244350, FM244344, DQ342360, DQ342359, DQ342358, DQ342356, DQ342355, DQ342354, DQ342353, DQ342351, DQ342350, AY226837, AY226836, LT222296, LT222295, LT222294, LT222292, LT222291, LT222290, LT222289, LT222288, LT222285, LT222283, LT222281, LT222278, LT222272, LT222271, LT222269, LT222268, LM652956, LM652955, LM652954, LM652953, LM652952, LM652951, LM652950, LM652949, LM652948, LM652947, LM652946, LM652945, LM652944, LM652943, HE583659, HE583656, HE583624, HE583623, HE583622, HE583620, HE583616.

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Centaurea lycaonica Boiss. & Heldr. Bitkisinin İnsan Servikal Kanser Hücre Hattında Sitotoksisitesinin MTT Testi ve xCELLigence Sistemi ile Değerlendirilmesi

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ÖZET

Centaurea L. cinsine ait birçok tür, dünyanın çeşitli bölgelerinde endemik olarak varlığını sürdürmekte ve halk hekimliğinde kullanımlarıyla literatürde yerini almaktadır. Söz konusu cinse ait bazı türlerin servikal kanser hücre hattı üzerindeki sitotoksisitesi incelenmiştir. Bu çalışmada Türkiye'ye endemik ve hakkında yok denecek kadar az sayıda çalışma bulunan *Centaurea lycaonica* türünün kök kısmından hareketle, 24 saat maserasyon yöntemiyle hazırlanmış diklorometan (CRD) ve metanol (CRM) ekstrelerinin 48 saatlik maruziyette insan servikal kanser hücre hattındaki (HeLa) sitotoksik etkisinin araştırılması amaçlandı. Bitki ekstresinden hazırlanan farklı konsantrasyonların hücre canlılığına etkisi çalışma prensipleri farklı olan MTT ve xCELLigence GZHA sistemi kullanılarak araştırıldı ve IC_{50} değerleri belirlendi. Sonuç olarak, CRD ve CRM ekstrelerinin MTT bulguları, xCELLigence analiziyle tutarlı olup HeLa hücrelerinde sitotoksik etkiye sahip olduğu bulundu. *C. lycaonica* türünün kanser tedavisinde yeni bir doğal kaynak olarak değerlendirilebileceği düşünüldü. Bu çalışmanın, Türkiye'ye endemik birçok türün biyolojik aktivite ve etki mekanizmalarının aydınlatılmasını teşvik edeceği düşünülmektedir.

Moleküler Biyoloji

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Anahtar Kelimeler
Centaurea lycaonica
Servikal kanser
HeLa
MTT
xCELLigence

Evaluation of Cytotoxicity of *Centaurea lycaonica* Boiss. & Heldr. Plant on Human Cervical Cancer Cell Line with MTT Test and xCELLigence System

ABSTRACT

Many species belonging to the genus *Centaurea* L. are endemic in various parts of the world and are used in folk medicine. The cytotoxicity of some species belonging to this genus on cervical cancer cell lines was investigated. In this study, it was aimed to investigate the cytotoxic effect of dichloromethane (CRD) and methanol (CRM) extracts prepared by 24 h maceration method on human cervical cancer cell line (HeLa) at 48 h exposure based on the root part of *Centaurea lycaonica*, which is endemic to Turkey and about which there are almost no studies. The effect of different concentrations of plant extracts on cell viability was investigated using MTT and xCELLigence RTCA systems, which have different working principles, and IC_{50} values were determined. As a result, MTT findings of CRD and CRM extracts were consistent with xCELLigence analysis and found to have a cytotoxic effect on HeLa cells. It was thought that *C. lycaonica* species could be evaluated as a new natural source in cancer treatment. It is thought that this study will encourage the elucidation of the biological activities and mechanisms of action of many species endemic to Turkey.

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GİRİŞ

Jinekolojik kanserler arasında önemli bir yere sahip olan serviks kanseri, yıllık 604.127 yeni vaka ve 341.831 ölümle Dünya'da kadınlar arasında en sık görülen dördüncü kanser türü olmuştur (Sung ve ark., 2020; Rajaram ve ark., 2021). Serviks kanseri; karsinojen türdeki insan papilloma virüsü (HPV) enfeksiyonları, serviks displazisi, geçirilen doğum sayısı, genetik faktörler, metabolik bozukluklar, ilk cinsel ilişki yaşı, çok partnerli cinsel yaşam tarzının benimsenmiş olması gibi birçok faktöre bağlı olarak gelişmektedir. Çoğu gelişmiş ülkede insidansı azaltmak amacıyla bireylere cinsel sağlık eğitimleri verilmekle beraber kapsamlı tarama testleri, etkin tanı yöntemleri ve tedavi stratejileri geliştirilmiştir. Buna karşın sağlık kaynaklarının sınırlı olduğu ve mevcut tedavi seçeneklerinin dahi karşılanamaz olduğu düşük ve orta gelirli ülkelerde, rahim ağzı kanseri ciddi bir halk sorunu olmaya devam etmektedir. Bu nedenlerle küresel anlamda yük oluşturan serviks kanserine karşı etkili tedavi yolu geliştirilmesi için çalışmalar devam etmektedir (Brown ve ark., 2012; Shaikh ve ark., 2023).

Kanserin seyrine göre ihtiyaç duyulan tedavi stratejisi belirlenerek cerrahi, radyoterapi ve kemoterapiden biri veya bunların bir kombinasyonu uygulanmaktadır. Çoğu zaman bu tedavi seçenekleri ağır yan etkilere sebep olmakta ve hastanın hayatta kalma şansını azaltmaktadır (Saklani & Kutty, 2008). Kemoterapi ilaçlarının dozlarını düşürmeye yarayacak bitkisel kaynaklardan elde edilen biyoaktif maddeler ve kemoterapötiklerden oluşan; doksorubisin ve resveratrol (Xu ve ark., 2017), sisplatin ve epigallokateşin gallat (Yuan ve ark., 2017), paklitaksel ve naringin (Jabri ve ark., 2019), doksorubisin ve rein (Wu ve ark., 2019), dosetaksel ve kersetin (Lu ve ark., 2020) gibi kombinasyonlar ve paklitaksel, dosetaksel, vinkristin, vinblastin gibi doğal bileşiklerin başlı başına bir antikanser ilaç olarak varlığını gösterdiği örnekler, araştırmacıları doğal kaynaklardan ilaç adayı moleküllerin keşfine yönlendirmiştir (Saklani & Kutty, 2008).

Yaklaşık 1600 cins ve 22000 tür ile Asteraceae, çiçekli bitki familyalarının en büyüğüdür. Etnobotanik çalışmalar sonucu terapötik etki görebilmek adına birçok türü halk arasında kullanılmaktadır (Lopes ve ark., 2021). Yapılan bir etnobotanik çalışmada, antikanser etkili 41 tür incelenmiş ve en çok üye ile listede bulunan ikinci botanik familyanın Asteraceae olduğu saptanmıştır (Gras ve ark., 2022). Asteraceae familyasının üyesi olan *Centaurea* L. cinsi, birçok türü ile dünyada geniş bir dağılım göstermektedir. *Centaurea*'nın bazı türleri halk arasında soğuk algınlığı, gastrointestinal sistem rahatsızlıkları, jinekolojik problemler ve daha birçok hastalığın tedavisinde kullanılmaktadır (Khammar & Djeddi, 2012; Fattaheian-Dehkordi ve ark., 2021). *Centaurea* türlerinin halk arasında kullanılmasını sağlayan yaygın etkilerinin, bitkinin içeriğindeki seskiterpen laktonlar, lignan bileşikleri ve flavonoidler gibi sekonder metabolitlerden kaynaklandığı düşünülmektedir (Koç ve ark., 2015).

Literatürde farklı *Centaurea* türlerine ilişkin çalışmalar (Kısa ve ark, 2024; Keser ve ark, 2020) bulunmasına rağmen türlerin kanser üzerindeki etkileri konusunda çok az sayıda çalışma bulunmaktadır. Bu çalışma ile Türkiye'ye endemik ve bilindiği kadarıyla henüz sitotoksik etkisi araştırılmamış *Centaurea lycaonica* Boiss. & Heldr. türünün kök kısmından hareketle hazırlanmış diklorometan (CRD) ve metanol (CRM) ekstrelerinin insan serviks kanser hücre hattı olan HeLa'da sitotoksik etkisi incelendi.

MATERYAL ve METOD

Bitkinin Toplanması ve Ekstrelerin Hazırlanışı

Türkiye'nin İç Anadolu Bölgesi'nde endemik olarak yetişen *C. lycaonica* türü, Konya-Seydişehir civarında bitkinin botanik özellikleri ve habitatına ait değerlendirmeler (Davis, 1970) yardımıyla 2022 yılının Temmuz ayında Prof. Dr. Osman TUGAY tarafından tanımlandı. Türe ait kök kısımları toplanarak gölgeli ve havalandırılmış ortamda kurutuldu. Kurutulmuş bitki materyali uygun şekilde öğütüldü ve kaba toz haline getirildi. Bitkisel materyalden 24 sa. maserasyon yöntemiyle önce diklorometan ekstresi (CRD), daha sonra kalan bitki üzerinden metanol ekstresi (CRM) hazırlandı (Artun & Karagöz 2021). Maserasyon yöntemi sayesinde çözücüler, bekletme süresi boyunca bitki materyaliyle temasında sitotoksik etkiden sorumlu olan aktif bileşenleri çözünmüş halde içerir hale getirildi. Ekstreler elde edildikten sonra süzgeç kâğıdı yardımıyla süzüldü. Süzüntü çözücüsü, rotavaporda 38°C'de düşük basınç altında kuruluğa kadar uçuruldu. Elde edilen konsantre, liyofilize edilerek çalışma anına kadar stabilitesi bozulmadan +4°C'de kuru halde saklandı.

Hücre Kültürü Çalışmaları

Çalışmada, HeLa (ATCC® CRM-CCL-2) hücre hattı kullanıldı. Hücreler, hücre kültürü laboratuvarında, 37ºC'de %5 CO_2'li ortamda çoğaltılarak, hücre kültüründe %10 Fetal sığır serumu (FBS), 100 IU ml penisilin/streptomisin çözeltisi içeren Dulbecco'nun modifiye edilmiş eagle besiyeri (DMEM) ile muamele edildi.

Hücre Canlılığının Ölçülmesi

CRD ve CRM ekstrelerinin potansiyel sitotoksisitesini end-point bir testle ve gerçek zamanlı ortaya koymak ve IC_{50} değerlerini belirlemek için hücre canlılığındaki değişim sırasıyla MTT ve xCELLigence gerçek zamanlı hücre analizi gerçekleştirildi.

3-(4,5-Dimetiltiyazol-2-il)-2,5-Difeniltetrazolyum Bromit (MTT) Yöntemi

Potansiyel sitotoksik etkisi araştırılan CRD ve CRM'nin, HeLa hücreleri üzerindeki etkisini göstermek ve IC_{50} değerlerini belirlemek amacıyla MTT testi uygulandı. Testin esası, mitokondrilerde bulunan dehidrogenazların aktivitesi nedeniyle, prolifere olan hücrelerin sarı renkteki MTT tuzunu suda çözünmeyen mavi formazan kristallerine indirgenmesine dayanmaktadır. Meydana gelen renk değişimi absorbans ölçümü ile değerlendirilmektedir. Formazan kristalleri, ekim ortamında çözünemediğinden indirgenme ürünlerini çözmek için dimetil sülfoksit (DMSO) kullanılmaktadır (Mosmann, 1983). HeLa hücrelerinin 1 x 10^4 hücre/plaka ekimi sağlandı ve 24 sa. inkübasyona bırakıldı. Pozitif kontrol olarak doksorubisin (1.00 µM) IC_{50} konsantrasyonunda (Delgado Waldo ve ark., 2023), kontrol grubu, belirlenen CRD konsantrasyonları (10, 30, 100, 180, 200, 300 µg ml^{-1}) ve CRM konsantrasyonları (10, 30, 100, 180, 200, 300 µg ml^{-1}) uygulandı. Hücreler 48 sa. boyunca ekstrelerle muamele edildi. Sürecin sonunda 0.5 mg/ml/kuyu olacak şekilde MTT çözeltisi eklendi ve 2 sa. muamele edildi. Formazan kristallerinin çözülmesi için DMSO eklendi ve çalkalayıcı üzerinde 1-2 dak. bekletildi. Mikroplaka okuyucu ile 570 nm dalga boyunda ölçüm yapıldı. Ekstre uygulanan kuyucuklardan elde edilen absorbans değerleri, kontrolün (%100) absorbans değerine göre oranlandı ve hücre canlılığı yüzde olarak ifade edildi.

Gerçek Zamanlı Hücre Canlılık Deneyi

xCELLigence Gerçek Zamanlı Hücre Analizi (GZHA) SP (Tek Plaka) ACEA Biosciences sistemi, gerçek zamanlı olarak hücre canlılığını boya veya başka bir kimyasal kullanmadan elektrik sinyalleri ile ölçen bir cihazdır. Cihaz; 96 kuyulu e-plaka, %5'lik CO_2 inkübatörü içinde bulunan bir istasyona sahip analizör ve CO_2 inkübatörünün dışında bulunan yazılım kontrol ünitesi bileşenlerinden oluşmaktadır. Sistemden e-plakaya sabit bir şekilde elektrik akımı gönderilir ve kuyucukların tabanında bulunan altın elektronik sensörler yardımıyla adherent hücrelerin elektrik akımına karşı gösterdiği direnç (empedans) analiz edilir. Hücre bağlanması ve yayılması nedeniyle empedansta gözlenen değişiklikler hücre indeksi (CI) olarak ifade edilir. Dolayısı ile empedans ölçümü; hücrelerin sayısı, canlılığı, morfolojisi ve hareketi gibi hücresel parametrelerin değişimi hakkında kalitatif ve kantitatif bilgi sunar (Oberg ve ark., 2020).

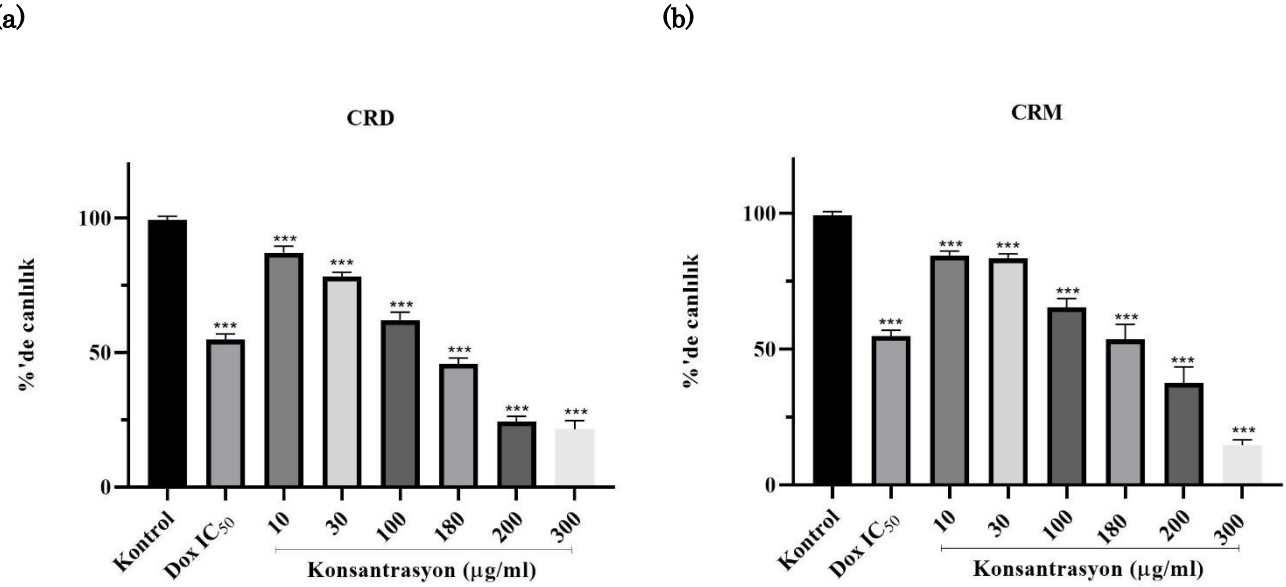
Bu çalışmada serviks kanseri hücre hattında potansiyel sitotoksisiteyi ölçmek ve değerlendirmek için hücre ekiminden 24 sa. sonra e-plaka kuyucuklarına kontrol grubu, IC_{50} konsantrasyonunda doksorubisin (1.00 µM), belirlenen CRD konsantrasyonları (10, 30, 100, 180, 240 µg ml^{-1}) ve belirlenen CRM konsantrasyonları (10, 30, 100, 180, 240 µg ml^{-1}) uygulandı. Zamana bağlı hücre indeksi (CI) profilleri, xCELLigence GZHA sistemi kullanılarak tespit edildi. Elde edilen hücre canlılığı analiz sonuçlarına göre ekstrenin aktivitesi yorumlandı.

İstatistiksel Analizler

Çalışma gruplarından elde edilen sonuçlar, GraphPad Prism 8.3.0 (Graph pad yazılımı, ABD) istatistiksel yazılımı kullanılarak analiz edildi. Sonuçların analizinde, deney ve kontrol grupları arasındaki anlamlılığı saptamada farklılıklara uygun olarak tek yönlü varyans analizi ile istatistiksel farklılığa sahip parametreleri saptamada post-hoc test olarak Dunnet testi kullanıldı. Veriler 3 ayrı deneyden hesaplanan ortalama ± ortalamanın standart hatası olarak verildi. $p<.05$ istatistiksel olarak anlamlı kabul edildi ve anlamlılık aralıkları *<0.05; **<0.01 ve ***<0.001 olarak belirlendi.

BULGULAR ve TARTIŞMA

Dünyada birçok bölgede endemik olarak varlığını sürdüren farklı *Centaurea* türleri bulunmaktadır. Türlerin endemik (Keser ve ark., 2020) oluşu bilimsel anlamda sınırlı sayıda çalışma bulunmasına neden olmuştur. Nitekim Türkiye'ye endemik *C. lycaonica* türüne ait literatürde başka sitotoksisite çalışmasına rastlanmamıştır. Bu çalışmada, *C. lycaonica* türünün kök kısmına ait diklorometan ve metanol ekstrelerinin HeLa hücre hattındaki sitotoksik etkisi araştırıldı. Söz konusu ekstrelerin farklı konsantrasyonlarının sitotoksik etki düzeyi MTT (Şekil 1) ve xCELLigence GZHA sistemi (Şekil 2 ve Şekil 3) kullanılarak verildi. MTT yönteminde ekstreler 10, 30, 100, 180, 200, 300 µg ml^{-1} konsantrasyonlarda 48 sa. süreyle uygulandı. CRD ve CRM'nin hücre canlılığını 100 µg ml^{-1} ($P<0.001$); 180 µg ml^{-1} ($P<0.001$); 200 µg ml^{-1} ($P<0.001$); 300 µg ml^{-1} ($P<0.001$) konsantrasyonlarda %70'in altına düşürdüğü görüldü. CRD ve CRM'nin 180, 200, 300 µg ml^{-1} konsantrasyonlarında, pozitif kontrol olan doksorubisinin IC_{50} değerinden daha fazla hücre canlılığını azalttığı gözlemlendi. HeLa hücresinde CRD'nin 48 sa. için IC_{50} değeri 111.5 µg ml^{-1}; CRM'nin 48 sa. için IC_{50} değeri 143.30 µg ml^{-1} olarak hesaplandı.



Şekil 1. HeLa hücrelerinin 48 saatlik CRD (a) ve CRM (b) maruziyeti sonrası MTT deney sonuçları
Figure 1. HeLa cells' MTT assay results after 48-hour exposure to CRD (a) and CRM (b)
Pozitif kontrol: Doksorubisin IC_{50}: 1.00 µM uygulandı.
Gruplar kontrole göre yüzde değişimi olarak verildi. Değerler GraphPad Prism 8.3.0 programında One-way ANOVA ve post-hoc Dunnett testi ile analiz edildi. p değeri <.05. Anlamlılık aralığı; ***<.001. Sonuçlar, ortalama ± ortalamanın standart hatası olarak sunuldu.
Positive control: Doxorubicin IC_{50}: 1.00 µM was applied.
*Groups were given a percentage change compared to the control. Values were analyzed by One-way ANOVA and post-hoc Dunnett test in GraphPad Prism 8.3.0 software. p value <.05. Significance range; ***<.001. Results are presented as mean ± standard error of the mean.*

xCELLigence GZHA sisteminde ekstreler 10, 30, 100, 180, 240 µg ml^{-1} konsantrasyonlarda 48 sa. süreyle uygulandı ve hücre profili izlendi. HeLa hücresinde CRD'nin 48 sa. için IC_{50} değeri 162.47 µg ml^{-1}; CRM'nin 48 sa. için IC_{50} değeri 177.01 µg ml^{-1} olarak hesaplandı. Hücre profillenmesi incelendiğinde ise bitki ekstrelerinin artan konsantrasyonlarda CI değerini azalttığı, xCELLigence sistemine ait deney sonuçlarının ve IC_{50} değerlerinin, MTT deney sonuçları ve IC_{50} değerleri ile tutarlılık gösterdiği görüldü. Cinse ait literatür incelemesi yapıldığında, CRD ve CRM'nin tespit edilen sitotoksik etkilerinin bitkideki terpenler (Huang ve ark., 2018), fenolik bileşikler ve flavonoid içeriğinden kaynaklandığı düşünülmektedir (Ayad & Akkal, 2019; Fattaheian-Dehkordi ve ark., 2021). CRD ve CRM'nin HeLa hücrelerinde sitotoksisitesinin gösterilmesi bu çalışmayla bir ilk teşkil etmektedir. Bu nedenle, tespit edilen IC_{50} değerleri ile doğrudan bir literatür karşılaştırılması yapılamadı. *C. calcitrapa* subsp. *calcitrapa* türünün yaprak kısmına ait metanol ekstresinin HeLa hücre hattındaki sitotoksik etkisinin incelendiği bir çalışmada 48 sa.'lik maruziyette IC_{50} değerinin 92.5 µg ml^{-1} olduğu tespit edilmiştir (Erol-Dayı ve ark., 2011). Artun ve Karagöz (2021) ise *C. hermannii*'nin yapraklarından hazırlanan %95'lik metanol ekstresinin HeLa hücre hattındaki potansiyel sitotoksik etkisini, MTT ve xCELLigence GZHA sistemi kullanılarak incelemiş ve metotlara göre sırasıyla 48 sa. için IC_{50} değerlerini 15.74 µg ml^{-1}; 18.3 µg ml^{-1} olarak bulmuşlardır. Sitotoksisitenin hangi mekanizmayla ilişkili olduğu araştırılmış ve Kaspaz 3, 7 ve 9 aktivitesindeki artışla ilişkili olabileceği sonucuna varılmıştır. *Centaurea* cinsi ile aynı kanser hücre hattında yapılan bu çalışmalar, çalışmada kullanılan aynı metotların uygulanması ancak 48 sa. için farklı IC_{50} değerlerinin elde edilmesi; kullanılan bitki türü ve kısımlarındaki farklılığa bağlı olduğu sonucuna varıldı. Bu çalışmadaki CRD ve CRM'ye ait IC_{50} değerlerindeki farklılığın nedeni ise, bitki materyalinin farklı çözücü ile muamele edilmesidir. Analiz sonuçları değerlendirildiğinde daha sitotoksik olan çözücünün diklorometan olduğu görülmektedir. Literatüre bakıldığında, bu etkinin sebebinin diklorometan ekstresindeki zengin seskiterpen lakton içeriği olduğu düşünülmektedir (Huang ve ark., 2018). IC_{50} değerlerindeki farklılığın bir diğer nedeni ise hücre canlılığının ölçüldüğü metotların farklı prensiplere dayanmasından ileri gelmektedir. MTT testi bir end-point test olarak hücre canlılığını ölçmeye yarayan hücreleri boya ve organik solventlerle muamele ederek spektroskopik veriler elde eden, eski ve güvenilir bir yöntemdir (Mosmann, 1983). xCELLigence GZHA sistemi ise mikroelektronik biyosensörler vasıtasıyla hücrelerin herhangi bir ajana maruz kaldığı andan tedavi sonlanana kadar olan tüm sürecin takibinin elektrokimyasal yolla, gerçek zamanlı olarak, bir boyanın kullanılmadığı, yeni ve daha çok verinin elde edildiği

bir yöntemdir (Oberg ve ark., 2020). Hücre ölümünün başlangıç zamanının ve hücre morfolojisindeki erken değişikliklerin daha net tespit edilebilir olmasının yanında ölçümün hücrelere zarar vermeden yapılabilir olması, sistemin MTT testine olan üstünlüğüdür (Atmaca ve ark., 2016). xCELLigence GZHA sisteminde CI, altın kaplama e plaka üzerindeki hücrelerin büyümesi, büzülmesi, ölümü, yapışması ve morfolojik değişikliklerine göre ölçülen elektriksel empedans değerini belirlediği için elektrot temas yüzeyindeki hücre yüzeyinin azalmasının nedenleri olan hücre sayısının azalması, hücrelerin büzülmesi ve yapışma kapasitelerinin azalması veya kaybolması hücresel indeksin azalmasına neden olmaktadır. xCELLigence ve MTT sonuçlarına göre, CRD ve CRM'nin hücre ölümünden önce hücrelerin küçülmesine veya yapışma yeteneğini kaybetmesine neden olabileceği düşünülmektedir.

SONUÇ ve ÖNERİLER

C. lycaonica türünün sitotoksik etkisini değerlendirmek üzere bitkinin kök kısmından hareketle diklorometan ve metanol ekstrelerinin farklı konsantrasyonları hazırlanıp HeLa hücrelerine 48 sa.'lik tedavi uygulandı. Sitotoksisite kantitatif olarak MTT ve xCELLigence GZHA yöntemleri ile tahlil edildi. Sonuç olarak metotlara göre sırasıyla, CRD'nin IC_{50} değerleri 111.50; 162.47 µg ml^{-1}, CRM'nin IC_{50} değerleri 143.30; 177.01 µg ml^{-1} bulundu. *C. lycaonica* için bu değerlendirme bilindiği kadarıyla literatürde ilk defa yapılmaktadır. Çalışmanın konusu olan türün, aktif bileşenlerine ait sitotoksik etki mekanizmasının belirlenmesi için daha fazla araştırmaya ihtiyaç vardır. Ayrıca bu çalışma ile kanser tedavisinde büyük bir öneme sahip olan doğal kaynaklardan elde edilen ilaç çalışmalarının ve endemik bitkilerin değerlendirilmesinin önemi vurgulandı. Çalışmanın, *Centaurea* türlerinin araştırılması, sitotoksik etki düzeylerinin belirlenmesi ve etki mekanizmalarının aydınlatılması gibi ileri düzeydeki çalışmaları teşvik edeceği düşünülmektedir.

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Antigenotoxic Effects of *Stachys viscosa* (Lamiaceae), a potential medicinal plant in *Drosophila melanogaster*

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ABSTRACT

This research aimed to examine the anti-genotoxic properties of *Stachys viscosa*, a plant of the Lamiaceae family, which has medicinal and aromatic properties and is widely used in cosmetics, food, and pharmaceutical industries. The *Drosophila* wing somatic mutation and recombination test (SMART), an *in vivo* tool for evaluating somatic mutation and recombination, was employed. The anti-genotoxic effect of the *S. viscosa* plant against mitomycin C (MMC) mutagen was investigated. The study evaluated doses of 5 mg, 15 mg, 45 mg, and 60 mg of *S. viscosa*, concluding that doses of 45 mg and 60 mg effectively mitigated DNA damage induced by MMC. At doses of 45 mg and 65 mg, the total number of spots caused by MMC was suppressed by 8.21% and 13.17%, respectively. The data collected indicate that the *S. viscosa* plant possesses certain anti-genotoxic properties.

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Potansiyel tıbbi bir bitki olan *Stachys viscosa*'nın (Lamiaceae) *Drosophila melanogaster*'da Antigenotoksik Etkileri

ÖZET

Bu araştırmada tıbbi ve aromatik özelliklere sahip olan, kozmetik, gıda ve ilaç endüstrilerinde yaygın olarak kullanılan Lamiaceae familyasına ait bir bitki olan *Stachys viscosa*'nın anti-genotoksik özelliklerinin incelenmesi amaçlanmıştır. Somatik mutasyon ve rekombinasyonu değerlendirmek için *in vivo* bir test olan *Drosophila* kanat somatik mutasyon ve rekombinasyon testi (SMART) kullanıldı. Yapılan çalışmada, *S. viscosa* bitkisinin Mitomisin C (MMC) mutajenine karşı anti-genotoksik etkisi araştırılmıştır. Çalışmada *S. viscosa*'nın 5 mg, 15 mg, 45 mg ve 60 mg'lık dozları değerlendirildi ve 45 mg ve 60 mg'lık dozların MMC'nin neden olduğu DNA hasarını etkili bir şekilde azalttığı saptandı. Araştırma neticesinde 45 mg ve 65 mg'lık dozlarda, MMC'nin neden olduğu toplam benek sayısının sırasıyla %8,21 ve %13,17 oranında baskılandığı tespit edilmiştir. Elde edilen veriler, *S. viscosa* bitkisinin belirli anti-genotoksik özelliklere sahip olduğunu göstermektedir.

Genetik

Araştırma Makalesi

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Stachys viscosa
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INTRODUCTION

Mutations are defined as spontaneous or induced alterations in genetic material, whereas mutagens are the physical or chemical agents that trigger these mutations. These mutagens are vital because they trigger processes that lead to somatic cell damage, such as the transmission of genetic damage to subsequent generations and cancer development.

The genotoxic effect, defined as damage to DNA, is the main subject of genetic toxicology as it is a cancer-initiating

process and the emergence of diseases associated with DNA damage (Sumer et al., 2009).

Many medicinal plants used for traditional purposes are essential in discovering new drugs. Many compounds are obtained from different parts of these plants. These include pectin, starch, flavonoids, coumarin, tannin, asparagine, and many amino acids (Şeker, 2019). Interest in these plants' therapeutic and anti-genotoxic effects, which have many metabolites with proven efficacy is increasing (Shabab et al., 2021). Therefore, the genotoxic effect caused by various mutagens may be reduced due to the anti-genotoxic effect of medicinal plants. Many plants are significant regarding their anti-genotoxic effect (Önen et al., 2017).

The Lamiaceae family attracts much attention due to its medicinal and aromatic character. They are widely used in the cosmetic, food, and pharmaceutical sectors, mainly thanks to the essential oils contained in the plant content. The biological and pharmacological activities of plants in the Lamiaceae family, which is a family wealthy in essential oil components, are being tried to be determined, and the essential oils in their content constitute an area of use in phytotherapy (Nilofar et al., 2024).

Turkey is acknowledged as a crucial center of biodiversity for the Lamiaceae family. The country hosts 603 species and 782 taxa (346 endemic) within this family. Extracts and essential oils from specific species of the Lamiaceae family have been utilized in traditional medicine to address various ailments, provide nourishment, and function as food preservatives for millennia. Species of *Stachys* L. are among those employed for such uses. *Stachys*, a notable genus in the Lamiaceae (Labiatae) family, exhibits significant taxonomic diversity and complexity. As a sub-cosmopolitan genus, *Stachys* demonstrates extensive distribution across the Mediterranean and Southwest Asia, subsequently extending to North and South America and North Africa. Nonetheless, it is absent in New Zealand and Australia. The genus has around 435 taxa and 370 species worldwide. The genus *Stachys* in Turkey includes 118 taxa and 91 species, of which 57 taxa are endemic, yielding an endemism rate of 48%. In Mediterranean locations, these plants have been employed in alternative medicine as herbal treatments and are ingested as wild tea, generally referred to as mountain tea. A multitude of chemical investigations have been performed on the volatile components of *Stachys* taxa. The volatile oil composition of these species is an important determinant in their use as tea in Anatolian ethnobotany. These species also comprise polyphenols, saponins, phenolic acids, glycosides, tannins, diterpenoids, flavonoids, essential oils, monoterpenes, and sesquiterpenes. The synergistic effects of the constituent compounds are believed to be the principal cause for the use of *Stachys* flowers and aerial parts as tea for therapeutic purposes in Anatolian culture. Some representatives of the genus have been recorded for their application as antibacterial and anti-inflammatory agents. Furthermore, studies suggest their capacity to offer antianxiety, antioxidant, and antinephritic effects (Satıl & Açar, 2020).

The ethnobotanical applications of 38 taxa of *Stachys*, including 29 species, have been recorded in Turkey, with *S. lavandulifolia* Vahl and *S. cretica* L. being the most commonly utilized species. In Turkey, *Stachys* species are frequently utilized as medical herbal infusions. Additionally, they are utilized as a powder for treating animal diseases, as a gargle for sore throat, and in the form of handkerchiefs and hair accessories (derived from leaves) for children. Research has identified approximately 40 different diseases and symptoms for which *Stachys* taxa are employed in treatment, with notable applications including addressing stomach issues, colds, coughs, and diabetes (Satıl & Açar, 2020).

Stachys species are widely employed in Anatolia and Iran for herbal therapy, frequently ingested as tea. *Stachys* taxa, commonly known as Mountain tea (above-ground parts in the form of infusion and decoction), are considered to have antibacterial and antifungal properties, as well as being used as a tonic for gastrointestinal disorders. The literature indicates their role in pain and inflammation inhibition, exhibiting anxiolytic properties, and serving as antibacterial, antinephritic, anticancer, anti-Helicobacter pylori, and antioxidant agents. Examples are *S. recta* L., utilized for wound healing, and *S. lavandulifolia*, applied for digestive issues. *S. officinalis* (L.) Trevis., *S. sylvatica* L., *S. recta,* and *S. palustris* L. are utilized in Hungarian folk medicine for their anti-inflammatory, anti-rheumatic, and antibacterial attributes. These plants are utilized as antiphlogistics, spasmolytics, diuretics, sedatives, and for the treatment of neoplastic illnesses. *Stachys* taxa are abundant in secondary metabolites, particularly diterpenoids, which demonstrate antibacterial, antifungal, antimycobacterial, and anti-Alzheimer properties (Satıl & Açar, 2020).

Many species in the genus *Stachys* are well known for treating a wide range of illnesses in traditional medicine across various cultures. In Italy, for example, *Stachys recta*, also known as "yellow woundwort," is called "erba della paura," a reference to its use in herbal teas for anxiety treatment. *S. lavandulifolia*, also called "Chaaye Koohi," and other *Stachys* species are widely used in Iran to treat gastrointestinal disorders, skin irritation, and stress. Although this common term may cause confusion with other herbal medicines from *Sideritis* species, herbal teas derived from *Stachys* species, commonly referred to as "mountain tea," are also widely used for treating skin and stomach disorders.

While *Stachys geobombycis* C.Y.Wu, also known as Dong Chong Xia Cao, is used as a tonic in Asia and Europe,

Stachys affinis Fresen. is utilized in Chinese traditional medicine for a variety of ailments, such as colds, heart disease, antioxidant qualities, and brain abnormalities. Species such as *S. acerosa* Boiss., *S. fruticulose* M.Bieb., *S. byzantine* K.Koch , and *S. lavandulifolia* have a variety of purposes in Iranian traditional medicine, including the usage of *S. sylvatica* to treat polycystic ovarian syndrome (PCOS). Furthermore, species like *S. cretica*, *S. lavandulifolia*, and *S. sylvatica* are used in Turkish traditional medicine to treat stomach and respiratory conditions; some species are also used to treat cardiac problems.

Infusions and decoctions of *Stachys* species are used in Greek and Italian traditional medicine to treat gastrointestinal issues, headaches, rheumatic pain, and neuralgia. Some species are even used in food; for instance, *S. palustris* is used in European cuisine as "mayday flour" in bread additions, and *S. affinis* tubers are consumed in China and Japan as "Chinese artichokes." There have also been documented veterinary use for *S. germanica* and *S. officinalis* (Tomou et al., 2020)

S. viscosa Montbret & Aucher ex Benth the native range of this species is N. & E. Türkiye to W. Transcaucasus. It is a subshrub and grows primarily in the temperate biome. It is utilized by the public as a nectar source for bees, an infusion for tea, forage, and for alleviating colds and intestinal issues (Satıl & Açar,2020).

Anti-genotoxic effects of the *Stachys* genus and Lamiaceae family members have been revealed in some studies (Rencuzogullari et al., 2012; Sevindik & Rencuzogullari, 2013; Kilic et al., 2018; Rasgele et al., 2021). In addition, the main subject of the study was a plant formerly called *S. laetivirens*, now classified as a synonym of *S. viscosa*. In a previous study, Sixty-one compounds were identified in the essential oil extracted from the aerial components of *S. laetivirens*, and the oil composition was found to contain 2.0% oxygenated monoterpenes, 12.9% sesquiterpene hydrocarbons, 18.6% diterpenes, 8.2% oxygenated sesquiterpenes, and 43.7% other components. The highest components were found to be nonacosan (23.1%) and phytol (17.9%). While sesquiterpenes are generally found in significant amounts in *Stachys* species, some may accumulate monoterpenes. However, the composition of *S. laetivirens* contains abundant straight-chain alkanes such as nonacosan and phytol (Duman et al., 2005).

Since the existence of mankind, the first area he turned to in order to solve his problems was nature itself. Ethnobotany, defined as the relationship between nature and plants, the most important part of nature, is the basic asset used today to solve problems, especially various diseases. Recently, diseases and problems such as genetic disorders and cancer have been occupying our day. The examination of these problems in various ways continues to increase. Mutagens that affect genetic structure are among the subjects of this research. It is one of the basic research materials in the field of genetics, as in many studies on medicinal plants.

Notwithstanding its therapeutic efficacy, *S. viscosa* has yet to be evaluated for its antimutagenic characteristics. *Drosophila melanogaster* (vinegar fly), a eukaryotic organism used in this study, is used in the determination of mutagenic, genotoxic, carcinogenic, and anti-genotoxic effects of many substances due to its easy and economical production and its genetically diverse lineages (Öz et al., 2023). Among the tests that use *Drosophila* as a model organism, one that has become quite popular in recent years is the Somatic Mutation and Recombination Test (SMART), which allows the recognition of a wide range of genetic issues such as deletions and point mutations, as well as the exact types of chromosomal errors such as mitotic recombination and gene conversion (Çetinkaya & Yurtsever, 2021; Çelik & Uysal, 2023). SMART is a superior method in that it provides more accurate results in genetically measuring the mutation caused by various substances in somatic cells. Compared to other tests, SMART has the advantage of being faster, more reliable, and more economical (Sarıkaya & Çakır, 2005; Yalçın et al., 2024).

The similar genetic characteristics of humans and *Drosophila* make *Drosophila* especially advantageous for such studies. Many substances that are carcinogenic to humans also give positive results in *Drosophila* tests. In addition, there is no need for metabolic activation to test for promutagens and procarcinogens (Rincon & Graf, 1995).

This research sought to examine the anti-genotoxic properties of *S. viscosa*, which has potential medicinal value, against the mitomycin-C (MMC)-induced genotoxic effect of *Drosophila* SMART.

MATERIAL and METHOD

Plant material

The aerial components of *S. viscosa* were collected from its habitat during the flowering season. The plant was desiccated in the shade for extraction research and stored in the Herbarium of Munzur University for identification and preservation purposes. MA 2045: *S. viscosa*, roadside limestone rocks around 5th km between Tunceli Merkez and Ovacık, May 2020. B7, Tunceli Merkez (Türkiye).

Plant extraction

The above-ground parts of *S. viscosa* were dried in the shade and ground in a mortar and pestle. Weighed 4 g of the samples obtained, and 40 mL of methanol (1/10 w/v) was added. They were kept in an incubator (Elekto-mag M 5040 P, Turkey) at 40 ºC for 24 hours. The resulting solutions were centrifuged (Universal320 R) at 9,000 rpm. The solvent was removed with the help of an evaporator. After determining the amounts of the extracts obtained, methanol extracts were obtained. The extracts were stored at +4 ºC until analyzed (Soldamlı, 2016).

Figure 1 *S. viscosa* general appearance in nature
Şekil 1. S. viscosa doğadaki genel görünümü

Drosophila Strains

Two genetically different strains of *Drosophil*a were used in the study. The *mwh/mwh* and *flare-3 strains* of *D. melanogaster* were utilized in the research (Rincon & Graf, 1995).

Anti-genotoxic Application

MMC (CAS No. 50-07-7), which has known genotoxic effects, was used as a positive control group in the study. In addition, the study utilized dosages of 5 mg, 15 mg, 45 mg, and 60 mg to assess the genotoxic and anti-genotoxic effects of *S. viscosa*.

To assess the anti-genotoxic effects of these dosages against MMC, a concentration of 0.05 mM MMC was employed. For this purpose, 5 mg, 15 mg, 45 mg, and 60 mg of powdered *S. viscosa* pieces were added to 0.5 g of prepared medium (Formula 4-24; Carolina Biological Supply) in sterile test tubes.

Implementation Procedure

The diagnostic technique was executed in accordance with a precise protocol, albeit with certain alterations (Graf et al., 1984). 72±4 h larvae developing from eggs obtained after 8 h of crossing of *mwh* males and *flr*^{3} virgin females were collected using 17% sodium chloride solution. These larvae were counted and placed in tubes containing group-specific medium. In this way, distilled water was the negative control, MMC was the positive control, and *S. viscosa* and MMC + *S. viscosa* groups were also formed.

Preparation and Microscopic Analysis of Wing Preparations

For microscopic analysis, only trans-heterozygous (*mwh flr+/mwh+flr*^{3}) individuals were used from larvae to adults in test tubes. The wings of adult flies were clipped and placed on slides on which Faure's solution (30 g of gum arabic, 20 ml of glycerol, 50 g of chloralhydrate, and 50 ml of distilled water) was added. The surfaces of the positioned wings were analyzed using a light microscope at 400X magnification. The observed spot types were documented as single spots and double spots (Rincon & Graf, 1995).

Statistical Analysis

The spots observed by microscopic scanning were recorded as large single spots, small single spots, and double spots. The single-type clones observed in the SMART test are formed by point mutation, deletion, translocation, chromosome loss or chromosome non-separation, and mitotic recombination in the DNA, while twin clones are formed only by mitotic recombination. For statistical analysis, the number of spots on each wing was compared with those in the control groups. The MICROSTA package program was used to evaluate the results, and a one-way Kastenbaum-Bowman test was performed. The percentage inhibition of wing spot frequency of *S. viscosa* by MMC was calculated using the following formula (Idaomar et al., 2002).

$$\frac{100(a-b)}{a}$$

According to the formula above, a- shows the spot frequency caused by MMC, and b- shows the spot frequency caused by MMC as a result of *S. viscosa* application.

RESULTS and DISCUSSION

In the study, 5 mg, 15 mg, 45 mg, and 60 mg doses of *S. viscosa* were used. Doses of 0.025mM, 0.05mM, and 0.1mM of MMC, which has antibacterial and antitumor fungal effects and is classified as an alkylating agent, were used as the positive control group. The MMC was directly dissolved in distilled water and used in the study. As a result of the statistical evaluations, while the genotoxic effect of MMC in *Drosophila* was detected, no significant difference was observed in all tested doses of *S. viscosa* compared to the control group (distilled water) for all spots. In the literature, many studies show that MMC, an antibiotic obtained from *Streptomyces caespitosus*, causes genotoxic effects in *Drosophila* (Niikawa & Nagase, 2007; Karabulut & Yesilada, 2014; El-Hefny et al., 2020). In the study, survival percentages were also calculated for each treatment group. All findings and statistical evaluations are presented in Table 1 and Table 2.

Table 1. SMART findings and statistical analysis results of MMC application groups
Çizelge 1. MMC uygulama gruplarına ait SMART bulguları ve istatistiki analiz sonuçları

Test Groups	Survival rate (%)	Number of Wings Inspected	Statistical analysis of the number of spots per wing *			
			Small single spot (1-2 cells) (m=2)	Large single spot (>2 cells) (m=5)	Twin spot (m=5)	Total spot Number of (m=2)
Distilled Water	98	110	0.14 (16)	0.02 (2)	0	0.16 (18)
MMC (mM)						
0.025	60	75	3.24 (243) +	3.39 (254) +	2.10 (158)+	8.73 (655)+
0.05	54	66	6.23 (411) +	7.17 (473) +	2.92 (193)+	16.32(1077)+
0.1	43	64	8.81 (564) +	9.05 (579) +	3.22 (206)+	21.08(1349)+

* One-way Kastenbaum-Bowman Test, +: positive (genotoxic), -: negative (not genotoxic), i: insufficient, m: multivariate factor, α=β=0.05, Distilled water are negative control, MMC is a positive control.

Table 2. SMART findings and statistical analysis results of S. viscosa application groups
Çizelge 2. S.viscosa uygulama gruplarına ait SMART bulguları ve istatistiki analiz sonuçları

Test Groups	Survival rate (%)	Number of Wings Inspected	Statistical analysis of the number of spots per wing *			
			Small single spot (1-2 cells) (m=2)	Large single spot (>2 cells) (m=5)	Twin spot (m=5)	Total spot Number of (m=2)
Distilled Water	98	110	0.14 (16)	0.02 (2)	0.009(1)	0.17 (19)
***S. viscosa* (mg)**						
5	97	104	0.13 (14) i	0.02 (2) i	0.009(1) i	0.16(17) i
15	93	97	0.12 (12) i	0.01 (1) i	0.00 (0) i	0.13(13) i
45	85	90	0.11(10) i	0.02 (2) i	0.01(1) i	0.14(13) i
60	70	84	0.12 (10) i	0.01 (1) i	0.01 (1) i	0.14 (12) i

* One-way Kastenbaum-Bowman Test, +: positive (genotoxic), -: negative (not genotoxic), i: insufficient, m: multivariate factor, α=β=0.05, Distilled water are negative control.

In order to determine the anti-genotoxic effect of *S. viscosa* against MMC, 5 mg, 15 mg, 45 mg, and 60 mg doses of *S. viscosa* were added to the prepared medium containing 0.05 mM MMC. In all groups, wings were scanned for the presence of spots. When spot numbers were examined, it was observed that all spot numbers decreased in the 45 mg and 60 mg MMC+ S. viscosa groups compared to the MMC groups. Except for the double spots observed in the 45 mg treatment group, these decreases had a statistically negative effect. In addition, the percentage of inhibition of the number of spots caused by MMC in *S. viscosa* was calculated by the simultaneous application of 45 mg and 65 mg doses of *S. viscosa* and 0.05 mM MMC. The administration of 45 mg and 60 mg doses of *S. viscosa* resulted in a reduction of 8.21% and 13.17%, respectively, in the total number of spots generated by MMC. The outcomes derived from the wing spot test are presented in Table 3.

Table 3. SMART findings and statistical analysis results of MMC+ S. viscosa application groups
Çizelge 3. MMC+ S. viscosa uygulama gruplarına ait SMART bulguları ve istatistiki analiz sonuçları

Test Groups	Survival rate (%)	Number of Wings Inspected	Statistical analysis of the number of spots per wing *				
			Small single spot (1-2 cells) (m=2)	Large single spot (>2 cells) (m=5)	Twin spot (m=5)	Total spot Number of (m=2)	Inh. %
MMC (0,05mM)	54	66	6.23 (411)	7.17 (473)	2.92 (193)	16.32(1077)	
MMC+*S. viscosa* (mg)							
5	55	64	6.33(405) i	7.29(466) i	3.11(199) i	16.72(1070) i	
15	54	61	6.34(387) i	7.51(458) i	3.11(190) i	16.97(1035) i	
45	55	64	5.51(353) -	6.80(435) -	2.70(171) i	14.98(959) -	8.21
60	57	66	5.12 (338) -	6.53(431) -	2.51(166) -	14.17 (935) -	13.17

* One-way Kastenbaum-Bowman Test, +: positive (genotoxic), -: negative (not genotoxic), i: insufficient, m: multivariate factor, α=β=0.05, MMC (0.05mM) is a positive control.

It is known that many plants that we frequently use in our daily lives are used in the treatment of many types of cancer and other somatic mutation-related diseases and show anti-genotoxic activity thanks to their components. The mutagenic effect due to the effect of various mutagens is reduced due to the anti-genotoxic effect of the components of these plants (Boldbaatar et al., 2014; Zor and Aslan 2020). Secondary metabolites found in the structure of organs such as leaves stems, and roots of plants are widely used in the protection and maintenance of the health of living things and the treatment of diseases due to their various biological activities. Many studies have shown that plant secondary metabolite products have protective effects in preventing genetic damage caused by various damaging factors, especially free radicals. Phytochemicals, natural substances contained in plants, are molecules used to produce plant-based drugs in drug development. Phytochemicals are found in medicinal and aromatic plants, vegetables and fruits, roots, leaves, and flowers. Phytochemicals, which are not the main food source consumed by humans, are natural compounds that give plants taste, color, and aroma (Vasanthi et al., 2012). This study examined the genotoxic and anti-genotoxic effects of *S. viscosa*, a potentially significant medicinal plant from the Lamiaceae family utilized in many cosmetic, culinary, and pharmaceutical applications, on *D. melanogaster* by the *Drosophila* wing spot test. Numerous studies investigate the antimutagenic properties of several plant extracts, utilizing *Drosophila* as a model organism (Radak & Andjelkovic, 2016). This work is the initial investigation of the anti-genotoxic impact of *S. viscosa* on *Drosophila* utilizing the wing spot test.

It has been shown in different test systems that the negative effects of MMC and promutagens that can be genotoxic as a result of metabolic activation can be prevented with various synthetic or natural molecules. These effects are considered to be antigenotoxic by preventing them from binding to DNA by inducing repair mechanisms, binding to genotoxins, or blocking their metabolism by inhibiting enzyme activities, and molecules that exhibit these activities are considered to be antigenotoxic agents or antimutagens (Laohavechvanich et al., 2006). In this context, we can say that *Stachys* species, which contain various biopolymers that are medically important, prevent breaks in DNA strands that occur due to the effects of various mutagens such as MMC and can also prevent point mutations that develop in parallel. In a study conducted with cell culture, it was shown that the cytotoxic effect of the essential oil contained in a species belonging to the Lamiaceae family on colorectal cancer cell lines was higher than its effect on healthy cell lines. In addition, as a result of apoptosis analysis, it was shown that the essential

oil showed a high apoptosis effect and induced apoptosis. As a result, it was reported that this species has the potential to be a therapeutic agent against colorectal cancer (Yadollahi, 2024). The anti-cancer properties of essential oils obtained from some Lamiaceae species against human cancer cells have been investigated. Studies have reported that the tested members of this species have anticancer, antioxidant, and antimutagenic properties (Kolumbayeva et al., 2022; Gezici et al., 2024). Additionally, in a study investigating the antigenotoxic effect of *Salvia verticillata* L. belonging to the Lamiaceae family using the Cytokinesis Block MicroNucleus (CBMN) assay, it was reported that *Salvia* showed a statistically significant antigenotoxic effect against MMC, a mutagenic agent. It can be said that the phenolic acids, flavonoids, and terpenes contained in the plant contribute to this effect (Stavropoulou et al., 2024).

CONCLUSIONS

This study determined that *S. viscosa* did not cause any genotoxic effect in *D. melanogaster*. In addition, statistical evaluations determined that 45 mg and 60 mg of *S. viscosa* showed anti-genotoxic effects by suppressing DNA damage induced by MMC in *Drosophila*. Accordingly, it can be said that the plant contains some anti-genotoxic factor(s). Members of the Lamiaceae family are extensively utilized for medicinal and aromatic purposes. The family showing aromatic properties has rich secondary metabolites. It also contains many species with antibacterial, anti-fungal, and antioxidant properties. In addition, studies on genotoxic effects are at the beginning level. Many different model organisms are used in genotoxicity and antigenotoxicity studies, especially in the field of Mendelian genetics. Among these organisms, *Drosophila* is the most widely used. With this *in vivo* study with *Drosophila*, one of the most commonly used model organisms in experimental studies due to its genetic characteristics, the anti-genotoxic activity of *S. viscosa* was investigated for the first time on *D. melanogaster*. In this context, we think the study should be evaluated in terms of public health. In order to better elucidate the subject of the study, support our findings, and clarify the mechanism of anti-genotoxicity, further studies such as different extractions and evaluation of different parts of the plant may be recommended. As a result, the pioneering of effective research on the evaluation and characterization of components obtained from the Lamiaceae family and having antigenotoxic effects makes it possible to apply these components to human health. Additionally, it may be recommended to conduct new studies with different *in vivo* and *in vitro* test systems that work with different mechanisms and different model organisms to elucidate the mechanism of the antigenotoxic effect of *S. viscosa* on *Drosophila*.

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors have declared no conflict of interest.

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Confirmation of the Natural Distribution of *Euphorbia condylocarpa* M.Bieb. (Euphorbiaceae: Sect. *Helioscopia*, Subsect.*Galarhoei*) in Türkiye

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ABSTRACT
This study; it is about the verification of the distribution of *Euphorbia condylocarpa* Boiss. & Heldr. (Euphorbiaceae), for which there is no reliable information about its existence in Türkiye. Because, the most important website regarding the distribution of plants distributed in Türkiye(Bizimbitkiler website) and the most up-to-date and comprehensive study called "Bizim Bitkiler Listesi (Damarlı Bitkiler)" do not accept the existence of this species in Türkiye. It is stated that the existence of this species in Türkiye needs to be confirmed. The closest species to the *E. condylocarpa*, which we focused on in order to eliminate doubts about its distribution in Turkey, is in tuberous form *Euphorbia apios* L.. It is distinguished from *E. apios* by the fact that the cauline leaves of *E. condylocarpa are* cordate–auriculate at the base and the number of axillary rays is more than 6 (to 30). In addition, the description of *E. condylocarpa*, the ecological information in the area where it develops, and some features of the species that differ from the known description are also emphasized.

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Türkiye'den *Euphorbia condylocarpa* M.Bieb. (Euphorbiaceae: Sect. *Helioscopia*, Subsect. *Galarhoei*) 'nın Tabii Yayılışının Teyidi

ÖZET
Bu çalışma; Türkiye'de varlığına dair güvenilir bilgi bulunmayan *Euphorbia condylocarpa* Boiss. & Heldr. (Euphorbiaceae)'nın Türkiye'de yayılışının doğrulanmasıyla ilgilidir. Zira, Türkiye'de yayılışı olan bitkilerin dağılışı ile ilgili en önemli web sitesi (Bizimbitkiler veb sitesi) ve en güncel ve kapsamlı çalışma olan "Türkiye bitkileri listesi (Damarlı bitkiler)" adlı çalışma bu türün Türkiye'de varlığını kabul etmemekte ve bu türün varlığının teyidinin gerektiğini belirtmektedirler. Türkiye'de yayılışı hakkındaki şüpheleri gidermek için üzerinde durduğumuz *E. condylocarpa*'ya en yakın tür tuberli formdaki *Euphorbia apios* L.'dir. *E. condylocarpa*'nın gövde yapraklarının tabanda kordat–aurikülat olmaları ve aksillar ray sayısının da 6'dan fazla (30'a kadar) olması ile *E.apios*'tan ayırt edilmektedir Ayrıca, *E. condylocarpa*'nın tanımı, geliştiği alandaki ekoloji bilgileri ve türün bilinen tanımından farklılık gösteren bazı özellikleri üzerinde de durulmuştur.

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 16.08.2024
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Anahtar Kelimeler
Euphorbia condylocarpa,
gijeletri Euphorbiaceae
Yayılış
Baskil
Elazığ

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INTRODUCTION
The Euphorbiaceae has around 8000 members of 340 genera worldwide (Radcliffe-Smith, 2001; Wurdack et al. 2004; Yang et al., 2012; Islam et al., 2019). Some of the members of this family (such as *Acalypha indica* L,

Croton bonplandianum Baill, *Euphorbia hirta* L, *E. thymifolia* L, *Jatropha gossypifolia* L, and *Ricinus communis* L) have significant medical uses, and some taxa such as *Ricinus communis* are widely cultivated due to their medical importance (Islam et al.,2019). Although members of this genus are distributed in various parts of the world; it has more diversity in the arid and semi-arid parts of the tropical and subtropical regions. *Euphorbia* L. genus is represented in Turkey with 107 members; it has a rich diversity and these taxa are considered as members of 2 subgenus (subg. *Chamaesyce* Raf. and sung. *Esula* Pers.) (Şafak Odabaşı, 2023).

Interesting tuberous *Euphorbia* L. (members of this genus are known as **sütleğen** in Turkish) specimens (Figure 1-3) were collected during the botanical trips carried out on 20.04.2024 on the stony steppe slopes and off the oak (*Quercus infectoria*_Oliv. subsp. *Ferris* (A.Kern) Meikle and *Q. libani* Oliv.) communities of the mountainous part north of the Odabaşı village of Baskil district(Elazığ/Türkiye). According to Walter (1962), divides the Irano-Turanian phytogeographic region of Türkiye into two parts, Baskil district; it is located in the forest area dominated by deciduous trees. The area where *Euphorbia* specimens were collected also includes steppe and rocky areas in the area between the locally destroyed oak communities. The distribution of 950 taxa was determined in the flora of Baskil district, where tuberous *Euphorbia* samples were collected (Behçet 2020). In addition to this flora, a significant part of which is determined to be composed of elements of the Irano-Turanian phytogeographic region, some new species have recently been published in the field for the scientific world (Behçet 1998; Behçet & İlçim 2018; Hamzaoğlu & Behçet 2022). In addition, in recent years, new records for Turkey (Yapar & Behçet 2022) and new taxon studies (Behçet et al. 2019; Behçet & Gülbasan 2024) have been published within the borders of Elazığ province where *E. cardophylla* Boiss. & Heldr. was collected.

These collected samples; according to the "Flora of Turkey and the East Aegean Islands and Flora of the USSR" identification keys, they are members of *Helioscopia* Dumort. Emend. Tutin section and *Galarhoei* (Haw.) Boiss. Ex Pax emend. Radcliffe –Smith. Subsection (Group B) (Radcliffe-Smith,1982; Prokhanov,1974). The fruits of our perennial herbaceous specimens are verrucose, the seed surfaces are smooth and many other features comply with the definition of *Euphorbia cardiophylla* Boiss. & Heldr.(Radcliffe-Smith,1982). However, in Öztekin (2012a and 2012b)'s current studies regarding the distribution of *Euphorbia* genus members in Türkiye, it is stated that *E. cardiophylla* is a synonym for *E. condylocarpa* M.Bieb. and the existence of *E. condylocarpa* in Türkiye should be confirmed. In addition, the website (Öztekin, 2012b) shows the distribution areas of each of the vascular plants distributed in Türkiye on a map; no distribution area is specified on the map given for *E. condylocarpa*. In this case, it is not clear whether *E. condylocarpa* is distributed in Türkiye and it is necessary to prove the existence of natural distribution of this species in Türkiye.

On the other hand, although the definition and information of localities of *Euphorbia condylocarpa* (which is included in the 7th volume of the Flora of Turkey as *E. cardiophylla* and in the 10th volume as *E. condylomata*) in7th the 10th volumes of Flora of Turkey (Radcliffe-Smith,1982; Davis et al., 1988) is given; Öztekin's (2012a) study evaluated the distribution of the said species in our country as suspicious. When you visit the website (Öztekin, 2012b) showing the distribution of plants growing in Türkiye, it is seen that there is no distribution map or information provided for the *Euphorbia cardiophylla* species in Türkiye. Also on the same website no distribution area is given for *Euphorbia condylocarpa* within the borders of Türkiye and regarding this species, the statement "Confirmation of its existence in Türkiye is required" is given, and there is a note "Fl. Taur.-Caucas. 1: 377 (1808)" regarding the distribution of this species. The fact that the existence of this species in Turkey is considered suspicious and requires confirmation may be due to the fact that specimens that fully reflect the characteristics of the species in question are not available in Türkiye or have not been seen. The reasons stated above; necessitates the elimination of doubts about the distribution of *Euphorbia condylocarpa* in Türkiye.

In this study, the main features of *Euphorbia condylocarpa* that distinguish it from its related species; it is evidenced by field and scan photographs (Figure 1-5). In addition, locality information and ecological characteristics of the species were given and doubts about the distribution of the species in Türkiye were eliminated.

MATERIALS and METHODS

Specimens of *Euphorbia condylocarpa* were collected from the Baskil district of Elazığ province in Türkiye (Figure 6). While describing *Euphorbia condylocarpa*, in addition to the description of the distribution of the species in the Flora USSR (Prokhanov, 1974), some variations seen in our samples (especially sometimes, in addition to the development of sterile branches on the stem, tuber sizes, tuber division and, although rare, flattening) are also presented by taking into account the samples we collected from Baskil. Also in the given definition; The characteristics of this species, which is known to be distributed in the Caucasus, in the Russian flora (Prokhanov, 1974) were compared with the photographs of live and dry *Euphorbia condylocarpa* specimens

on the Gbif website (2024). Photographs of specimens were taken in the field, scanning device(*hp*), and morphological observations were made using an Olympus SZ51 stereo microscope. The herbarium specimens are deposited in BIN (the Herbarium of Bingöl University).

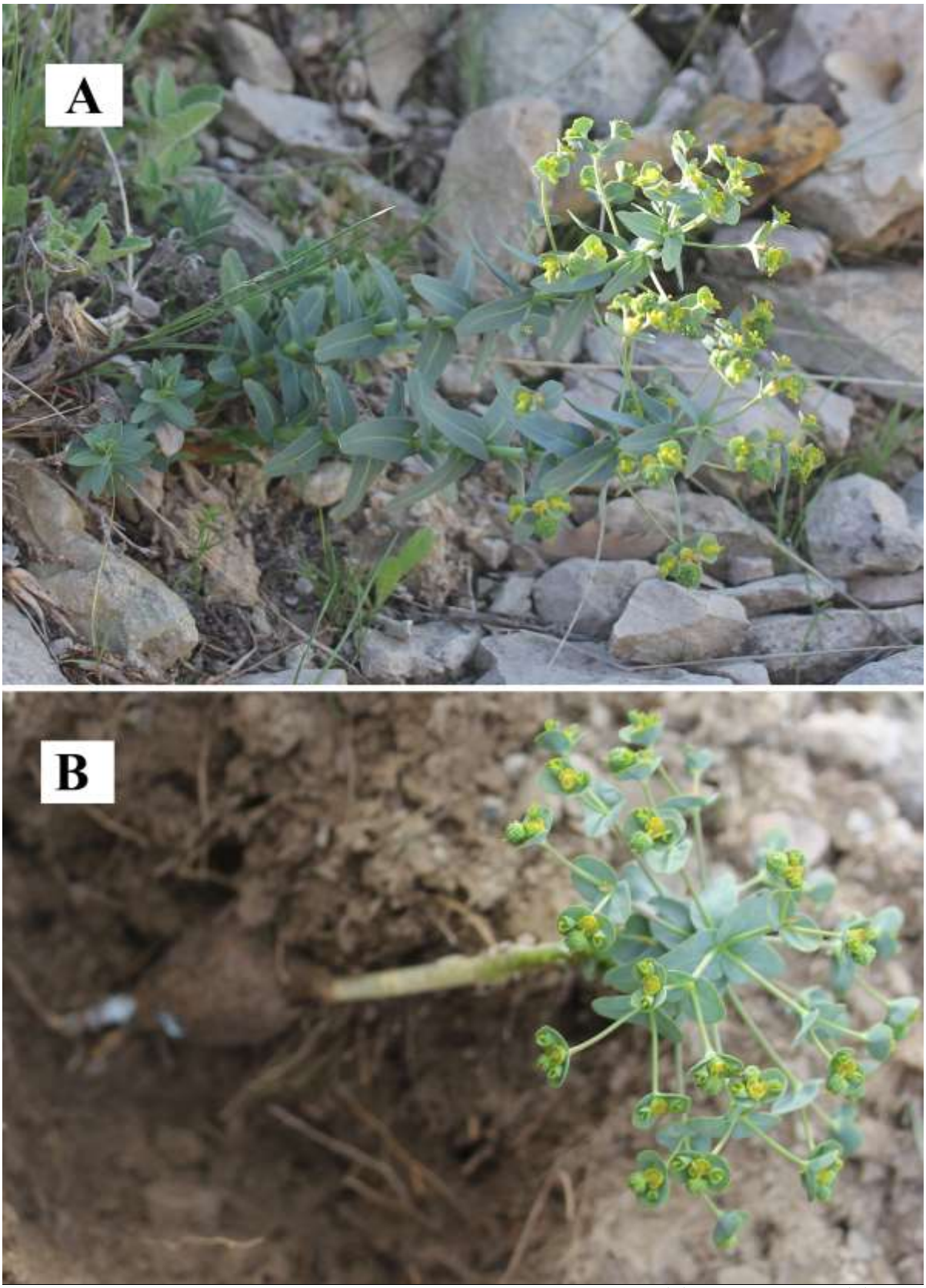

Figure 1. View of *Euphorbia condylomata* in its original habitat (A) and with tuber (B)
Şekil 1. Euphorbia condylocarpa 'nın orijinal habitatında(A) ve yumrulu(B) görünümü

Figure 2. Scanned habit of *Euphorbia condylocarpa* with a single flattened tuberous and chordate-based leaf (from BIN 12197).

Şekil 2. Euphorbia condylocarpa'nın taranmış tek yumrulu ve kalpsi tabanlı yaprağa sahip olan habitusu (BIN 12197'den)

These tuberous *Euphorbia* specimens collected from Baskil were found to be very similar when compared to the *Euphorbia condylocarpa* images on the Gbif website (Figures 4 and 5).

RESULTS and DISCUSSION

Euphorbia. condylocarpa M. Bieb. Fl. taur.-cauc. I (1808) 377, et III, 328; Ldb. Fl. Ross. Ill, 567; Boiss. In DC. Prodr. XV, 2, 126; Fl. Or. IV, 1102.- *E. amplexicaulis* Ledeb Fl. Ross. Ill (1849—1851) 567.—

Tithymalus condylocarpus (M. Bieb.) Klotzsch & Garcke in Abh. Akad. Berl. 1859(1860)78, nomen altera. - Ic: Boiss. Ic. Euph. Tab. 77.

Type: in Leningrad (LE).

Syn.: Homotypic synonym

Tithymalus condylocarpus (M.Bieb.) Klotzsch & Garcke in Abh. Königl. Akad. Wiss. Berlin 1859: 78 (1860)

Heterotypic synonyms

Euphorbia amplexicaulis Ledeb. In Fl. Ross. 3: 567 (1850), nom. illeg.

Euphorbia cardiophylla Boiss. & Heldr. İn P.E.Boissier, Diagn. Pl. Orient. 12: 107 (1853)
Tithymalus amplexicaulis Klotzsch & Garcke in Abh. Königl. Akad. Wiss. Berlin 1859: 80 (1860)
Tithymalus cardiophyllus (Boiss. & Heldr.) Klotzsch & Garcke in Abh. Königl. Akad. Wiss. Berlin 1859: 78 (1860)

Figure 3. Scanned habit of *Euphorbia condylomata* with sterile branches, chordate-based leaf and 3-tube red: A-General view of habitus B-Fruits and glands appearances in the inflorescence part (from BIN 12197)
Şekil 3. Euphorbia condylocarpa'nın taranmış çiçeksiz dallı, kalpsi tabanlı yaprağa sahip olan ve 3 yumrulu habitusu: A-Habitusun genel görünümü B- Çiçek durumundaki meyve ve glandların görünümleri (BIN 12197'den)

Figure 4. Photograph of fresh specimens of *Euphorbia condylomata* (from GBIF 2024)
Şekil 4. Euphorbia condylocarpa'nın taze örneklerine ait fotoğraf (GBIF 2024'den)

Figure 5. Image of *Euphorbia condylomata* in the Herbarium of Moscow State University (MW1007566) (from GBIF 2024)

Şekil 5. Moskova Devlet Üniversitesi Herbaryumundaki Euphorbia condylocarpa'nın görüntüsü (MW1007566) (GBIF 2024'den)

Description: Perennial. **Rootstock** is tuberous, tuber entire or **branching, 1–6 cm wide**, those that develop in the rock area are **flattened due to bilateral** rock compression. **Stems:** 10–45(54) cm high, 2–8 mm thick, l–several, often branched, prostrate, decumbent-ascending or ± erect, **with** or without **sterile branches**, glabrous, thinner at the base, densely leafy, with internodes 3–6(rarely 10) mm long. **Leaves:** basal leaves are scarious, deciduous, the basalmost squamiform; cauline leaves sessile, dilated-cordate and amplexicaul at base, ovate-oblong, oblong, elliptic-oblong or linear-oblong, (0.5–)1.3–3.5(–5) cm long, (4)5–16(–18) mm wide, obtuse or acute, serrate. **Inflorescence:** paniculate; bearing above 6–40 axillary peduncles 1–4.5 cm long, terminal peduncles 1–3 cm long, often inconspicuous; axillary peduncles many, like the terminal, bifurcate; raylet leaves ovate-rhombic to transversely ovate, oblongtriangular or rhombic-ovate, (3)8–18 mm long, (3)5–13(–16) mm wide, usually more or less serrate or entire, obtuse, sometimes abruptly cuspidate, often more or less reddish. **Cyathium:** subglobular-turbinate, ca. 1.5 mm long, 2 mm in diameter, glabrous, with short broad transversely oblong lobes; nectaries 5, transversely elliptic; styles 0.5–1 mm long, nearly free, cleft. **Schizocarp** trilobate, short-stalked, subglobulose

3–4.5x3.5-5 mm, covered with shortly cylindrical or conical green or purplish warts, glabrous. **Seeds** compressed ovate, ca.2.5 mm long, brown, smooth. Fl.3-4-Fr. 4-6.

Forests and shrubby formations, stony and rocky slopes.- Gen. distr.: Iran, Türkiye. Described from the mineral source Narzan (Kislovodsk) in the foothills of the Caucasus.

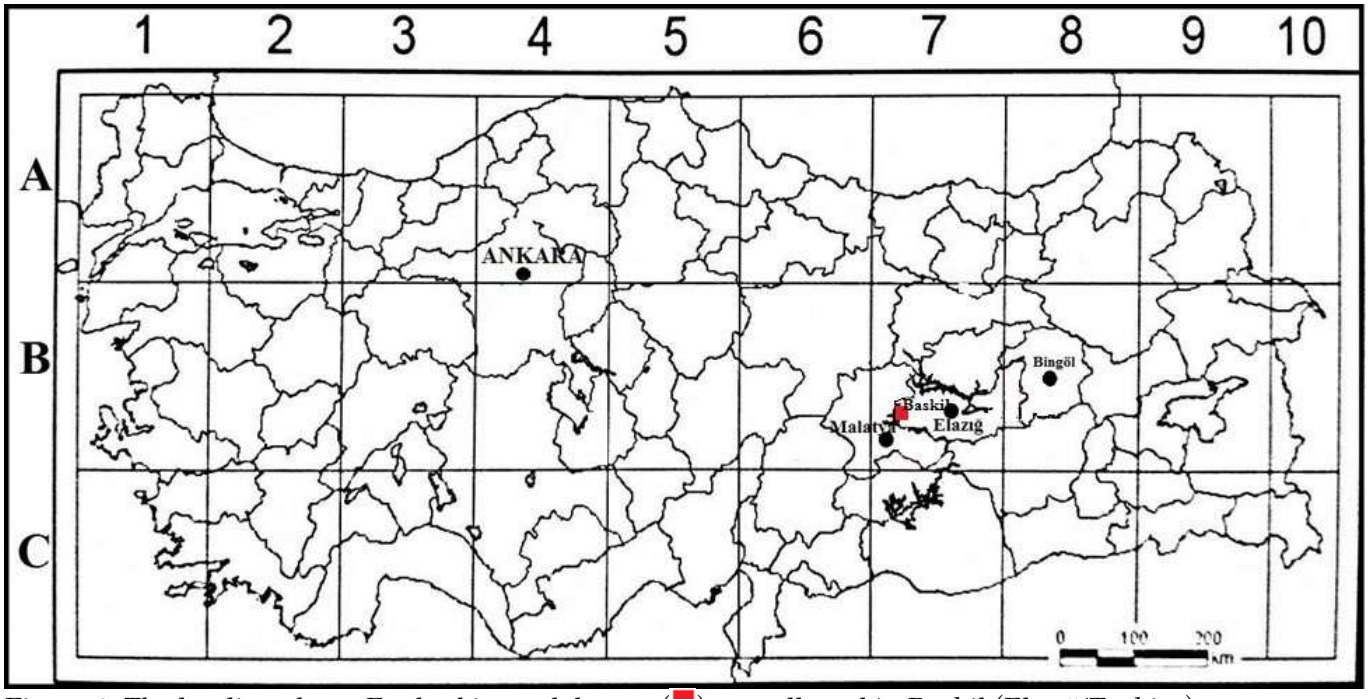

Figure 6. The locality where *Euphorbia condylomata* (■)was collected in Baskil (Elazığ/Türkiye)
Şekil 6. Euphorbia condylocarpa (■)'nın Baskil (Elazığ/Türkiye)'de toplandığı lokalite

Flowering: March-April

Fruiting: April-June

Distribution: North Caucasus, Transcaucasus, Iran, Iraq, Türkiye (POWO, 2024).

Type: in Leningrad (LE).

Specimens examined: *Euphorbia. condylomata*: Türkiye, B7 square: Elazığ, Baskil district, the mountainous part north of the Odabaşı village, on the stony steppe slopes and off the oak(*Quercus infectoria*_Oliv. subsp. *Ferris* (A.Kern) Meikle and *Q. libani* Oliv.) communities, 1500-1600 m, 20.04.2024, *L.Behçet* 21188, BIN 12197.

Ecological preferences: In the area where *Euphorbia condylomata* grows at 1500-1600 m on the mountain slopes north of Odabaşı village; there is a distribution of oak communities (*Quercus infectoria* Oliv. subsp. *veneris* (A.Kern.) Meikle and *Q. libani* Oliv.) and the shrub-like *Cerasus macrocarpa* (C.A.Mey.) Boiss. subsp. *microcarpa and Cotoneaster nummularius* Fisch. &C.A.Mey., *Ficus carica* L. subsp. *rupestris* Browicz. In these communities where *E. condylocarpa* develops and in the steppe areas between them; other important plants that grow alongside spiny or xerophytic taxa such as *Acantholimon acerosum* (Willd.) Boiss., *Helichrysum plicatum* (Nab.) P.H.Davis & Kupicha, *Marrubium parviflorum* Fisch. & C.A.Mey. subsp. *parviflorum*, *Noaea mucronata* (Forssk.) Asch. & Schweinf are: *Alyssum menicoides* Boiss., *A. simplex* Rudolph, *Arabis montbretiana* Boiss., *Astragalus lanigerus* Desf., *Bromus tectorum* L.,*Carlina involucrata* subsp. *libanotica* (Boiss.) Meusel & Kästner, *Centaurea virgata* Lam., *Cerastium dichotomum* L. subsp. *dichotomum, Clypeola jonthlaspi* L., *Crepis foetida* L. subsp. *commutata* (Spreng.)Babcock, *Crocus cancellatus* Herb. subsp. *damascenus* (Herb.)B. Mathew, *C. pallasi* Goldb., *Draba verna* L., *Erodium cicutarium* (L.) L'Herit subsp. *cicutarium, Euphorbia macroclada* Boiss., *Geranium rotundifolium* L., *Holosteum umbellatum* L. var. *glutinosum* (M.Bieb.) Gay, *Muscari neglectum* Guss. ex Ten., *Myosotis refrecta* Boiss. subsp. *refracta*, *Saxifraga tridactylites* Sm. *Ranunculus isthmicus* Boiss. subsp. *stepporum* P.H.Davis, *Viola occulta* Lehm., *Valeriana dioscoridis* Sm., *Taraxacum pseudonigricans* Hand. -Mazz., *Thalictrum isopyroides* C.A.Mey., *Thlaspi perfoliatum* L.

Members of the *Euphorbia* genus are called **sütleğen** in Turkish due to the milk-like white latex secretion they contain in their tissues. Tuber root feature is not a well-known feature in the members of this genus, which has many annual and perennial members. Therefore, *E. condylocarpa* is an interesting plant with its globose root

feature. Although *E. candyllocarpa* is similar to *E. apios* L., which is known to be distributed in Türkiye, with its tuberous root structure, verrucose fruit characteristics, and the ability to produce flowers and fruits between March and June; it differs from cauline leaves in that they are cordate-auriculate at the base and have a higher number of axillar rays (to 30).

In the collected samples, the tuber width is 1–6 cm (not 1–4 cm wide), the tuber shape is rarely flattened due to compression (not only globose), and the tubers are sometimes branched (not continuously entire) and there are sterile shoots on their stems (not always sterile branches absent) differ from the known definition of *E. candyllocarpa* (Figure 3). *E. candyllocarpa* is distinguished from the *E. apios* species, which is known to be distributed in Türkiye, as follows:

–Cauline leaves ± rounded at base; axillary rays rarely more than 3 .....**apios**

–Cauline leaves cordate-auriculate at base; axillary rays to 30 ..... **candyllocarpa**

We hope that there will be no doubt or hesitation with this study about the distribution of this plant in Türkiye, whose detailed characteristics we have given.

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Cabernet-Sauvignon Üzüm Çeşidinde Abiyotik Streslerin Primer / Sekonder Metabolitler ve Resveratrole Etkisi

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ÖZET

Araştırma; 41° 01' 11.15" N enlem ve 27° 40' 18.00" E boylamda ve denizden 60 m yüksekte ve 15 yaşındaki Cabernet-Sauvignon/110R omcaları kurulmuş ve iki yıl süreyle yürütülmüştür. Bağın dikim aralık ve mesafesi 2.6×0.9 m olup, asmalar çift kollu kordon Royat terbiye şekline sahiptir. Araştırma bağda, 3 farklı fenolojik dönemde (ben düşme, ben düşme-hasat ve hasat) 5 gün süre ile sabah ve akşam olmak üzere, Kontrol dahil 4 abiyotik stres uygulaması (Darbe, Yaprak Yaralama, UV-C) yapılmıştır. Yaprak Yaralama bir kez ve yapraklara çubuk ile vurularak gerçekleştirilmiştir. Darbe uygulaması plastik çekiç kullanılarak, UV-C uygulaması da günde iki kez 1 dakika süreyle yapılmıştır. Sonuçta abiyotik stres uygulamalarının primer metabolitlerden; SÇKM (23.69° Brix) ve TA (7.32 g L^{-1}) açısından önemli farklılık oluşturmadığı; sekonder metabolitlerde (toplam tanen, toplam antosiyanin, toplam fenolik madde, resveratrol) artış yönünde etkisi olduğu belirlenmiştir. Ayrıca toplam polifenol indeksini artırıcı etki gösterdikleri saptanmıştır. Resveratrol açısından, sırasıyla UV-C (0.35 mg kg^{-1}) ve Yaprak Yaralama (0.27 mg kg^{-1}) uygulamalarının etkileri diğerler iki uygulamadan (Darbe ve Kontrol) yüksek olduğu kaydedilmiştir.

Bahçe Bitkileri

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Anahtar Kelimeler
Stres
Fitoaleksin
Resveratrol
UV-C ışını
Şıra

Effect of abiotic stresses on primary / secondary metabolites and resveratrol in cv. Cabernet-Sauvignon

ABSTRACT

The research was located at latitude 41° 01' 11.15" N and longitude 27° 40' 18.00" E, at an altitude of 60 m above sea level, with 15-year-old Cabernet-Sauvignon/110R vines over two years. The vineyard has a planting distance of 2.6×0.9 m, and the vines are trellised to double cordon Royat. In the vineyard, 4 abiotic stress applications (Shock Action, Leaf Injury, UV-C) including the Control were applied twice a day (morning and evening) for 5 days during 3 different phenological stages (Veraison, Veraison-Harvest, and Harvest). The Leaf Injury was performed once by striking the leaves with a rod. The Shock Action was carried out using a plastic hammer, and the UV-C was applied twice a day for 1 minute. As a result, it was determined that the abiotic stress did not cause significant differences in primary metabolites such as Total Soluble Solids (23.69°Brix) and Total Acidity (7.32 g L^{-1}) but had an increasing effect on secondary metabolites (total tannin, anthocyanin, TPC, resveratrol). Additionally, it was found that they had an enhancing effect on the TPI. In terms of resveratrol, the effects of UV-C (0.35 mg kg-1) and Leaf Injury (0.27 mg kg^{-1}) were noted to be higher than the other two (Shock Action and Control).

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INTRODUCTION

Grape ripening, from veraison to harvest, involves significant changes in berry composition, including primary metabolites (sugars, organic acids) and secondary metabolites (phenolic compounds, taste-active molecules, aroma precursors, and aromas) (van Leeuwen et al., 2022). Traditionally, ripeness is determined by measuring Total Soluble Solids (TSS), Total Acidity (TA), or pH of the grape juice.

Temperature, water, light, and CO_2 concentration are key abiotic factors that interact with vine and berry development in a manner dependent on the genotype (Keller, 2010; Ferrandino et al., 2023). Rienth et al. (2021) reported that abiotic factors control the synthesis and degradation of primary and secondary metabolites, either directly through biosynthetic pathways or indirectly through vine physiology and phenology.

Secondary metabolites are low molecular weight phenolic compounds that, while not essential for plant life, help defend against abiotic and biotic stress (Billet et al., 2018; Valletta et al., 2021). These include bioactive compounds like anthocyanins, organic acids, tannins, and flavonoids. Secondary metabolites categorized into phenolic compounds, terpenoids, and nitrogen compounds. Their levels vary based on factors such as variety, ripeness, climate, and post-harvest processing.

Phenolic compounds are important indicators of grape berry and wine quality (Candar, 2023a). Gindri et al. (2021) highlighted the importance of anthocyanins in grapes and wine. Moreover, red grape anthocyanins determine the final color of wine, which is a key factor in assessing its quality (Iland et al., 2004; Kennedy, 2010). Xavier Machado et al. (2021) found that grape remains (seeds, skins, etc.) contain about 70% of total phenolic compounds (TPC), including high levels of anthocyanin, gallic acid, catechin, epicatechin, and *trans*-resveratrol. Environmental factors, especially climatic extremes, can negatively impact the phenolic content of grape varieties. Luzio et al. (2021) speculated that some extent, the rise in secondary metabolites enhances the quality, aroma profiles, and antioxidant capacity of berries, must, and wine.

Valletta et al. (2021) found that stilbenes, including resveratrol (3, 4', 5-trihydroxystilbene), act as phytoalexins and are crucial for plant defense against phytopathogens (Del-Castillo-Alonso et al., 2016; Billet et al., 2018). Resveratrol is a natural phenolic compound produced by plants under stress. Grapes contain more resveratrol than any other natural source. Resveratrol is known to have two isomers: E-*trans* and Z-*cis*. The resveratrol found in plants is mostly the -*trans* isomer. While it is present in the highest amount in the berry skin, it is proportionally less in grape juice and wine (Hasan & Bae, 2017). The resveratrol concentrations in grapes vary by climate and vegetation period, with high levels found in Cabernet-Sauvignon. Such stresses enhance stilbene biosynthesis and accumulation (Valletta et al., 2021). Additionally, resveratrol is a phytoalexin linked to resistance against biotic stresses like *Botrytis cinerea* and *Plasmopara viticola* (Langcake & Pryce, 1977; Ferrandino et al., 2023).

Candar, (2023b) stated that wounding is one of the abiotic stress factors. At the same time, Candar (2023a) examined the impact of ten different human-made woundings on the leaves of Cabernet Sauvignon grapevines on the accumulation of grape berry metabolites. The research concluded that wounding treatments have the potential to diversify the phenolic compound profile and can be used for the management of these compounds compared to the control group. Climate change models provide unclear predictions about solar radiation of different wavelengths reaching Earth's surface. Ultraviolet (UV) radiation (100–400 nm) is crucial for the physiology of plants, mammals, humans, and ecosystems due to its high energy and impact (Ballaré et al., 2011). Ultraviolet (UV) rays impact plant morphology and physiology. UV-C light (100-280 nm), which does not reach the biosphere, stimulates the accumulation of phytoalexins in vine leaves and berries (Langcake & Pryce, 1977). Del-Castillo-Alonso et al. (2016) noted that UV temporarily affected phenolic components in berry skin during the growing season. Gindri et al. (2021) found that post-harvest UV-C application to Cabernet-Sauvignon grapes boosted secondary metabolite production and increased resveratrol in treated leaves. Post-harvest UV-C light treatment elevated phenolic compounds in organic grape juice, enhanced antioxidant capacity at low doses, and increased trans-resveratrol content in irradiated grapes. The cv. Cabernet-Sauvignon has high resveratrol production potential, concentrated in the skin for fungal resistance. Resveratrol production in the skin negatively correlates with berry development stages (Jeandet et al., 1991).

In this study, abiotic stress was applied to living vines. These stresses included shock action, leaf injury, and UV-C abiotic stress applications, which were applied under field conditions during three different phenological development stages (veraison, veraison-harvest, and harvest) for 5 days before harvest. The chemistry of grape berries, primary and secondary metabolites, including resveratrol, was examined.

MATERIAL and METHOD

Site selection and plant material

The research was conducted in the vineyards, located at 41° 01' 11.15" N latitude and 27° 40' 18" E longitude, at

an altitude of 60 m above sea level. The study involved 15-year-old Cabernet-Sauvignon/110R vines. The vineyard's planting distance is 2.6×0.9 m, and the vines are trained in a double-cordon Royat system.

The research was set up using a Randomized Block Design. Four different stress applications (Control, Shock Action, UV-C, and Leaf Injury) were applied to the Cabernet-Sauvignon/110R graft combination vines during 3 different phenological development stages (Veraison, Veraison-Harvest, and Harvest). These applications were conducted with 3 replications, and each plot contained 3 vines. Homogeneity was ensured among the selected vines (Figure 1).

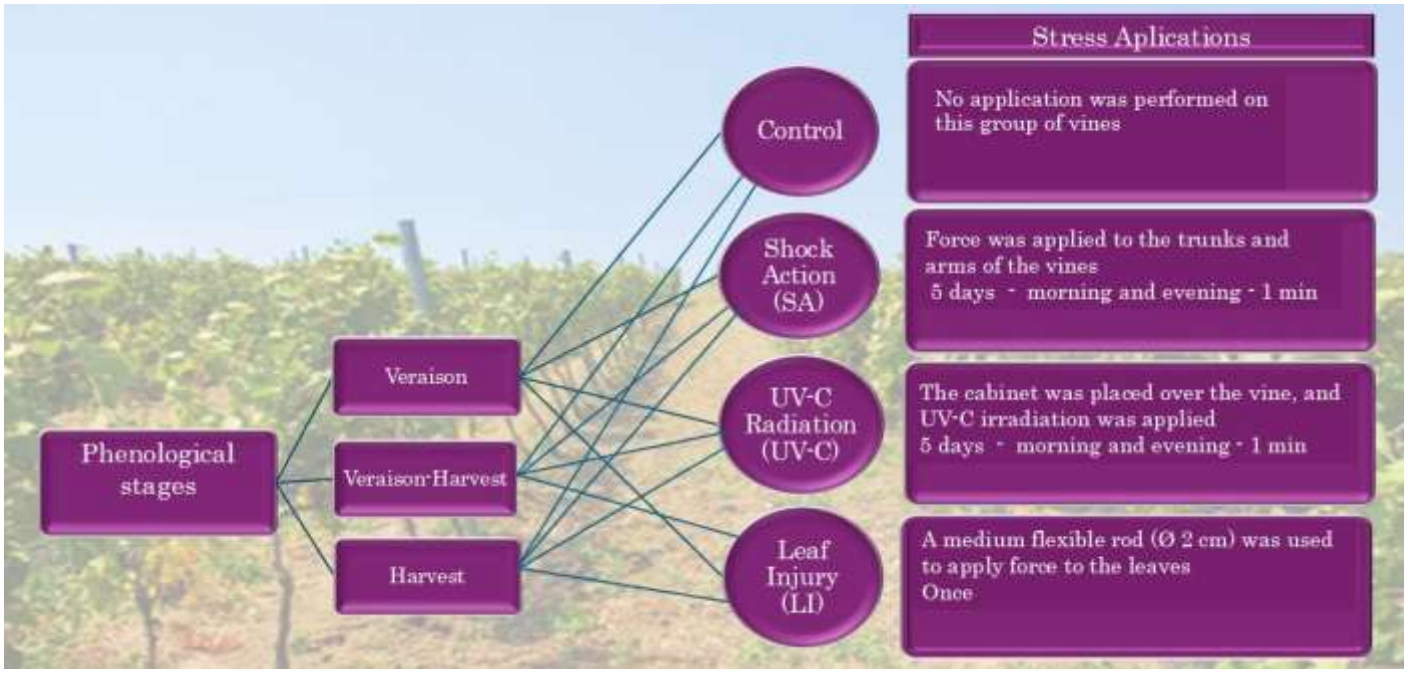

Figure 1. Experimental plan
Şekil 1. Deneme planı

Phenological stages

The stress applications were carried out during the following phenological stages (Coombe, 1995):

Veraison (V) stage: The onset of color change in the clusters and berry softening (EL35),

Veraison-Harvest (V-H) stage: Continued berry softening and color change, approaching harvest (EL35-EL38),

Harvest (H) stage: Berry ripening (EL38).

Harvest was manually conducted on 24.09.2017 and 27.09.2019.

Stress applications

The Shock Action and UV-C light abiotic stress applications were performed twice daily (morning and evening) for 5 days, while the leaf injury application was done once. In this study, shock action and leaf injury, selected as abiotic stress factors, were chosen because they are among the physiological interactions used to enhance grape quality (especially resveratrol accumulation) (Del-Castillo-Alonso et al., 2021; Bahar et al., 2024b).

Control (C): No application was performed on this group of vines.

Shock Action (SA): Force was applied to the trunks and arms of the vines in the vineyard using a plastic-covered hammer. However, the force was not strong enough to damage the vines, only to shake them. In this way, it was applied for 5 days during the veraison, veraison-harvest, and harvest periods. The Shock Action application was performed twice a day, in the morning and evening.

UV-C Radiation (UV-C): A rectangular cabinet with a 254 nm, 30-watt UV-C lamp was used. The cabinet had five sides covered with a light-impermeable membrane. The cabinet was placed over the vine, and UV-C irradiation was applied. The UV-C application was performed twice a day, in the morning and evening. The UV-C cabinet was held over the vine for 1 minute. In this way, it was applied for 5 days during the veraison, veraison-harvest, and harvest periods.

Leaf Injury (LI): A medium flexible rod (Ø 2 cm) was used to apply force to the leaves on both sides of the vine

once, aiming to shred some leaves. This application, aimed at breaking into pieces irregularly, was performed once during the veraison, veraison-harvest, and harvest periods.

Grape chemistry and maturity indexes

To determine grape composition, standard measurements of TSS, TA, and pH were performed (Cemeroğlu, 2007). Sugar concentration and maturity indices like TSS/TA and pH^2x°Brix were calculated (Blouin & Guimberteau, 2000).

Secondary metabolites

After removing the seeds, the grapes were crushed, centrifuged, and filtered. Total anthocyanin content was measured using the pH differential method (Cemeroğlu, 2007), total tannin content at 760 nm with the Folin-Denis reagent, and total phenolic content (TPC) at 765 nm with the Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), converted to gallic acid equivalent (Waterhouse, 2002; Kurt et al., 2023). TPI was read at 280 nm (INRA, 2007). Resveratrol was detected using HPLC with a fluorescence detector, and concentration was calculated using LC Solutions software, with a calibration graph (R^2=0.999).

Trial design and statistical analysis

Statistical data analysis was conducted using JMP 17. Analysis of variance (ANOVA) was employed to assess the significance of differences between treatments, and significant differences were further categorized using the LSD test. All results are expressed as the mean of three replications with ± standard error (SE).

RESULTS and DISCUSSION

Total Soluble Solids (TSS) (°Brix)

It has been determined that the TSS value of 2017 (23.97±0.13°Brix) is greater than that of 2019 (23.40±0.20°Brix). When examined in terms of Applications Main Effect (AE), the values are ranked from highest to lowest as C (24.00±0.24°Brix), UV-C (23.69±0.22°Brix), SA (23.64±0.22°Brix), and LI (23.42±0.32°Brix). According to the Phenologic Stage Main Effect (SE), this ranking is V-H (23.58±0.21°Brix), V (23.71±0.23°Brix), and H (23.77±0.21°Brix). The results are in line with the findings of Bahar et al. (2024c) (23.50°Brix to 25.25°Brix), Bahar et al. (2018) (23.13°Brix), Cebrián-Tarancón et al. (2024) (23.60°Brix), and Bindon et al. (2013) (23.01°Brix). However, the research findings contradict those of Bahar & Yaşasin (2010) (21.16°Brix), Antalick et al. (2015) (22.70°Brix), Jiang et al. (2013) (19.86-22.41°Brix), and Chapman et al. (2005) (25.30°Brix); it is thought that this difference may be due to location, soil, etc. On the other hand, Bramley (2005) and Tisseyre et al. (2008) reported that year-to-year variations in TSS values are not an effective parameter for determining grape quality in the following season. The results of this study are consistent with these findings.

Total Acidity (TA) (g L^{-1})

It has been determined that the total acidity was 7.62±0.09 g L^{-1} in 2017 and 7.02±0.14 g L^{-1} in 2019. According to the SE, the TA values are ranked in descending order as V (7.43±0.16 g L^{-1}), V-H (7.36±0.17 g L^{-1}), and H (7.16±0.19 g L^{-1}). According to the AE, the values are ranked in ascending order as LI (7.19±0.21 g L^{-1}), C (7.25±0.16 g L^{-1}), UV-C (7.31±0.18 g L^{-1}), and SA (7.53±0.18 g L^{-1}). Bahar et al. (2024c) reported that the highest TA value was obtained from LI (8.10 g L^{-1}), similar to the study. Similarly, Chapman et al. (2005) found a value of 6.93 g L^{-1}. On the other hand, Antalick et al. (2015) recorded this value as 4.40 g L^{-1}, and Cebrián-Tarancón et al. (2024) as 5.80 g L^{-1}, which are considerably lower than the findings of this study. Additionally, Bindon et al. (2013) found it to be between 8.30-5.30 g L^{-1}, Bahar & Yaşasin (2010) found it to be 8.64 g L^{-1}, and Jiang et al. (2013) found it to be between 6.3-11.9 g L^{-1}. It is thought that this difference may be due to location, climate, year, etc. However, as noted by Tisseyre et al. (2008), the TA value was also not found to be an effective parameter for determining grape quality in the following season.

pH

In terms of AE, it was found that the C had a pH value of 3.28±0.02, while the others had a value of 3.27±0.01. Regarding SE, it was observed that the V and H periods had a value of 3.26±0.01, while the V-H period had a value of 3.30±0.01. The research findings are consistent with those of Bahar et al. (2018) 3.33; Bahar et al. (2024c) 3.31; Bahar & Yaşasin (2010) 3.39; Bindon et al. (2013) 3.18-3.48; and Jiang et al. (2013) 3.10-3.40. However, the results are not consistent with those researchers who found a pH value of 3.58 (Cebrián-Tarancón et al., 2024), 3.41-3.53 (Candar, 2023a), 3.69 (Antalick et al., 2015), and 3.69 (Chapman et al., 2005). This discrepancy may be due to

climate, location, training system, etc. The changes in TSS, TA, and pH values observed in the research were noted to be consistent with the findings of Trought & Bramley (2011) and Baluja et al. (2012), who reported that these parameters are influenced by phenological development stages (V, V-H).

Sugar Concentration (g L^{-1})

The Year Main Effect (YE) was found to be significant for sugar concentration, with the sugar concentration in 2017 determined to be 237.78±1.60 g L^{-1} and the value in 2019 to be 231.62±2.52 g L^{-1}. In similar studies conducted on the Cabernet Sauvignon variety, the sugar concentration was determined to be 205.70 g L^{-1} by Bahar & Yaşasin (2010), 231.10 g L^{-1} by Bahar et al. (2018), and 251.58 g L^{-1} by Bahar et al. (2024c). The differences between the findings of the researchers and the results of this study can be attributed to variations across the years.

Sugar Per Berry (mg $berry^{-1}$)

There was also a difference in the sugar per berry between the years, with a higher value in 2017 (88.73±2.06 mg $berry^{-1}$) compared to 2019 (81.35±2.41 mg $berry^{-1}$). In line with the research findings, Bahar et al. (2024c) reported that the average amount of sugar per gram of berry in the Cabernet Sauvignon variety ranged between 88.22 mg $berry^{-1}$ and 103.00 mg $berry^{-1}$.

Sugar Per Gram of Berry (mg 1 g $berry^{-1}$)

In terms of AE, the values were ranked as LI 77.21±1.27 mg 1 g $berry^{-1}$, SA 77.97±0.85 mg 1 g $berry^{-1}$, UV-C 78.84±0.98 mg 1 g $berry^{-1}$, and Control 79.47±0.98 mg 1 g $berry^{-1}$. Similarly, Bahar et al. (2024c) reported that the average amount of sugar per gram of berry ranged between 75.73 mg 1 g $berry^{-1}$ and 83.84 mg 1 g $berry^{-1}$. Korkutal et al. (2019) found values of V 86.50 mg $berry^{-1}$, Half-Maturity 78.75 mg $berry^{-1}$, and Before Maturity 85.91 mg $berry^{-1}$, which are in line with the research findings.

Maturity Indexes

TSS/TA (g L^{-1})

The TSS/TA values for stress applications were numerically ranked in ascending order as SA 3.15±0.07 g L^{-1}, UV-C 3.25±0.08 g L^{-1}, LI 3.28±0.12 g L^{-1}, and Control 3.32±0.09 g L^{-1}. Regarding the application periods x application interaction, the highest value was obtained from the H x LI interaction at 3.42±0.09 g L^{-1}, and the lowest value from the V x LI interaction at 3.12±0.08 g L^{-1}. The combination with the highest value in the S x A x Y interaction was H x LI x 2019 (3.77±0.11 g L^{-1}). Bahar et al. (2024c) found that this value ranged between 2.97-3.44 g L^{-1}, which is within a similar range to the study.

pH^2x°Brix (g L^{-1})

When considering a pH^2x°Brix value above 260 g L^{-1} as full maturity (Blouin & Guimberteau, 2000), C (257.88±4.80 g L^{-1}) is the closest value. This result is parallel with Candar (2023a) as 259.86 g L^{-1}. This is followed by the UV-C (253.62±2.58 g L^{-1}), SA (252.25±4.13 g L^{-1}), and LI (250.78±4.13 g L^{-1}) applications. Bahar et al. (2018) reported this value to be 255.93 g L^{-1}, and Bahar et al. (2024c) found it to range between 247.97 g L^{-1} and 265.84 g L^{-1}, which aligns with the research findings.

Seconder Metabolites

Total Anthocyanin Content (mg kg^{-1})

Abiotic stress applications at different stages for Cabernet Sauvignon had statistically significant effects on the total anthocyanin content, considering YE, SE, and S x A, S x A x Y, and S x Y interactions (Table 1). The difference between the trial years was found to be statistically significant. In 2019, the total anthocyanin content (1479±75.33 mg kg^{-1}) was found to be higher than in 2017 (1306±56.19 mg kg^{-1}). The research findings are in line with the observation of Moreno-Olivares et al. (2024) that the experimental years influenced total anthocyanin. In terms of Stage Main Effect (SE), significant differences in total anthocyanin content were observed among the phenological development stages where abiotic stress applications were performed. In H stage (1576±102.44 mg kg^{-1}) has the highest anthocyanin content same as Baluja et al. (2012) findings. The research results align with those of other researchers; in general, anthocyanin content increases rapidly during the first 3-4 weeks following veraison, then stabilizes or undergoes slight changes around harvest (Holt et al., 2010). For the S x A interaction, the highest value was found in the H x SA interaction (1821±303.20 mg kg^{-1}). V x LI (1056±79.89 mg kg^{-1}) and V x SA (1071±68.53 mg kg^{-1}) had the lowest total anthocyanin values. The highest value obtained from the S x A x Y interaction was 2489±109.39 mg kg^{-1} (H x SA x 2019). The lowest value was obtained from V x LI x 2017 (978±54.96

mg kg^{-1}) interaction. In the S x Y interaction, the highest value was obtained in the H x 2019 combination (1844±149.01mg kg^{-1}). The other interactions were in the same significance group. These values align with the findings of Bahar et al. (2024a) 1094 mg kg^{-1}, Bahar et al. (2024c) 1043.841 mg kg^{-1}, and Bindon et al. (2013) 1.37-1.87 mg g^{-1}.

Total Tannin Content (g kg^{-1})

The effect of abiotic stress applications applied at different growth stages was found to be statistically significant in terms of YE, AE, SE, S x A, and A x Y interactions (Table 2). The total tannins in 2019 (7.00±0.20 g kg^{-1}) was found to be significantly higher than in year 2017 (4.53±0.14 g kg^{-1}). The findings of Ortega-Regules et al. (2008) that tannin concentration varies across years is consistent with our research. It was determined that the application (AE) that increased the total tannin the most was SA (6.35±0.51 g kg^{-1}). While the UV-C application followed this, the other two applications, LI and C, were in the same significance group. When SE was examined, the H (6.06±0.34 g kg^{-1}) was recorded as the period with the highest total tannin value. The V (5.53±0.32 g kg^{-1}) had the lowest total tannin value. In terms of the S x A interaction, H x UV-C had the highest value at 6.80±0.63 g kg^{-1}, while V x LI had the lowest value at 4.55±0.78 g kg^{-1}. On the other hand, for the A x Y interaction, the SA x 2019 interaction had the highest (8.38±0.19 g kg^{-1}), all applications in 2017 recorded the lowest values. The obtained results are consistent with the findings of Bahar et al. (2024c) 3.23 g kg^{-1}-4.26 g kg^{-1}, Korkutal et al. (2019) 8475.20 mg kg^{-1} in the V period, and Bindon et al. (2013) 3.26-4.15 mg g^{-1}. However, it conflicts with the findings of Jiang et al. (2013) (2.3-5.3 g L^{-1}), which may be due to the research location (China).

Total Phenolic Index (TPI)

In terms of TPI, the effects of YE, AE, SE, and S x Y are statistically significant. Accordingly, it was observed that the TPI value for 2019 (7.95±0.32) was higher than the value for 2017 (7.13±0.45). In terms of AE, abiotic stress applications were grouped together (SA 8.24±0.35; LI 7.98±0.32; and UV-C 7.74±0.30). C (6.21±0.22) has the lowest TPI value. According to SE, H had the highest TPI value at 8.23±0.34. V-H and V stages followed this stage. When the S x Y interaction was examined, it was determined that the V x 2017 interaction (5.32±0.12) had the lowest TPI. For the Cabernet Sauvignon, Blouin & Guimberteau (2000) reported a TPI value of 13.30; Bahar et al. (2024a) 6.00; Bahar et al. (2024c) 9.76; and Bahar et al. (2018) between 5.31-6.87. The obtained TPI values align with the findings of researchers other than Blouin & Guimberteau (2000).

Total Phenolic Content (TPC) (mg kg^{-1})

In cv. Cabernet Sauvignon, statistical differences in TPC were found between YE, AE, SE, A x Y, and S x Y interactions (Table 3). The TPC for 2017 (3414±86.99 mg kg^{-1}) is lower than that for 2019 (3889±136.47 mg kg^{-1}). Ramos et al. (2024) reported that changes in temperature and rainfall can also affect grape phenolic content, and therefore grape quality. It has been suggested that the difference in TPC between the years may have resulted from this. In terms of AE, SA (3939±213.19 mg kg^{-1}) and UV-C (3771±152.36 mg kg^{-1}) are in the same importance group. The LI (3578±184.05 mg kg^{-1}) application is in the second importance group, while C is in the last importance group (3318±77.43 mg kg^{-1}). In terms of SE, H had the highest value (3937±171.43 mg kg^{-1}), and V had the lowest value (3301±135,43 mg kg^{-1}). The findings of Bahar et al., (2024c) at 3268.99 mg kg^{-1} for TPC are in line with the research. In terms of the S x Y interaction, SA x 2019 (4577±288.35 mg kg^{-1}) had the highest TPC value, while C x 2017 (3235±116.44 mg kg^{-1}) had the lowest TPC value. It should not be overlooked that the H x 2019 interaction (4418±263.84 mg kg^{-1}) also had the highest TPC value.

Resveratrol (mg kg^{-1})

It was found that only the AE has a statistically significant effect on resveratrol concentration (Table 4). The UV-C abiotic stress application (0.35±0.06 mg kg^{-1}) was determined to be the most effective in increasing the resveratrol value in the Cabernet Sauvignon variety. The research findings are consistent with the finding that UV-C and leaf wounding treatments were effective in enhancing *trans*-resveratrol levels in Cabernet Sauvignon at harvest time (Bahar et al., 2024c). This was followed by LI (0.27±0.05 mg kg^{-1}), while C (0.07±0.02 mg kg^{-1}) and SA (0.05±0.02 mg kg^{-1}) were in the third importance group. The findings of Romero-Pérez et al. (1999), which reported 0.50 mg L^{-1} *trans*-resveratrol and 0.06 mg L^{-1} *cis*-resveratrol in red grape juice, are consistent with the research. Çaylak et al. (2009) recorded the resveratrol content in Marmara Region wines as 0.252 mg L^{-1}. In 2017, resveratrol values ranged between 0.08-0.28 mg kg^{-1} among the applications, while in 2019, resveratrol values ranged between 0-0.42 mg kg^{-1}. Candar (2023a) reported that *trans*-resveratrol ranged between 0.36-3.59 mg kg^{-1}. Specifically, it was determined that leaf wounding applied 15 days before harvest increased the *trans*-resveratrol content by 35.78% compared to the Control group. Numerically, the high value in SE was recorded for V-H (0.22±0.04 mg kg^{-1}).

Table 1. The effects of abiotic stresses applied during different growth stages on the total anthocyanin content in the cv. Cabernet-Sauvignon
Çizelge 1. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam antosiyanin miktarı üzerine etkileri

Stage	Apps	S x A x Y int.			A x Y int.			S x Y int.		
		2017	2019	S x A		2017	2019	AE		2017	2019	SE
V	C	1539±391.76 BCDE	1208±129.04 CDE	1373±198.78 **BCD**	C	1294±160.49	1291±82.58	1293±87.55	V	1225±109.83 B	1168±58.59 B	1197±61.16 **b**
	SA	1142±127.72 CDE	1000±45.54 DE	1071±68.53**D**								
	UV-C	1242±95.55 CDE	1332±71.54 CDE	1287±57.04 **CD**								
	LI	978±54.96 E	1135±150.81 CDE	1056±79.89 **D**								
V-H	C	1274±291.91 CDE	1423±163.03 BCDE	1348±153.19 **BCD**	SA	1222±72.67	1559±237.24	1391±127.08	V-H	1385±91.42 B	1426±85.89 B	1405±61.48 **ab**
	SA	1371±168.60 CDE	1187±59.04 CDE	1279±89.79 **CD**								
	UV-C	1576±209.90 BCDE	1369±177.23 CDE	1473±130.33 **ABCD**	UV-C	1446±96.21	1576±126.28	1511±78.61				
	LI	1319±26.48 CDE	1723±162.98 BC	1521±116.76 **ABC**								
H	C	1070±123.04 DE	1244±159.21 CDE	1157±98.00 **CD**								
	SA	1154±45.79 CDE	2489±109.39 A	1821±303.20 **A**	LI	1261±106.62	1491±117.79	1376±82.00	H	1307±92.54 B	1844±149.01 A	1576±102.44 **a**
	UV-C	1520±164.86 BCDE	2028±37.83 AB	1774±136.56 **AB**								
	LI	1485±256.43 BCDE	1617±137.20 BCD	1551±133.33 **ABC**								
YE		1306±56.19 b	1306±75.33 b									

YE p<0.1=134.1977; S x A x Y intr. p<0.1=620.1465; S x A intr. p<0.1=438.5098; S x Y intr. p<0.01=310.0732; SE p<0.01=286.5193
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 2. The effects of abiotic stresses applied during different growth stages on the total tannin content in the cv. Cabernet-Sauvignon
Çizelge 2. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam tanen miktarı üzerine etkileri

Stage	Apps	S x A x Y int.				A x Y int.				S x Y int.		
		2017	2019	S x A		2017	2019	AE		2017	2019	SE
V	C	4.90±0.50	6.52±0.15	5.71±0.43 *ABCD*	C	4.76±0.27 C	6.09±0.20 B	5.43±0.23 **B**	V	4.41±0.26	6.66±0.38	5.53±0.32 b
	SA	4.61±0.28	8.19±0.51	6.40±0.84 *AB*								
	UV-C	4.92±0.43	6.00±0.43	5.46±0.36 *ABCD*								
	LI	3.19±0.09	5.91±1.08	4.55±0.78 *D*								
V-H	C	4.03±0.44	5.75±0.49	4.89±0.48 *CD*	SA	4.31±0.19 C	8.38±0.19 A	6.35±0.51 **A**	V-H	4.44±0.25	6.98±0.34	5.71±0.33 ab
	SA	4.04±0.22	8.35±0.05	6.20±0.96 *ABC*								
	UV-C	4.18±0.21	6.22±0.23	5.20±0.47 *BCD*	UV-C	4.84±0.23 C	6.8±0.38 B	5.81±0.32 **AB**				
	LI	5.50±0.60	7.62±0.38	6.56±0.57 *AB*								
H	C	5.36±0.12	6.03±0.30	5.69±0.20 *ABCD*								
	SA	4.30±0.48	8.62±0.38	6.46±1.00 *AB*	LI	4.21±0.40 C	6.73±0.41 B	5.47±0.41 **B**	H	4.76±0.24	7.37±0.34	6.06±0.34 a
	UV-C	5.42±0.12	8.18±0.31	6.80±0.63 *A*								
	LI	3.95±0.42	6.64±0.15	5.30±0.63 *BCD*								
YE		4.53±0.14 **b**	7.00±0.20 **a**									

YE $p<0.1=0.5135$; S x A x Y intr. $p<0.1=1.4502$; A x Y intr. $p<0.1=1.1840$; AE $p<0.1=0.8372$; SE $p<0.5=0.4159$
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 3. The effects of abiotic stresses applied during different growth stages on the TPC in the cv. Cabernet-Sauvignon
Çizelge 3. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam fenolik madde miktarı üzerine etkileri

Stage	Apps	S x A x Y int.			A x Y int.			S x Y int.		
		2017	2019	S x A		2017	2019	AE		2017	2019	SE
V	C	3194±336.40	3277±125.04	3236±161.57	C	3235±116.44 C	3401±101.03 BC	3318±77.43 *b*	V	3285±212.04 **C**	3316±178.03 **C**	3301±135.43 *b*
	SA	3259±96.93	3738±453.33	3499±233.33	SA	3302±91.48 BC	4577±288.35 A	3939±213.19 *a*				
	UV-C	3144±165.79	3430±57.19	3287±101.25	UV-C	3454±113.61 BC	4088±246.18 AB	3771±152.36 *a*	V-H	3500±116.03 **BC**	3933±145.21 **AB**	3717±101.56 *ab*
	LI	3543±889.70	2820±503.46	3181±484.89	LI	3666±2859.58 BC	3491±241.19 BC	3578±184.05 *ab*				
V-H	C	3127±136.47	3299±142.10	3213±96.06								
	SA	3474±200.21	4494±79.03	3984±247.49								
	UV-C	3538±215.80	3843±210.29	3690±150.98								
	LI	3862±237.10	4099±129.71	3980±131.91								
H	C	3383±115.26	3626±220.20	3505±123.66					H	3457±106.70 **BC**	4418±263.84 **A**	3937±171.43 *a*
	SA	3171±163.21	5499±77.31	4335±526.62								
	UV-C	3680±60.35	4993±156.06	4336±302.79								
	LI	3594±359.24	3553±126.70	3574±170.60								
YE		3414±86.99 **b**	3889±136.47 **a**									

YE $p<0.01=406.4150$; A x Y intr. $p<0.01=812.8301$; SE $p<0.01=497.7547$; AE $p<0.5=439.8252$; S x Y intr. $p<0.5=538.6740$
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 4. The effects of abiotic stresses applied during different growth stages on the *trans*-resveratrol levels in the cv. Cabernet-Sauvignon
Çizelge 4. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde resveratrol miktarı üzerine etkileri

Stage	Applications	S x A x Y int.			A x Y int.				S x Y int.		
		2017	2019	S x A		2017	2019	AE		2017	2019	SE
V	C	0.05±0.05	0.00±0.00	0.03±0.02	C	0.08±0.02	0.06±0.03	0.07±0.02 B	V	0.16±0.07	0.20±0.08	0.18±0.05
	SA	0.05±0.05	0.00±0.00	0.03±0.02								
	UV-C	0.26±0.25	0.52±0.26	0.39±0.17								
	LI	0.26±0.14	0.27±0.01	0.26±0.06								
V-H	C	0.15±0.02	0.14±0.07	0.15±0.03	SA	0.10±0.04	0.00±0.00	0.05±0.02 B	V-H	0.20±0.04	0.25±0.07	0.22±0.04
	SA	0.08±0.07	0.00±0.00	0.04±0.03								
	UV-C	0.37±0.08	0.46±0.09	0.42±0.05	UV-C	0.28±0.08	0.42±0.09	0.35±0.06 A				
	LI	0.20±0.08	0.39±0.21	0.29±0.11								
H	C	0.05±0.04	0.05±0.04	0.05±0.03								
	SA	0.18±0.11	0.00±0.00	0.09±0.06								
	UV-C	0.20±0.02	0.28±0.13	0.24±0.06	LI	0.21±0.07	0.32±0.09	0.27±0.05 AB	H	0.15±0.05	0.15±0.07	0.15±0.04
	LI	0.18±0.17	0.29±0.24	0.24±0.13								
YE		0.17±0.032	0.20±0.04									

YE $p<0.01=0.2521$
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

CONCLUSION

-Abiotic stresses have been observed to have a greater effect on increasing secondary metabolites than on primary metabolites.

-For total tannins: SA (6.35±0.51 mg kg^{-1}), UV-C (5.81±0.32 mg kg^{-1}), and LI (5.47±0.41 mg kg^{-1}) provided the highest values, while C (5.43±0.23 mg kg^{-1}) gave the lowest value. If an increase in total tannins is desired, these three applications can be used.

-In terms of total anthocyanins: UV-C (1511.64±78.61 mg kg^{-1}), SA (1391.05±127.08 mg kg^{-1}), and LI (1376.51±82.00 mg kg^{-1}) were found to be more effective than C (1293.38±87.55 mg kg^{-1}).

-For increasing TPC: SA (3939.75±213.19 mg kg^{-1}) and UV-C (3771.82±152.36 mg kg^{-1}) were found to be more effective than the other.

-Regarding *trans*-resveratrol: The UV-C (0.35±0.06 mg kg^{-1}) application was found to have higher values compared to LI (0.27±0.05 mg kg^{-1}) and the other applications.

When evaluated by phenological stages:

-H stood out with the highest values for total tannins (6.06±0.34 mg kg^{-1}), total anthocyanins (1576.34±102.44 mg kg^{-1}), TPC (3937.92±171.43 mg kg^{-1}), and TPI (8.32±0.39).

-For resveratrol, the V-H (0.22±0.04) showed high values.

As a result, in Tekirdağ conditions, Shock Action application is recommended 5 days before the Harvest to increase total tannins, TPC, and TPI. Additionally, UV-C and Leaf Injury applications are also considered viable. To increase total anthocyanins, UV-C application is recommended 5 days before harvest. For resveratrol increase, it is suggested to perform UV-C and Leaf Injury treatments during the Veraison-Harvest period. These research results are considered useful in determining the applications for increasing the important bioactive compound *trans*-resveratrol.

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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 declare no conflict of interest.

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Assessment of Genetic Diversity and Relationships among Gypsophila and Silene Species from Türkiye based on SRAP Markers

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ABSTRACT

Gypsophila is a member of the Caryophyllaceae family and its genus consists of approximately 150 species. Several species are grown commercially, including herbal medicine and food. Its most common use is as a cut flower worldwide. Gypsophila species are native and widely distributed in Türkiye, the main genetic resource center. In this study, *Gypsophila* L. genotypes were first collected from native areas in Türkiye. Secondly, genetic diversity using molecular markers provided valuable information for breeding programs and strategies of germplasm conservation. Sequence-related amplified polymorphism (SRAP) as a molecular marker was used to determine diversity and relationships among 41 Gypsophila (Caryophyllaceae) genotypes including 13 species (*G. viscose*, *G. simonii*, *G. venusta*, *G. bicolor*, *G. simulator*, *G. bitlisensis*, *G. germanicopolitana*, *G. perfoliata*, *G. acrostic*, *G. eleganas*, *G. paniculata* and *G. aucheri*) and two *Silene* types (*S. vulgaris* L. and *Silene* spp.) as outgroups. Results revealed that twenty primer combinations produced 153 scorable fragments, and all markers showed 100% polymorphism for 43 genotypes. The cophenetic correlation (r = 0.80) between the Dice similarity matrix and the corresponding dendrogram obtained by the SRAP marker revealed good compliance. The *Gypsophila* and *Silene* species were grouped according to subspecies and by region. Results indicated that SRAP markers were useful for investigating diversity and relationships among *Gypsophila* L. germplasm. Additionally, this data could be used to develop new *Gypsophila* L. varieties in the breeding program.

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Türkiye'deki Gypsophila ve Silene Türleri Arasındaki Genetik Çeşitlilik ve İlişkilerin SRAP Belirteçleri Kullanılarak Değerlendirilmesi

ÖZET

Cipsofilya, Caryophyllaceae familyasının bir üyesi olup cinsi yaklaşık 150 türden oluşur. Bazı türleri, bitkisel ilaç ve gıda gibi çeşitli kullanımlar için ticari olarak yetiştirilmektedir. Dünya çapında en yaygın kesme çiçek olarak kullanılmaktadır. Gypsophila türleri ana genetik kaynak merkezi olan Türkiye'de yaygın olarak bulunmaktadır. Bu çalışmada, ilk olarak Türkiye'deki yerel bölgelerden *Gypsophila* L. genotipleri toplandı. İkinci olarak, moleküler belirteçler kullanarak genetik çeşitlilik, ıslah programları ve germplazmayı koruma stratejileri için değerli bilgiler elde edildi. Moleküler belirteç olarak dizi ilişkili çoğaltılmış polimorfizm (SRAP), 13 tür içeren (*G. viscosa*, *G. simonii*, *G. venusta*, *G. bicolor*, *G. simulatrix*, *G. bitlisensis*, *G. germanicopolitana*, *G. perfoliata*, *G. arrostii*, *G. eleganas*, *G. paniculata* ve *G. aucheri*) 41 Gypsophila (Caryophyllaceae) genotip ve iki *Silene* tipin (*S. vulgaris* L. ve *Silene* spp.) genetik farklılık ve akrabalık durumunu belirlemek amacıyla kullanılmıştır. Sonuç olarak, 20 primer kombinasyonundan 153 skorlanabilir fragment üretilmiş ve ayrıca tüm belirteçlerr 43 genotip için %100 polimorfizm göstermiştir. Kofenetik korelasyon r değerleri (r ≥ 0,80) hesaplanarak, SRAP belirteçlerinin oluşturduğu dendrogramların

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önemi bilgiler sunmuştur. *Gypsophila* ve *Silene* türleri alt türlere ve bölgelere göre gruplandırılmıştır. Sonuçlar, SRAP belirteçlerinin *Gypsophila* L. genotipleri arasındaki çeşitliliği ve ilişkileri araştırmak için yararlı olduğunu göstermiştir. Ayrıca, bu veriler ıslah programında yeni *Gypsophila* L. çeşitlerinin geliştirilmesinde de kullanılabilir.

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INTRODUCTION

The genus Gypsophila is a member of the Caryophillaceae and includes about 150 species. The main diversification centers of the Gypsophila genus are in the Caucasus, the Transcaucasian region (northern Iraq and northern Iran), and particularly in the Eastern part of Türkiye (Barkoudah, 1962; Madhani et al., 2023). The feature of these regions is temperate or warm temperate regions in the Northern Hemisphere, especially the Mediterranean region and Near East (Ataslar et al., 2009; Intrieri et al., 2010). Gypsophila L. is the third largest genus of the Caryophyllaceae family in Türkiye and 60 taxa belonging to 56 species grow naturally (Korkmaz and Özçelik, 2011a). Gypsophila species are seen as one of the important alternatives in the cut flower industry and product diversification (Karagüzel and Ortaçeşme, 2000; Korkmaz and Özçelik, 2011b).

The genus Gypsophila contains several ornamental species, of which *G. paniculata* L. is the most important species used in cut flower production worldwide (Zvi et al., 2008; Madhani et al., 2023). *G. paniculata* L. is one of the indispensable elements of bouquets and arrangements in fresh and dry-cut flowers in the domestic market (Karagüzel, 2003). Gypsophila has male sterility, so in the classical breeding program, new varieties are obtained artificially from wild species through *in vitro* vegetative propagation and the selection of clonal variants. Another method is based on open pollination of wild plants (Bogani et al., 2012).

Knowing the genetic structure and germplasm diversity found in Gypsophila's germplasm can provide valuable information for Gypsophila breeding programs to tackle a variety of traits and select new cultivars for conservation purposes (Calistri et al., 2014). For successful breeding, it is crucial to have prior knowledge of the genotypes, their origin, genetic variability, and relationships. Molecular markers may prove precious in supporting Gypsophila germplasm development through characterization of the genetic diversity.

DNA molecular markers are used to evaluate plant diversity, plant breeding, phylogenetic and systematic analyses (Kanayama et al., 2007; Martínez-Nieto et al., 2013; Bolger et al., 2014, Jin et al., 2022). DNA-based markers can be detected at all stages of plant development, contain the entire genome, and can provide large amounts of information; they are highly polymorphic and evaluation to easy and simple (van Zonneveld et al., 2014; Serrote et al., 2020). In ornamental plants, molecular markers are an extremely effective tool for genetic characterization and variety conservation (Mahmood et al., 2013). Studies on the genetic variation of the genus Gypsophila have focused on Gypsophila varieties and their wild ancestors. Random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) analysis have been used to identify genetic variations between Gypsophila species and their genetic structure (Rady, 2006; Barakat and El-Sammak, 2011; Lachmayer, 2009). Calistri et al. (2014) investigated the genetic relationship of five Gypsophila spp., sixty-two *G. paniculata* L. within their native range and thirteen commercial hybrid strains using a combination of 63 AFLPs, ISSRs, 64 TRAP, and 65 cpSSRs. As a result, they determined that the markers used were dominant. Initially, Korkmaz and Doğan (2015) were found to be correlated with geographic and phytogeographic regions of genetic diversity of fourteen Gypsophila species from Türkiye using RAPD and ISSR markers. Jin et al. (2022) constructed a genome-wide InDel marker system of *G. paniculata* L. following genome resequencing of another white-flowered wild-type accession.

SRAP markers have been recognized as important molecular marker systems for gene tagging and mapping in Brassica (Li and Quiros, 2001). These PCR-based markers target open reading frames in genomic sequences, generating a set of codominant markers per amplification using forward and reverse primers. SRAP markers are more consistent and reproducible than RAPDs and require less labor and time than AFLP markers (Lia and Quiros, 2001; Budak et al., 2004). For this reason, it has widely been used to evaluate the genetic diversity and population structure of species (Bargish and Rahmani 2016; Kelemen et al., 2018; Akçali Giachino, 2023).

Türkiye is a major diversity center for Gypsophila, as are many other important plant species. Gypsophila is among one of the most important varieties of cut flowers in Türkiye, where it is grown in more than 250 decare greenhouses. Currently, its cultivation is spreading (Anonymous, 2018). The cut flower sector has many problems; one of them is production material, which is imported. Improvement of a new variety that is suitable for Türkiye's

ecological condition. Therefore, the project named "Cut Flower Breeding Program; conducted to generate gene pools of Carnation and *Gypsophila* L." was carried out for the exploration of Gypsophila germplasm. Gypsophila genetic materials were examined for their potential for use as ornamental plants, and seeds and herbarium samples were collected from different locations that may hold rich genetic diversity (Kaya et al., 2012).

This study aimed to evaluate SRAP to determine the genetic variability and relationships within and among Gypsophila and Silene species from different regions (Eastern Anatolia, Middle Anatolia, and the Mediterranean) of Türkiye to provide further insight and develop useful strategies for its conservation and evaluation in a breeding program.

MATERIALS and METHODS

Plant Materials

Fourty-one genotypes of Gypsophila accessions (Caryophyllaceae) including 13 species (*G. Simonis, G. viscose, G. venusta Fenzl., G. bicolor, G. simulatrix, G. bitlisensis, G. germanicopolitana, G. perfoliata, G. acrostic, G. eleganas, G. paniculate* and *G. aucheri*) and two *Silene* types (Caryophyllaceae) including *Silene vulgaris* L. and *Silene* spp. as outgroups were evaluated in this study (Table 1). Numbers of species were given in Figure 1 as *G. simonii* 14, *G. viscose* 1, *G. venusta* 3, *G. bicolor* 4, *G. simulatrix* 2, *G. bitlisensis* 4, *G. germanicopolitana* 1, *G. perfoliata* 3, *G. acrostic* 4, *G. eleganas* 1, *G. paniculata* 1, *G. aucheri* 1, and *Silene vulgaris* 2. details of original locations of collected genotypes.

DNA Extraction and SRAP Experimental Design

Seeds of the collected Gypsophila genotypes were germinated under greenhouse conditions in March 2008 at Batı Akdeniz Agricultural Research Institute, Antalya, Türkiye (36º55'46,30" N and 30º58'47,96" E, altitude 10 m). After 4–6 weeks, the young leaf parts were collected and genomic DNA was isolated using the modified CTAB method developed by Doyle and Doyle (1990). The resulting DNA concentration was measured in a 1% agarose gel stained with ethidium bromide, compared with the known concentration of Lambda DNA (0.5µg/µl, Fermantas).

SRAP analysis was performed as described by Li and Quiros (2001) with some modifications. A total of 36 different SRAP primer combinations were employed using six forward and six reverse primers (Table 2), of which 20 pairs produced clear and reproducible bands. The PCR amplifications were carried out using a thermal cycler (Eppendorf Mastercycler Gradient) in reaction volumes of 15 µl containing 15 ng of genomic DNA and 0.2 µM each of forward and reverse primers, 100 mM of dNTPs, 2 mM of $MgCl_2$, 10 x Taq buffer and 1 unit Taq DNA Polymerase (Biorun), and ddH_2O. PCR reactions were performed under the following conditions: 5 min of denaturing at 94 ºC and 5 cycles of three steps: 1 min of denaturing at 94 ºC, 1 min of annealing at 35 ºC and 2 min of elongation at 72 ºC. In the subsequent 34 cycles; 1 min of denaturing at 94 ºC, 1 min of annealing at 47 ºC, 1 min of elongation at 72 ºC, 1 cycle of 5 min at 72°C. PCR products were separated on 2.5% agarose gel 1X in TAE buffer at 100 V for 3 h. A 100 bp DNA ladder was used as a molecular standard. The gels were stained in ethidium bromide solution (0.5 µg/ml) and then photographed under UV light using the Kodak GelLogic200 Image Analysis System.

Figure 1 Location of sampling site of genotypes
Şekil 1. Genotiplerin elde edildiği bölgeler

Table 1. Gypsophila and Silene species and location of 43 genotypes evaluated in this study
Tablo 1. Çalışmada kullanılan 43 adet Gypsophila ve Silene türleri ve lokasyonları

Genotype	Taxon name	Location
1	*Gypsophila viscose* Muray	Ankara-Şereflikoçhisar
2	*Gypsophila simonii* Hub. Mor	Sivas – Gürün
3	*Gypsophila simonii* Hub. Mor	Sivas – Gürün
4	*Gypsophila venusta* Fenzl.	Erzurum -Aşkale - Tercan
5	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Erzurum - Aşkale
6	*Silene* spp.	Nevşehir-Ürgüp
7	*Gypsophila simulatrix* Bornm. & Woron	Konya – Ereğli
8	*Silene vulgaris* (Moench) Garcke	Sivas-Refahiye location
9	*Gypsophila simonii* Hub. Mor	Erzincan - Refahiye
10	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
11	*Gypsophila simonii* Hub. Mor	Erzincan - Refahiye
12	*Gypsophila simonii* Hub. Mor	between Yozgat-Boğazlayan and Sarıkaya
13	*Gypsophila germanicopolitana* Hub.-Mor	between Kayseri and Kırşehir
14	*Gypsophila* spp.	Artvin-Hopa
15	*Gypsophila bitlisensis* Bark.	Kars- Digor
16	*Gypsophila arrostii* Guss.	Konya-Beyşehir
17	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
18	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Van- Gürpınar -Başkale
19	*Gypsophila simonii* Hub. Mor	Erzincan-Tercan
20	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
21	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
22	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
23	*Gypsophila simonii* Hub. Mor	Yozgat-Boğazlayan
24	*Gypsophila simonii* Hub. Mor	Sivas- Zara
25	*Gypsophila perfoliata* L. var. *perfoliate*	Konya-Ereğli
26	*Gypsophila perfoliata* L. var. *perfoliate*	Konya-Ereğli
27	*Gypsophila perfoliata* L. var. *perfoliata*	Konya-Ereğli
28	*Gypsophila arrostii* Guss.	Konya- Beyşehir
29	*Gypsophila arrostii* Guss.	Konya- Beyşehir
30	*Gypsophila arrostii* Guss.	Antalya- Elmalı
31	*Gypsophila simulatrix* Bornm. & Woron	Konya-Ereğli
32	*Gypsophila elegans* Bieb.	Konya-Karapınar
33	*Gypsophila venusta* Fenzl.	between Konya- Seydişehir and Bozkır
34	*Gypsophila venusta* Fenzl.	between Konya- Seydişehir and Bozkır
35	*Gypsophila paniculata* L.	Isparta-University
36	*Gypsophila arrostii* Guss.	between Isparta-Eğirdir and Senirkent
37	*Gypsophila simonii* Hub. Mor	Yozgat-Boğazlayan
38	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Ağrı- Doğu Beyazıt
39	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
40	*Gypsophila simonii* Hub. Mor	Kırşehir
41	*Gypsophila simonii* Hub. Mor	between Van- Gürpınar and Gevaş
42	*Gypsophila bicolor* (Freyn & Sint.) Grossh	between Van- Gevaş and Tatvan
43	*Gypsophila aucheri* Boiss.	Erzincan-Tercan

Table 2. Sequence of SRAP primers used in this study
Tablo 2. Çalışmada kullanılan SRAP primerlerin baz dizilimi

Primer	Forward primer Sequence (5'–3')		Reverse primer Sequence (5'–3')
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTCGA
ME4	TGAGTCCAAACCGG CC	EM6	GACTGCGTACGAATTCCA
ME7	TGAGTCCTTTCCGGTCC	EM8	GACTGCGTACGAATTCAC
ME8	TGAGTCCTTTCCGGTGC	EM11	GACTGCGTACGAATTCTA
ME11	TGAGTCCTTTCCGGAAC	EM12	GACTGCGTACGAATTCTC
ME13	TGAGTCCTTTCCGGAAG	EM15	GACTGCGTACGAATTGAT

Data Scoring and Analysis

All clear and reproducible PCR products were scored as the presence (1) and absence (0) of a band for Gypsophila genotypes. The total number of bands, polymorphic bands, *Gypsophila* species-specific bands, and the average number of bands per primer were calculated. The statistical assessments, which are the evaluation method and variability formula, PIC (Polymorphic Information Content), MI (Marker Index), PI (Primer Index), and EMR (Effective Multiplex Ratio) were calculated to determine the polymorphism information and discriminating ability of each primer combinations (Table 3). The discriminatory ability of each SRAP marker was determined by calculating the PIC. PIC values were estimated according to the formula described by Smith et al. (1997). PIC= 1-Σ (fi^2), where fi^2 is the frequency of the i th allele. EMR is calculated as EMR = np × ß, where np is the total number of polymorphic loci per primer and ß is the rate of polymorphic loci from their total number (Powell et al.,1996; Nagaraju et al., 2001). MI is a statistical parameter used to estimate the total utility of the maker system. MI is the product of the polymorphism information content value and effective multiplex ratio. MI was calculated using the formula MI = PIC × EMR (Zitouna et al., 2015). The SRAP primer index was calculated by summing the PIC values of all loci amplified with the same SRAP primer combination, PI = PIC × total bands (Anderson et al., 1993; Ghislain et al., 1999; Rajwade et al., 2010).

The genetic similarity coefficient was calculated using the procedures in the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc version 2.02) (Rohlf, 2000). The similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average), using the SAHN function of the NTSYS to illustrate the genetic relationships among the germplasm studied. The representativeness of the dendrogram was determined by estimating the cophenetic correlation for the dendrogram and comparing it with the similarity matrix using Mantel's matrix correspondence test (Mantel, 1967). Principal Coordinate Analysis (PCoA) was obtained with the use of Dice's coefficient (Dice, 1945) to confirm associations among 43 species, and a two-dimensional plot (2D) was constructed.

RESULTS

The 41 Gypsophila (Caryophyllaceae) germplasm lines, including 13 species and 2 *Silene* (Caryophyllaceae) types, were analyzed using 20 different combinations of SRAP primers. For all that, 16 SRAP primer combinations produced only monomorphic DNA bands. The 20 primer combinations generated a total of 153 DNA fragments with distinct scoreable polymorphic bands and showed 100% polymorphism. The number of bands scored per primer ranged from 4 bands (Me4Em12 and Me4Em15) to 13 bands (Me3Em6) and 12 bands (Me4Em3), with 7.65 fragments for primers average (Table 3). The size of the amplified products generated using different primer combinations ranged from 200 bp to 1800 bp. The gel view obtained from the Me3Em12 primer combination is given in Figure 2.

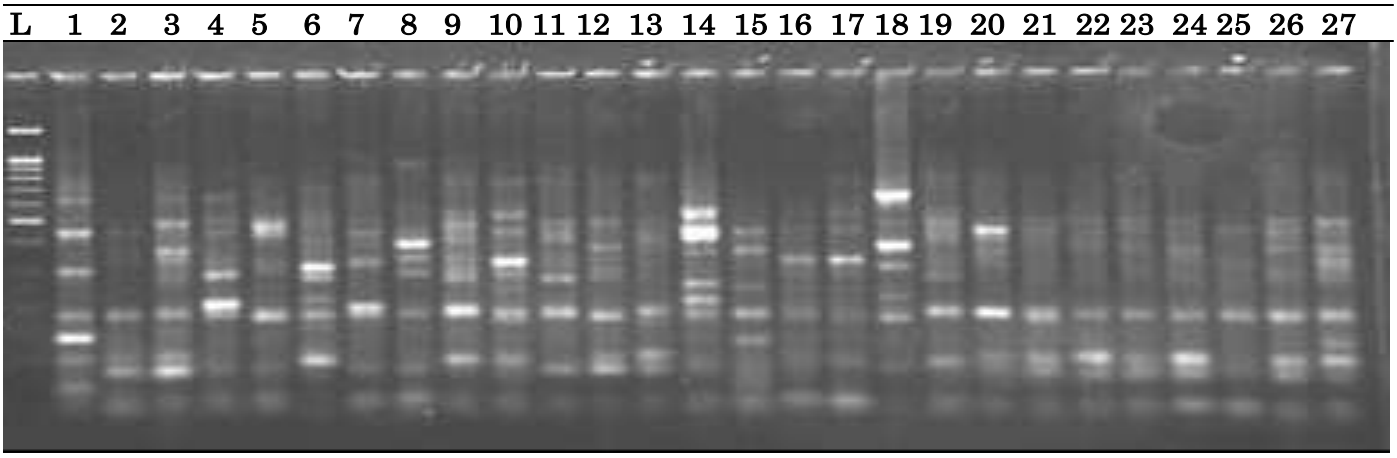

Figure 2. Gel view obtained from Me3Em12 primer combination. L: ladder DNA, 1-27: genotypes
Şekil 2. Me3Em12 primer kombinasyonundan elde edilen jel görünümü. L: DNA ladder, 1-27: genotipler

The informative and discriminating power of the PIC, EMR, MI, and PI values of markers at individual SRAP primer combinations were calculated (Table 3). The PIC values for 20 primer combinations ranged from 0.67 (Me7Em3) to 0.98 (Me8Em8 and Me7Em123), with a mean of 0.88 (Table 3). The EMR value ranged from 4.00 to 13.00 and the MI and PI values ranged from 3.35 to 9.96. The maximum EMR (13.00), MI, and PI (9.96) values were observed for the primers Me3Em16 and Me4Em3, respectively, whereas minimum scores for EMR (4.00) at

Me4Em12 and Me4Em13, MI and PI (3.35) at Me7Em3 SRAP primers combinations were recorded. Overall, the 15 SRAP primers used in this study showed an average EMR value of 7.64 and a mean of 6.72 scores for MI and PI (Table 3).

Table 3. Details of data produced by screening Gypsophila and Silene genotypes using SRAP markers
Tablo 3. Gypsophila ve Silene genotiplerinin ayrımında kullanılan SRAP belirteçlerin verileri

Primer Combinations	Total DNA fragments	Number of Polymorphic fragments	Percentage of polymorphism	PIC	EMR	MI	PI
ME3EM15	5	5	100.00	0.95	5.00	4.75	4.75
ME3EM3	7	7	100.00	0.94	7.00	6.58	6.58
ME3EM6	13	13	100.00	0.73	13.00	9.49	9.49
ME4EM12	4	4	100.00	0.89	4.00	3.56	3.56
ME4EM15	4	4	100.00	0.91	4.00	3.64	3.64
ME4EM3	12	12	100.00	0.83	12.00	9.96	9.96
ME4EM6	11	11	100.00	0.87	11.00	9.57	9.57
ME7EM12	7	7	100.00	0.98	7.00	6.86	6.86
ME7EM3	5	5	100.00	0.67	5.00	3.35	3.35
ME7EM6	8	8	100.00	0.95	8.00	7.60	7.60
ME8EM11	7	7	100.00	0.82	7.00	5.74	5.74
ME8EM12	10	10	100.00	0.96	10.00	9.60	9.60
ME8EM3	8	8	100.00	0.85	8.00	6.80	6.80
ME8EM8	7	7	100.00	0.98	7.00	6.86	6.86
ME11EM3	10	10	100.00	0.85	10.00	8.50	8.50
ME11EM6	9	9	100.00	0.93	9.00	8.37	8.37
ME13EM15	7	7	100.00	0.82	7.00	5.74	5.74
ME13EM3	6	6	100.00	0.88	6.00	5.28	5.28
ME13EM6	8	8	100.00	0.83	8.00	6.64	6.64
ME13EM8	5	5	100.00	0.96	5.00	4.80	4.80
Total	153	153	-	-			
Average	7.64	7.64	100.00	0.88	7.64	6.72	6.72

PIC: Polymorphic Information Content, EMR: Effective Multiplex Ratio, MI: Marker Index, PI: Primer Index

According to the genetic relationship analysis, Dice's genetic similarity coefficient of 41 Gypsophila genotypes and two Silene species indicated a high genetic variation among the genotypes, ranging between 0.31 and 0.85. However, the genetic closeness of the two varieties was observed between genotypes 25 and 27, including *G. perfoliata* L. both of which belonged to the same location of origin (Konya). The dendrogram created using UPGMA cluster analysis showing the general genetic relationship between genotypes is given in Figure 3.

UPGMA clustering was used to determine the rare germplasms of Gypsophila species found in different locations of Türkiye and the relationships between genotypes. UPGMA cluster analysis showed the presence of two major clusters (I and II) (Figure 3). Forty-one Gypsophila and two Silene genotypes were clustered separately. Group I is divided into two branches and the second branch is divided into four subgroups (1A, 1B, 1C, and 1D). There were no identical genotypes on the dendrogram (Figure 3). Sub-group 1A consisted of two genotypes (1 and 14), of which genotype 1 was identified as *G.viscosa* Muray and 14 number genotype were selected to Artvin, was determined as *Gypsophila* spp, two types were located on the border of the dendrogram and their genetic distance was 0.415 from other Gypsophila species (Figure 3). Sub-group 1B included in *G. simonii*, *G. perfoliata* var. *perfoliata* L. and *G. germanicopolitan* species. All *G. simonii* types were collected from different regions in Türkiye: *G. perfoliata* var. *perfoliata* L. and *G. germanicopolitan* species, collected from nearer regions, Konya and Kayseri, respectively. Sub-group 1C was formed by seven *Gypsophila* species, which were *G. venusta, G. bitlisensis, G. bicolor, G. simulatrix* from Ulukışla, *G. arrostii* L. and *G. paniculata* L. species from Isparta. Sub-group 1D included four genotypes of *G. arrostii* L. which were selected from nearby locations in Konya, Isparta, and Antalya, and the other *G. perfoliata* L. var. *perfoliata*, *G. simulatrix,* and *G. elegans* species that were collected from Konya formed a joint group of clusters. Additionally, sub-group 1D comprised *G. bitlisensis* and *G bicolor* species that were collected to Erzurum and Van, respectively. The genetic distance among Gypsophila species ranged from 0.58 to 0.85, and genetic diversity among the individuals of sub-cluster 1B is lower than that of the other sub-groups individuals. The greatest similarity was observed among two types (26 and 27) of *G. perfoliata* var. *perfoliata* L. collected from the same location in Konya.

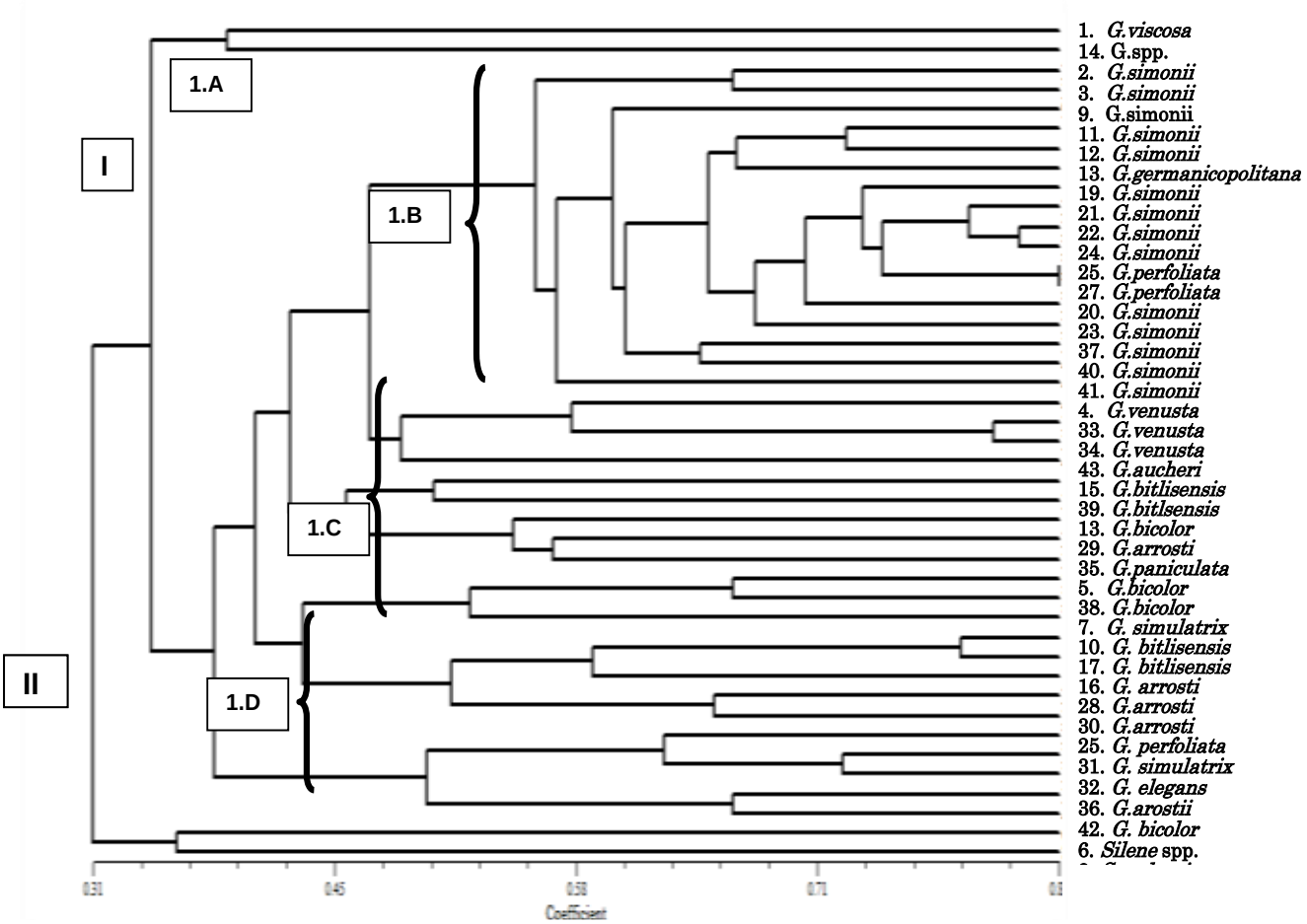

Figure 3. UPGMA dendrogram based on similarity matrix constructed from the 153 SRAP markers amplified for the 41 Gypsophila (Caryophyllaceae) and the two Silene accessions

Şekil 3. 153 adet SRAP belirteciyle 41 Gypsophila (Caryophyllaceae) ve 2 Silene türleri arasındaki benzerlik indeksini gösteren UPGMA yöntemiyle elde edilmiş dendrogram

Interestingly, the other *G. perfoliata* type (25) was shown to be dissimilar and take place in the sub-group 1D. The genetic distance of the same genotype was nearer *G. simulatrix* and *G. elegans* than the other two *G. perfoliata* types. *Gypsophila simonii* (22 and 24 number) were placed close to each other, with a genetic distance of 0.81. The reason is that these two genotypes were collected in Kayseri and Sivas, which are near provinces. However, it was noted that the genetic dissimilation of genotype 41 selected from Van was quite far from the other *G. simonii* types. The genetic distance of all genotypes selected from different localities representing *G. simonii* species ranged from 0.58 to 0.81. High similarity was observed between 33 and 34 number genotypes (*G. venusta* Fenzl), which were selected for the same location. Similarly, the genetic distance between 10 and 17 numbers (*G. bitlisensis* Bark.) was 0.78. Both genotypes were collected from Erzurum, the near location. *G. paniculata* L. is widely used in commercial cut flower production and is the primary source of commercial varieties. Clustering analysis showed the nearest genetic similarity of *G. paniculata* was determined to be *G. arrostii* L. and *G. bicolor* species from Konya and Van placed in the same sub-group, 1C. In this study, Silene spp. (6 numbers, from Nevsehir) and Silene vulgaris (8 numbers, from Sivas) were used as outgroups, and the genetic similarity of both was determined to be very low. Gypsophila and Silene taxa were distinguished and the genetic similarity (0.31) was very low, as expected.

Cluster analysis was supported by high bootstrap values and confirmed by PCoA analysis (Figure 3). The cophenetic correlation was determined as $r \geq 0.80$. Figure 3 shows the distribution of the different species of Gypsophila and Silene according to the two principal axes of variation using principal coordinates analysis (PCoA). PCoA analysis showed that the first and second principal components accounted for 12.01% and 36.18% of the total variation, respectively (Figure 4). The classification of all species derived from PCoA was similar to the result of the UPGMA analysis. Substantial dispersion of Gypsophila species in the PCoA plot and the result of the UPGMA analysis indicate high genetic diversity among different species of Gypsophila. The genetic diversity among two Silene types (*Silene vulgaris* L. and Silene spp.) was very high (0.35).

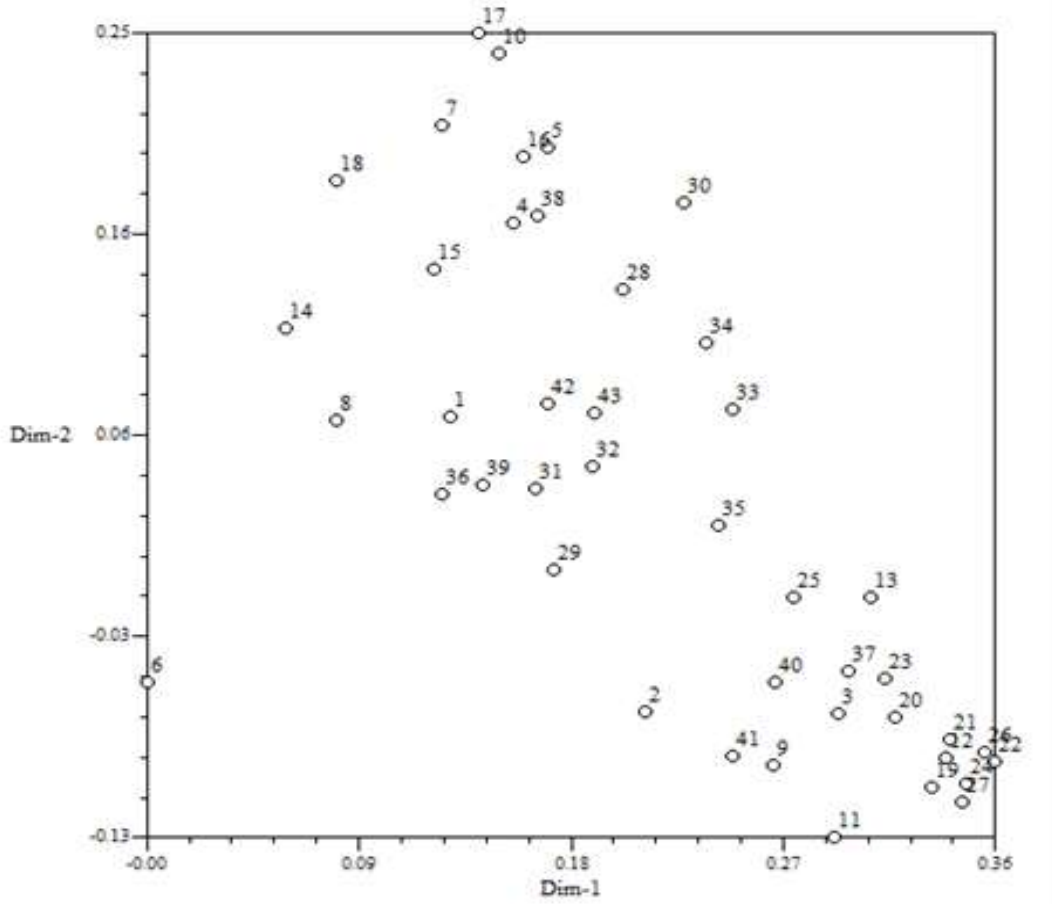

Figure 4. Diagram showing the relationships among 41 Gypsophila and two Silene accessions based on principal coordinates analysis using SRAP

Şekil 4.SRAP analizi sonucunda 41 Gypsophila ve 2 Silene türlerinin principle koordinat analizi (PCoA) sonucu göstermiş olduğu akrabalık dağılım deseni

DISCUSSION and CONCLUSIONS

In our study, genetic diversity within and between species and genotypes of the genus *Gypsophila* L. was evaluated using SRAP molecular markers. The findings of our study revealed that SRAP was found to be effective in assessing the genetic variation in 41 *Gypsophila* species and 2 *Silene* species from different locations in Türkiye. The results show that SRAP markers can be used in developing varieties, understanding relationships, and creating germplasm collections. It also shows that SRAP markers can be used in developing varieties, understanding relationships, and creating germplasm collections.

The 153 markers were found using 20 SRAP primers analyzed in this study and presented valuable information about genetic variations in Gypsophila species/germplasms originating from diverse geographical locations. SRAP markers allowed the obtaining of highly polymorphic fragments. This result demonstrated a good choice of method for the analysis of Gypsophila species with 20 SRAP markers revealed an average of 100% polymorphism, which was too high compared to earlier studies using a different marker system. Percentage of polymorphism (100 %) was found to be higher than in other SRAP-based studies, e.g. 95.76 % for Dianthus accessions (Xiao et al., 2008), 93 % for coffee species (Mishra et al., 2011) and 71.90 % for *Silene* species (Bargish and Rahmani, 2016). In a previous study of Gypsophila species in Türkiye, Korkmaz and Doğan (2015) and Kołodziej et al. (2018) studied the genetic diversity and relationships among the accessions were determined using RAPD and ISSR markers. Similar results were presented by Korkmaz and Doğan (2015) who found 92.7, 93.8, and 92.9% polymorphism for the 14 species based on RAPD, ISSR, and RAPD + ISSR data, respectively. The other study based on the RAPD and ISSR markers showed 80.31% and 95.86% polymorphic products, respectively (Kołodziej et al., 2018). Calistri et al. (2014) used AFLP, ISSR, cpSSR, and TRAP for the analysis of the genetic distance of 5 Gypsophila wild species from Europe and Asia and 13 commercial hybrids with similar phenotypes and reported that the higher number of polymorphic products was (96.3%) for ISSR markers.

The percentage of polymorphic fragments, as well as gene diversity, showed a high range of variability in the analyzed Gypsophila and Silene accessions. The high polymorphism of selected SRAP markers provides a unique

opportunity to study genetic variation and relatedness of Gypsophila germplasm. Thus, the assessment of genetic variance among Gypsophila species in Türkiye has a greater importance in Gypsophila breeding programs and in situ conservation. Another important point is to cross genotypes in highly diverse and distinct clusters to increase the opportunity for the over-segregation of alleles at various loci (Souza and Sorrells, 1991).

The number of polymorphic markers analyzed is important to detect true relationships between taxa. Dudley (1994) suggested that when numbers reach 50 to 100 markers, results are consistent with pedigree information. One hundred fifty-three SRAP bands have been obtained to determine the relationship between and within the Gypsophila species. For this reason, the numbers of markers and informative markers number were over the suggested range by Dudley (1994).

In this study, the average values of PIC, EMR, MI, and PI, were 0.88, 7.64, 6.72, and 6.72, respectively. PIC value shows the discrimination ability of the marker depending on the number of known alleles and their frequency distribution. PIC values were higher due to markers with equal distribution in the population (Botstein et al., 1980). High, medium, or low polymorphism is expressed by PIC >0.5, 0.5 > PIC > 0.25, and PIC < 0.25, respectively (Xie et al., 2010).

PIC results were within a relatively narrow range, indicating a uniform distribution of SRAP polymorphisms among the genotypes collected; this is a desirable trait for their use in genetic diversity analyses (Al-Faifi et al., 2013). All PIC values in this analysis were found to be higher than 0.5, thus indicating that the observed polymorphism was high. On the other hand, except for Me7Em3 primer combinations, the other 19 primer combinations could be considered highly informative in determining genetic diversity. Overall, the 20 SRAP primer combinations used in this study showed that EMR, MI, and PI values are higher in Me3Em6, Me4Em3, Me4Em6, and Me8Em12 primers. The average values of PIC, EMR, MI, and PI, were higher than >0.5 for PIC and >5.0 for others. The higher value of EMR and MI explains that the selected marker system is a more efficient primer-marker and suitable method (Chesnokov and Artemyeva, 2015). Diversity parameters such as the polymorphism rate, PIC, EMR, MI, and PI had high values, indicating high variability of the tested population. High levels of diversity may increase the adaptation of Gypsophila genotypes to a wide range of environments (Nagl et al., 2011). Additionally, a high genetic variation of Gypsophila germplasm should be considered as a background for breeding programs

Based on the SRAP marker system, the genetic distance between Gypsophila and Silene accessions/species of distinct geographical regions (Southeast Anatolia, the Middle and the Mediterranean regions of Türkiye) was revealed. The dendrogram delineated the genetic distance of two taxa which formed two major clusters. This sub-cluster formed in the dendrogram was mainly displaying the genetic structure and grouped in the different climatic zones. The emergence of high polymorphism can be explained by accessions/species in different climatic zones by changing selection pressure throughout the evolution process (Mishra et al., 2011). The genetic structure and geographic distribution of species appear to greatly influence levels of genetic diversity (Hamrick and Godt, 1989). *G. simonii* types were collected from different regions in Türkiye and grouped into the same cluster (subgroup 1B). However, it is noteworthy that the genetic variation within *G.simonii* species is quite high and the germplasm pool may help improve new varieties.

The dendrogram derived from SRAP data showed that the species *G. viscosa* Muray from Ankara and Gypsophila spp. from Artvin differed significantly from the 13 Gypsophila species. The distinctiveness of these species was associated with its geographic distribution. Ankara and Artvin have diverse topography (particularly altitude) and the wide range of climatic and ecological conditions during the growing period correlate with altitude. Higher variation of both germplasms may be created due to higher mutation rates and/or selection pressure in those regions. Mhiret and Heslop-Harrison (2018) noted that molecular markers can group *Linum usitatissimum* L. accessions by both altitude and region, indicating a lack of gene flow across the country and/or selection of specific genotypes in each environment. Similarly, Korkmaz and Dogan (2015) used RAPD and ISSR markers and reported that the genetic distance of *G. glomerata* and *G. muralis* with 14 *Gypsophila* species correlated with their different phytogeographic regions.

The dendrogram delineated that the clusters of *Gypsophila* species were closely related to their geographic origins and surrounding geographic environments. *G. simonii* included 14 genotypes, *G. venusta* (3 genotypes), *G. germanicopolitana* and *G. perfoliata* (2 types) collected along the nearer cities (Konya, Kayseri, Kırşehir, Sivas, Yozgat, Ercincan, and Erzurum) in the Middle Anatolia region formed one major cluster except one genotype (41) from Van, which is located in the South-eastern Anatolia. The seven locations in the Middle Anatolia part of Türkiye have relatively similar climates and altitudes; therefore, it can be said that geographic distance and environmental factors including local climates may impact the genetic differentiation of the native Gypsophila populations.

Characterization of the genetic structure of *Gypsophila* L. can be very useful in establishing breeding strategies

that enable selection. Kaya et al. 2019, developed the named GA8, which is a new Gypsophila type as cut flowers using a selection of clones from *G. arrosti* in Türkiye and the cultivation performance of this type has been investigated. Clustering analysis showed that the genetic distance of *G. paniculata* L. among *G. arrostii* L. is nearer than other Gypsophila species.

Clustering analysis was supported by high bootstrap values and confirmed by PCoA analysis (Figure 4). Also, the significance of the resulting dendrograms was confirmed by calculating the cophenetic correlation ($r \geq 0.80$). In statistics, the cophenetic correlation coefficient is a measure of how faithfully a dendrogram preserves the pairwise distances between the original unmodeled data points. Thus, it is a measure of how faithfully the tree represents the dissimilarities among observations (Rohlf and Fisher, 1968). PoCA helps in analyzing genetic variation among plant species and determining the most important variables contributing to variation (Chesnokovand Artemyeva, 2015). The classification of all species derived from PCoA was similar to the result of the UPGMA analysis. Substantial dispersion of Gypsophila species in the PCoA plot and the result of the UPGMA analysis indicate high genetic diversity among different species of Gypsophila. Similar results have been reported by Korkmaz and Doğan (2015). The Gypsophila and Silene species were grouped according to subspecies and by region. Silene spp. separated from Gypsophila species, while *Silene vulgaris* was closer to *G. viscosa* Murav. This result may reveal a high genetic relationship between *Gypsophila* and *S. vulgaris* studied. The genetic diversity among two Silene types (*Silene vulgaris* and Silene spp.) was very high (0.35). Similar results were reported for 13 different Silene species in Iran. Their results revealed sufficient level of genetic distance (0.10 to 0.52) (Bargish and Rahmani, 2016). The genetic diversity among two Silene types (*S. vulgaris* and Silene spp.) was very high (0.35). Similarly, Bargish and Rahmani (2016) reported that the genetic distance of 13 different Silene species from Iran was from 0.10 to 0.52.

To our knowledge, this is the first study to use SRAP markers to analyze genetic variation between and within Gypsophila and Silene species. The results provide a more detailed understanding of the genetic diversity and evolutionary relationships of the above-mentioned Gypsophila and Silene species and may be a useful tool for plant breeding and the conservation of genetic resources. The most important step for the breeding program to be successful is to work with the right genotypes. Additionally, genetic resources are national treasures of countries. UPGMA cluster analysis showed that most conspecific accessions tend to have high genetic similarity and cluster into the same group or subgroups. While *G. simonii* and *G. perfoliata* were found to be the most closely related species, *G. viscosa* appeared to be a separate species. Finally, using more genotypes in different species may provide more understandable results. The germplasm in this study should be a valuable source in Gypsophila breeding.

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Conflict of interest

The author declares that they have no conflict of interest.

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Evaluation of the Effects of Grafting and Vermicompost Applications on the Morphophysiological Properties of Eggplant under Drought Stress with Principal Component Analysis

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ABSTRACT

Drought is one of the important abiotic stresses that significantly affect plant growth and development process. The use of grafted plants and vermicompost application creates significant potential for tolerance to drought stress. The scope of the study; consists of the evaluation of the effects of grafting and vermicompost applications on the morphophysiological properties of eggplant under drought stress by principal component analysis (PCA). In the experiment, different amounts of vermicompost-V (0, 1, 2, 3%) were applied to the grafting and non-grafting plants under different levels of drought stress conditions (Control; 100%; mild stress-MS; 70% and severe stress-SS; 30% irrigation) at the greenhouse. In terms of traits studied, the first two of the components had 94.39% of the total variation in the grafted plants. PCA showed that 'V(3%)+MS' and 'V(2%)+MS' were in significant and positive correlations with SPAD, leaf area-LA, relative water content-RWC, shoot dry weight-SDW, and root fresh weight -RFW. 'V(%3)+MS' and 'V(%2)+MS' applications; shoot length-SL correlated positively and weakly with stomata conductivity-g_s, shoot fresh weight-SFW, shoot diameter-SD, and root dry weight-RDW. Based on the relationships between the variables; in general, the correlations of all the examined parameters with each other were found to be significant and positive. Especially; the positive and significant correlations between SPAD and LA and RWC, SDW and RFW, g_s and SL and SFW, and RDW and SD were obvious. Consequently, the use of grafted plants and V treatments in eggplants under MS conditions improved the morphophysiological parameters of the plants and increased their tolerance to stress. Therefore, it can be seen as an effective strategy for sustainable agricultural practices.

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Aşılama ve Vermikompost Uygulamalarının Kuraklık Stresi Altındaki Patlıcanın Morfofizyolojik Özellikleri Üzerine Etkilerinin Temel Bileşen Analizi ile Değerlendirilmesi

ÖZET

Kuraklık, bitki büyümesini ve gelişim sürecini önemli ölçüde etkileyen önemli abiyotik streslerden biridir. Aşılı bitki kullanımı ve vermikompost uygulaması kuraklık stresine tolerans sağlama bakımından önemli potansiyel oluşturmaktadır. Çalışmanın amacı; aşılı bitki kullanımı ve vermikompost uygulamalarının kuraklık stresi altında patlıcanın morfofizyolojik özellikleri üzerine etkilerinin temel bileşen analizi (PCA) ile değerlendirilmesidir. Sera koşullarında gerçekleştirilen çalışmada aşılı ve aşısız bitkiler kuraklık stresi altında farklı seviyelerde (%0, 1, 2, 3) solucan humusu-V içeren koşullarda yetiştirilmiştir (kontrol: %100, hafif stres-MS: %70 ve şiddetli stres-SS: %30 sulama). İncelenen

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özellikler açısından, bileşenlerin ilk ikisi aşılanmış bitkilerdeki toplam varyasyonun %94.39'unu oluşturmaktadır. PCA, 'V(%3)+MS' ve 'V(%2)+MS'nin, SPAD, yaprak alanı-LA, bağıl su içeriği-RWC, sürgün kuru ağırlığı- SDW ve kök kuru ağırlığı-RFW ile anlamlı ve pozitif korelasyon içinde olduğunu göstermiştir. 'V(%3)+MS' ve 'V(%2)+MS' uygulamaları; sürgün uzunluğu-SL, stoma iletkenliği-g_s, sürgün yaş ağırlığı-SFW, gövde kalınlığı- SD ve kök kuru ağırlığı-RDW ile pozitif ve zayıf korelasyon sergilemiştir. Genel olarak incelenen tüm özelliklerin birbirleriyle ilişkilendirilmeleri, anlamlı ve pozitif bulunmuştur. Özellikle SPAD, LA ve RWC; SDW ve RFW; g_s, SL ve SFW arasındaki ve RDW ile SD arasındaki pozitif ve güçlü korelasyonlar dikkat çekici olmuştur. Sonuç olarak patlıcanlarda aşılı bitki kullanımı ve hafif stres koşullarında vermikompost uygulamaları, bitkilerin morfofizyolojik özelliklerini olumlu yönde etkilemiş ve strese karşı toleranslarını artırmıştır. Bu nedenle sürdürülebilir tarım uygulamaları için etkili bir strateji olarak görülebilir.

Vermikompost
Abiotik stress
PCA

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INTRODUCTION

Drought stress; it occurs when the precipitation amount is insufficient and evaporation is more than water intake (Rao et al., 2006). Plant growth slows when soil moisture decreases, and as drought stress increases, crop yield quality suffers, eventually leading to plant mortality (Ashraf & Harris 2005). Water starvation in plant cells results in decreased soluble nutrient density, decreased turgor pressure, changes in cell volume, and degeneration of the structures of several physiological and molecular components in proteins (Wahab et al., *2022).* Morphological and physiological changes such as a reduction in leaf area, a decrease in stomata number, relative water content, stomatal conductivity and a decrease in chlorophyll content occur in many vegetable species under drought stress (Ors et al., 2021; Sousa et al., 2022; Wassie et al., 2023).

After potatoes and tomatoes, eggplant is the third most significant vegetable in the Solanaceae family in terms of production (Sekara et al., 2007). Although eggplant is a moderately drought-tolerant vegetable, it is known that limited water applications can cause drought stress, metabolic activities can be impaired in case of prolonged drought, plant growth and yield are adversely affected, and even irreversible plant damage occurs (Jifeng et al., 2009; Kurniawati 2014). The use of tolerant varieties is seen as a permanent solution for reducing yield and quality losses in drought stress conditions for economically important cultural plants like eggplant. Tolerance to drought stress is controlled by many genes which complicate breeding efforts to develop drought-tolerant varieties (Toppino et al., 2022; Villanueva et al., 2023). However, there are effective strategies to reduce the negative effects of drought stress on plants, such as grafting on rootstocks with high drought resistance and the use of organic materials such as vermicompost (V) (Boyaci & Ellialtioglu, 2018; Ebrahimi et al., 2021).

It is crucial to graft on rootstocks with a high ability to withstand drought conditions and increase water use efficiency (WUE) to reduce production losses. It was determined that the use of grafted plants on rootstocks with high resistance to stress in eggplant under limited water conditions gave positive results on crop yield and quality and it was seen that it gave positive results as a curative application (Kıran et al., 2019a). However, in cases where the severity of drought stress is high this application alone may be insufficient. In this situation, the usage of organic materials such as that support to increase in the water-holding capacity of the soil can be effective in plant drought tolerance (Kıran, 2019b; Istanbuli et al., 2020).

Drought stress is a multi-faceted stress factor that directly affects plant growth and development physiology, As well as the plant the plant cell organ, and the whole organism. Conventional methods for statistically evaluating a vast amount of data relating to morphological, physiological, and biochemical characteristics in order to understand plant responses to stress conditions involve useful knowledge for each variable studied. However, these methods may be insufficient to explain the state of the relationship between two or more features and to make real

interpretations based on this. Therefore, a multivariate statistical analysis method such as Principal Component Analysis (PCA) is needed in order to make the variables easier to interpret.

In the completed study, the effects of grafted plant and vermicompost applications on the development of eggplants grown under drought stress conditions were examined in terms of morphological and physiological characteristics, and the positive effect of the grafting and vermicompost interaction on stress tolerance was determined (Kıran et al., 2023). In this study, the effect of the positive interaction of grafting and vermicompost on some morphological and physiological characteristics of eggplant grown under drought stress was evaluated by principal component analysis (PCA).

MATERIALS and METHOD

The study was conducted between April and July 2020, under naturally lighted glasshouse conditions at the Ankara-Soil, Fertilizer and Water Resources Central Research Institute (Ankara, Türkiye). The temperature and humidity control was provided automatically (23-25 ^{o}C temperature. 50% to 55% relative humidity). Local variety Aydın Siyahı, having long shaped and black colored fruits, as scion source and a commercial rootstock Köksal F_1 (Yüksel Seeds Co., Türkiye) were used as plant material. The grafting process was carried out in a private nursery Antalya Tarım Co., Türkiye). The seedlings at the 3-4 true leaf stage were planted in polyethylene pots with a size of 39×35 cm and a volume of 35 L containing clay texture soil (organic matter: 0.51% available phosphorus: 16.7 kg ha^{-1} available potassium: 546.7 kg ha^{-1}, EC: 0.94 dS m^{-1}, pH: 7.90). Fifteen days before planting V was weighed at 1%, 2% and 3% (w/w) of the soil weight in the pot and mixed to a depth of 10-15 cm.

Vermicompost (V) (organic matter: 39.2%, total phosphorus: 0.21%, total potassium: 0.94%, EC:5.62 dS m-1, pH:6.62) was obtained from Ekosol Farming & Livestock Company, Manisa, Türkiye. Stress applications were initiated 10 days after planting the seedlings. The experiment was conducted in random plots with three replications per treatment with five plants per replication adopting the factorial (Factor 1: grafted plant, Factor 2: V application, Factor 3: drought stress) experimental. Accordingly, the subjects were as follows: 1. irrigation level-drought stress: bringing the current moisture to the field capacity (Control: 100%), applying 70% (mild stress-MS) and 30% (severe stress-SS) of the water, 2. grafting (G), usage of non-grafted plant, use of the grafted plant, 3. V application at levels of 0 (control), 1%, 2%, 3%. According to the results of soil analysis, each pot received 10 kg of phosphorus and 7 kg of nitrogen per 0.247 acre in the form of triple superphosphate (TSP) and urea before planting. During the flowering and the beginning of harvest, urea fertilization was continued in each pot in the form of 3 kg of nitrogen per 0.247 acre.

Measurements of plant growth

Shoot (stem+leaves)fresh weight (SFW) and dry weight (SDW) (g plant^{-1}), At the end of the production period the fresh weight of the shoot and leaves were weighed on a 1 1000-1 precision digital scale to measure the green part's wet weight (g) and after drying in an oven set at 65^{o}C the green part dry weight (g) was detected. Shoot length (cm) (SL), At the end of the growing period, the shoot height of the plants in all application groups from the root collar to the growth tip of the plant was measured in cm using a tape measure. The shoot diameter (SD) of the plants in all treatment groups was measured using a digital caliper. The mean shoot thickness (mm) value was obtained by averaging the shoot thickness measurements taken from a point just above the graft site half the plant height and the uppermost tip node. Leaf area (cm^2) (LA) at the date of the second fruit harvest in the growing period the 3rd and 4th leaves of each plant were taken backward from the shoot tip and measured with the Licor LI-3000A (Li-Cor Lincoln, USA) model leaf area meter and the 'leaf area/plant' value was obtained.

Measurements related to the physiological properties of the plant

Leaf chlorophyll content was measured using a SPAD chlorophyll meter (Minolta, SPAD 502, Osaka, Japan). SPAD values of the uppermost fully expanded leaves were determined by reading the 3rd leaf from the top of each plant. Stomatal conductance (g_s) with a handheld steady-state portable porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA) was used to measure stomatal conductivity. It was obtained by measuring between 13.00-14.00 with a fifteen-day interval on the same leaves that were randomly selected during the experiment. Leaf proportional water content (RWC) was measured using the method described by Dhanda & Sethi (1988).

Data analysis

Principal component analysis (PCA) was applied with the MS Excel XLSTAT program to determine the relationships between the investigated parameters.

RESULTS and DISCUSSION

The Principal component analysis is an analysis method that identifies the direction of the relationship between variables and transforms the original variables in a dataset into less unrelated variables, making them more easily interpretable (Faloye et al., 2024). In this study, the drought stress tolerance of grafted and vermicomposted eggplant plants was evaluated under drought stress conditions. In results of the first 2 basic components for morphological and physiological characteristics are given in Table 1. Accordingly, the first component accounted for approximately 94.40% of the total variation and 96.49% of it could be explained by the first two components. In other words, 96.49% of the total variance of ten variables concentrated on two variables (the principal component). In addition, principal components with a value greater than 1 according to their eigenvalues were considered important. Figure 1 shows the scree plot of ten principal components. This scree plot consists of decreasing eigenvalues that provide visualization of the principal components.

Principal component loads are given in Table 2, and the loading plot created for the plant morphological and physiological characteristics of the first two principal components is given in Figure 2a. The loading plot is interpreted according to the correlation between each variable and each principal component. Accordingly, there is a positive correlation between the close (narrow-angle) variables. There is a negative correlation between the variables that are 180^0 discrete. In addition, there is no correlation between the variables with an angle of 90^0 between them, that is, the variables are independent. According to this, although it is seen that all variables have close effects on each other, it is understood that especially the SDW, SFW, and RWC variables affect the first basic component more. However, the second principal component is further defined by the LA, SD, RDW, and SPAD. There is a negative correlation between PC2 and SDW, LA, RFW, SPAD, and RWC, and a positive correlation between PC2 and the rest (Table 2).

Table 1. Total variance explained for the first two principal components
Çizelge 1. İlk iki temel bileşen için açıklanan toplam varyans

Component	Eigenvalue	% of explained variance	Cumulative variance (%)
1	9.12	91.23	91.23
2	3.19	3.190	94.42

Table 2. Coefficients and loadings of the first two basic components
Çizelge 2. İlk iki temel bileşenin katsayıları ve yükleri

Trait	PC1	PC2
SFW	0.323	0.190
SDW	0.328	-0.084
SL	0.318	0.169
SD	0.313	0.455
LA	0.314	-0.467
RFW	0.316	-0.061
RDW	0.308	0.423
SPAD	0.313	-0.443
g_s	0.306	0.152
RWC	0.323	-0.316

SFW: shoot wet weight, SDW: shoot dry weight, SL: shoot length, SD: shoot diameter, LA: leaf area, RFW: root fresh weight, RDW: root dry weight, SPAD: Chlorophyll SPAD value, g_s: stomatal conductivity, RWC: Relative water content

According to the Bi-Plot, in grafted plants, all treatments of V in the MS condition appear to be correlated with all variables studied. The combination of 'V (3%) + MS' showed a strong and positive correlation with the SPAD, LA, and RWC values. The proximity of this combination especially to SPAD and LA; indicates that 3% V application in MS medium of grafted plants supports strong increases in plant chlorophyll content and LA. Indeed, the results obtained indicate that the leaf's chlorophyll content decreases due to the damage in chloroplasts with the increase in the severity of drought stress, and grafting and V application reduce the losses in leaf chlorophyll content (Kazeminasab et al., 2016; Kıran, 2019b). Greater preservation of chlorophyll pigment, RWC, and LA in grafted plants under drought stress conditions has been reported by Jiao et al., (2023). The delay in the destruction of photosynthetic pigments by V application could be explained as the reduction of oxidative stress with the preservation of osmotic potential in the plant due to the improved water efficiency in the plant. However, the combination of 'V(2%) + MS'; and RWC had positive and very strong correlations with SDW and RFW. This indicates that the application of 2% V in MS medium has a positive effect on the plant growth of the plant. Thanks to the strong root structures of the grafted plants, the leaf water content is preserved by taking more water and nutrients from the stress condition, resulting in SDW and RFW increases that are encouraged. As a matter of fact, the Bi-plot indicates RFW increases of grafted plants in MS medium. The properties of the root that could play an

active role in water and ion uptake are directly proportional to the root genotypic properties and total root surface area (Zhang et al., 2020).

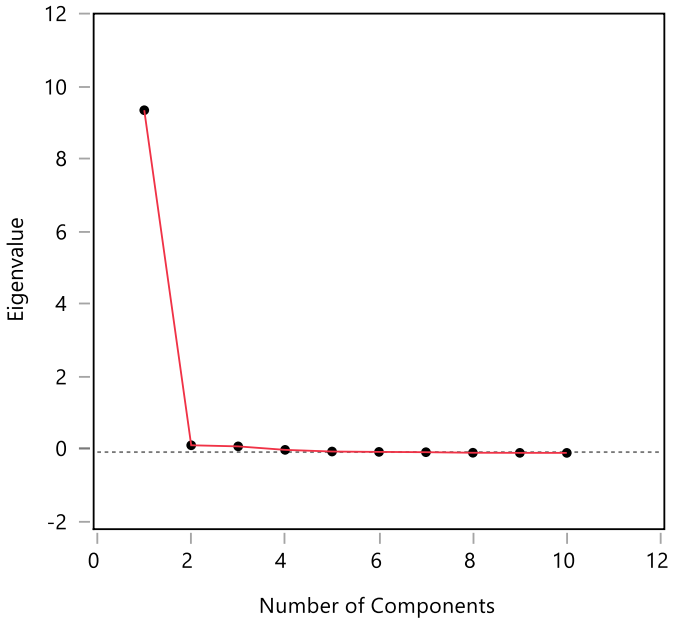

Figure 1. Scree plot of the PCA model
Şekil 1. PCA modelinin eğim grafiği

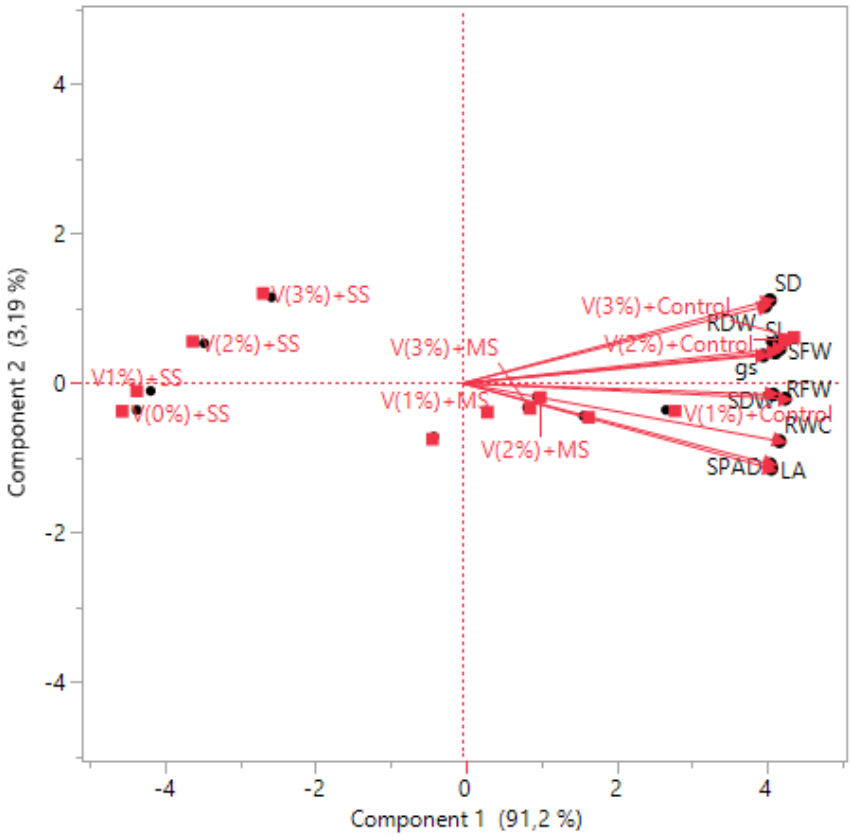

Figure 2. Morphological and physiological features in the Bi-plot explained by the two principal components in grafted plants. SFW: shoot wet weight, SDW: shoot dry weight, SL: shoot length, SD: shoot diameter, LA: leaf area, RFW: root fresh weight, RDW: root dry weight, SPAD: Chlorophyll SPAD value, g_s: stomatal conductivity, RWC: Relative

Şekil 2. Aşılı bitkilerdeki iki ana bileşenle açıklanan Bi-grafikteki morfolojik ve fizyolojik özellikler. SFW: sürgün yaş ağırlığı, SDW: sürgün kuru ağırlığı, SL: sürgün uzunluğu, SD: sürgün çapı, LA: yaprak alanı, RFW: kök taze ağırlığı, RDW: kök kuru ağırlığı, SPAD: Klorofil SPAD değeri, g_s: stoma iletkenliği, RWC: Bağıl su içeriği

It was also noted that V supports plant growth due to its positive effect such as containing growth hormones such as auxin and cytokinin, which increase plant growth, and provide nitrogen fixation (Wong et al., 2020). Similar results were reported by Ghaffari et al. (2022). The acute angles between all variables examined show that the variables are in strong and positive relationships with each other. In particular, the relationships between SPAD and LA, SDW and RFW, g_s and SFW and SL, RDW and SD were found to be quite strong and positive. Growth and development parameters are morphological phenomena that occur with cell growth, elongation, and differentiation (Osakabe et al., 2014). It is a fact that these events occurring at the whole cellular level are related to the loss of turgor that occurs due to the decrease in the relative water content in drought conditions. However, it is stated that the reduction in leaf area leads to a decrease in gas exchange due to a decrease in stomatal opening (Sousa et al., 2022). It is known that grafted plants develop different mechanisms such as accumulating solutes compatible with drought stress tolerance (Munns & Tester, 2008), transmitting some hormones from rootstock roots to the pen (El-Mog et al., 2022), and especially that high root volume is effective in water and nutrient uptake. It is stated that V helps to provide environmental conditions that affect tolerance to stress by increasing water use efficiency in arid conditions, causing changes in soil structure and texture, soil reaction, macro and micronutrient content, and organic matter content (Demir, 2019, 2020; Demir & Kıran, 2020).

CONCLUSIONS

In this study, the drought tolerance of grafted and vermicomposted eggplant plants was evaluated depending on some morphological and physiological parameters, and the positive effect of vermicompost and grafting was confirmed in the current study (Kıran et al., 2023). It is seen that each variable examined in drought tolerance has a different importance. However, PCA, by evaluating morphological and physiological characteristics as independent groups, showed that the variance observed in drought tolerance of grafted and vermicomposted plants could be explained by all variables examined. In addition, the close and positive relationships between morphological and physiological characteristics observed according to PCA confirmed that morphological changes under drought stress are directly related to physiological processes that affect plant growth. As a result, it has been shown that principal component analysis can be used as an effective method to evaluate the effects of grafted plant use and V applications on the drought stress tolerance of eggplant.

Author's Contribution

Author contributions SK designed the study, methodology, and project management, SK, ZD, HFB, KA, SZ, and ŞK performed the experiment and collected and analyzed data. SK wrote and edited the first draft, and ZD, HFB, KA, ŞK, and ŞŞE supported and corrected it. All authors read and confirmed the draft text of the manuscript.

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Statement of Conflict of Interest

The authors of the article declare that they do not have any conflict of interest.

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Topraksız Tarımda Hıyar Yetiştiriciliğinde Farklı Kompost Materyallerinin Yetiştirme Ortamı Olarak Kullanım Olanaklarının Araştırılması

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ÖZET

Çalışmanın amacı salça ve meyve suyu fabrika atıklarının kompost haline getirilerek topraksız tarımda hıyar yetiştiriciliğinde Hindistan cevizi lifi ve perlit yerine alternatif yetiştirme ortamı olarak kullanılıp kullanılamayacağının belirlenmesidir. Bu amaçla domates, elma ve üzüm atıklarından elde edilen kompostun serada topraksız tarım koşullarında hıyarın bitki gelişimi, verim ve kalite özelliklerine etkileri araştırılmıştır. Denemede kompost materyalleri, Hindistan cevizi lifi ve perlit ile farklı kombinasyonlarda karıştırılarak yetiştirme ortamları hazırlanmıştır. Çalışmada kontrol uygulaması dahil toplam 9 ortam tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak denenmiştir. Ortamlar hacim hesabına göre (1) 2:1– Hindistan cevizi lifi: perlit (kontrol), (2) 2:1– domates kompostu: perlit, (3) 2:1– elma kompostu: perlit, (4) 2:1– üzüm kompostu: perlit, (5) 1:1:1:1– domates: elma: üzüm kompostu: perlit, (6) 1:1:1– domates kompostu: Hindistan cevizi lifi: perlit, (7) 1:1:1– elma kompostu: Hindistan cevizi lifi: Perlit, (8) 1:1:1– üzüm kompostu: Hindistan cevizi lifi: perlit, (9) 1:1:1– domates-elma-üzüm kompostu: Hindistan cevizi lifi: perlit kombinasyonlarından oluşmuştur. Çalışma sonucunda kompost materyallerinin farklı oranlardaki karışımları hıyarda verim ve kalite açısından kontrol (Hindistan cevizi lifi: perlit) ortamı ile kıyasladığında, üç uygulama; (1:1:1) domates-elma-üzüm kompostu: Hindistan cevizi lifi: perlit, (2:1) elma kompostu: perlit ve (1:1:1:1) domates: elma: üzüm kompostu: perlit kombinasyonları verim açısından öne çıktığı ancak kompost ortamlarının kalite parametreleri üzerine etkisinin olmadığı görülmüştür.

Bahçe Bitkileri

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Kalite
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Topraksız tarım
Verim

Investigation of the Possibilities of Using Different Compost Materials as Growing Media in Cucumber Cultivation in Soilless Culture

ABSTRACT

The aim of the study was to determine whether tomato paste, and fruit juice factory wastes could be used as an alternative growing medium to cocopeat and perlite in the soilless cultivation of cucumber. To this end, the effects of compost made from tomato, apple, and grape wastes on plant growth, yield, and quality characteristics of cucumber under soilless culture conditions in a greenhouse were investigated. In the experiment, growing media were prepared by mixing compost materials with cocopeat, and perlite in different combinations. In the study, a total of 9 media including the control treatment were tested with 3 replicates according to the randomized experimental design. The media were (1) 2:1 cocopeat: perlite (control), (2) 2:1 tomato compost: perlite, (3) 2:1 apple compost: perlite, (4) 2:1 grape compost: perlite, (5) 1:1:1:1:1 tomato: apple: grape compost: perlite, (6) 1:1:1 tomato compost: cocopeat: perlite, (7) 1:1:1:1 apple compost: cocopeat: perlite, (8) 1:1:1:1-grape compost: cocopeat: perlite, (9) 1:1:1:1 tomato-apple-grape compost: cocopeat: perlite combinations. As a result of the study, when the mixtures of compost materials in different ratios were compared with the control (cocopeat: perlite) medium in terms of yield

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and quality of cucumber, three treatments; (1:1:1:1) tomato-apple-grape compost: cocopeat: perlite, (2:1) apple compost: perlite, and (1:1:1:1:1) tomato: apple: grape compost: perlite combinations were found to be prominent in terms of yield but compost media had no effect on quality parameters.

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GİRİŞ

Hızlı nüfus artışı ve artan teknolojik faaliyetler nedeniyle, geleneksel yöntemlere kıyasla daha yüksek verim ve daha kaliteli gıdalar üretmek için çözümler geliştirmek önemlidir. Bu bağlamda topraksız tarım sistemleri alternatif çözümler arasında yer bulmuş, kentsel yaşam ortamlarına da uygun tarım modelleri olarak ortaya çıkmaktadır.

Gerekli su ve besin maddelerinin bitki yaşamını kolaylaştıracak miktarlarda kök ortamına verilmesi şeklinde tanımlanan topraksız tarım, ortam (substrat) kültürü veya su kültürü (hidroponik) olmak üzere iki farklı şekilde yapılmaktadır (Gül, 2008). Kullanım kolaylığı ve daha az teknik bilgi gerektirmesi gibi nedenlerle ortam kültürü su kültürüne göre daha fazla kullanılmaktadır. Ortam kültüründe bitki büyümesini desteklemek için kum, perlit, turba, vermikülit, Hindistan cevizi lifi ve kaya yünü gibi ortamlar kullanılmaktadır ve bu ortamlar, besin kaynağı olmaktan ziyade bitkiler veya bitki kökleri için bir yetiştirme ortamı görevi görür (Raffar, 1990; Anonim, 2022b).

Son yıllarda, dünyada özellikle sürdürülebilir tarım, organik tarım ve çevre dostu tarım uygulamalarına olan ilgi artmıştır. Sürdürülebilir tarımsal faaliyetler sonucu elde edilen ürün için en değerli koşulların başında ise toprak verimliliği gelmektedir (Yalçın & Çimrin, 2019). Bu nedenle, kompost kullanımının önemi daha da artmıştır. Kompost, mikroorganizmalar tarafından stabilize edilen biyokimyasal olarak ayrıştırılabilir organik bir materyaldir. Toprağın fiziksel, kimyasal ve biyolojik yapısını düzenler ve ayrıca verim ve kaliteyi önemli ölçüde artırmaktadır (Kartal, 2023). Kompost, tarımsal üretimde toprağa organik madde olarak katkı sağlamanın yanında, aynı zamanda sebze yetiştiriciliğinde, topraksız tarım yöntemlerinde ve fide üretiminde torf yerine kullanılabilmektedir. Kompost üretiminde, hayvansal atıkların yanı sıra sebze ve meyve işleme fabrikalarından elde edilen katı meyve artıkları gibi çeşitli organik atıklarda kullanılmaktadır. Bu nedenle, dünya genelinde farklı meyve ve sebze atıklarını kompost olarak değerlendiren işletmeler bulunmaktadır (Sayara ve ark., 2020).

Türkiye'de torf ve Hindistan cevizi lifi, topraksız tarımda ve fide üretiminde yaygın olarak kullanılmakta ama yüksek maliyeti ve dışa bağımlılığı önemli bir sorun olarak öne çıkmaktadır. Ayrıca, kaliteli torf yataklarının küresel olarak sınırlı olması, rezervlerin giderek azalması ve özellikle son yıllarda topraksız yetiştiricilikte ortam olarak torfa olan talebin artması nedeniyle, yüksek kaliteli, düşük maliyetli alternatif substratların kullanımına doğru bir yönelim olmuştur. Bundan dolayı, doğal kaynaklardan elde edilen organik atıklar ve geri dönüştürülebilir organik atıklar, komposta dönüştürüldükten sonra torf için potansiyel ikame olarak önerilmektedir (Baran ve ark., 1995).

Çeşitli organik atıklardan elde edilmiş olan kompostlar, fiziksel, kimyasal ve biyolojik yönden çok iyi olduğu düşünülen Sphagnum torfuna alternatif olarak önerilmektedir. Bugüne kadar çok sayıda organik materyal kompost olarak denenmiş ve kullanılmıştır ve ayrıca, taze meyve-sebze işleme fabriklarından ortaya çıkan posaların kompost yapımında bitkisel atık olarak da kullanıldığı görülmektedir (Sayara ve ark., 2020).

Topraksız tarımın gelişimi, organik yetiştirme ortamlarına olan ilginin de artmasına neden olmuştur. Organik ortamların yetiştiricilikte kullanılması ile birçok avantaj sağlamaktadır. Organik ortamların, kolay temin edilebilmesi, düşük maliyetli olması ve en önemlisi kullanıldıktan sonra geri dönüşümünün kolay ve çevre dostu olması gibi avantajları bulunmaktadır (Raviv, 2013; Atzori ve ark., 2020). Avrupa Birliği ülkelerinde 2013 yılında, topraksız tarım kapsamında toplam 34.6 milyon m^3 organik ortam kullanılmıştır. Bu miktarın %75.1 torf, %7.9 kompost ve %17'sini diğer organik ortamlar oluşturur. Ayrıca, kokopit (Hindistan cevizi lifi) gibi lifli ortamların kullanımında da bir artış görülmektedir (Schmilewski, 2017; Massa ve ark., 2020).

Bu çalışmada topraksız tarım koşullarında sebze (hıyar) yetiştiriciliğinde ortam olarak dünya ve Türkiye'de en çok tercih edilen maliyeti yüksek olan torf ve Hindistan cevizi lifi yerine alternatif ortam olarak maliyeti düşük ve ayrıca sürdürülebilir çevre dostu kompostun topraksız yetiştiricilikte ortam olarak kullanılabilirliği amaçlanmıştır.

MATERYAL ve METOD

Bu çalışma 2021 yılında Tokat Gaziosmanpaşa Üniversitesi Araştırma ve Uygulama Merkezi içerisinde bulunan ısıtmalı ve tam otomasyonlu serada yürütülmüştür. Çalışmada kompost yapımında kullanılacak atık materyallerin belirlenmesi için 2019 yılında bir ön kompost çalışması yapılmıştır. Bu çalışmada Tokat'ta meyve suyu üreten Dimes Gıda San. ve Tic. A.Ş. fabrikasından domates, üzüm, elma ve şeftali atıkları temin edilerek Stoffella & Kahn (2001) ile Diacono & Montemurro (2019)'nun yöntemine göre kompostlaştırma yapılmıştır. Çalışma sonunda domates, üzüm ve elma atıklarının kompost yapımına uygun olduğu, şeftali atıklarının ise kompostlaşmaya uygun olmadığı belirlenmiştir. Bu sonuçlardan hareketle araştırmada üzüm, elma ve domates atıklarının kullanılmasına karar verilmiştir. Kompost yapımında kullanılacak olan materyaller fabrikadan 2020 yılında temin edilmiş ve kompost domates, elma ve üzüm posaları, taze ahır gübresi, sönmüş toz kireç, buğday samanı, üre gübresi, toprak ve su karışımından oluşturulmuştur.

Kompostlama sürecinde kullanılan malzemeler, hacim ölçümlerine uygun olarak hesaplanmış ve kullanılmıştır. Domates, üzüm ve elma posaları ayrı ayrı 2 m^3 olarak ölçülmüş ve üzerine 200 dm^3 yanmış ahır (inek) gübresi, 100 dm^3 saman (kurutulmuş balya formunda), 5 kg üre ve 5 kg sönmüş toz kireç ilave edilmiştir. Bu malzemeler beton zemin üzerine yığın haline getirilip, materyaller homojen şekilde birbirleri ile karıştırılmıştır. Karıştırma esnasında materyalin nem içeriği %50 oluncaya kadar sulama yapılmıştır. Materyal (kompost) beton zemin üzerinde homojen hale geldikten sonra bir metre yüksekliğe yığılmış ve üzeri şeffaf plastik örtü ile örtülmüştür. Daha sonra kompost yığınının durumuna göre ilk haftalarda iki veya üç daha sonraki haftalarda bir veya iki defa karıştırılmış, nem içeriği %50 olacak şekilde sulanmış ve tekrar üzeri örtülmüştür. Kompostun sıcaklığı 50 cm derinlikte günlük olarak ölçülmüş ve bu işlem, kompostun sıcaklığı kararlı bir dengeye ulaşana kadar 22 haftalık bir süre boyunca sürdürülmüştür. Son olarak kompost %20 nem içeriğine ulaşana kadar kurutulmuş ve depolanmıştır.

Yürütülen çalışmada yetiştirme ortamı olarak Hindistan cevizi lifi (Sera Marketim, Aksu/Antalya) ve perlit (Genper, Sarıyer/İstanbul) kullanılmıştır. Kullanılan perlitin ve Hindistan cevizinin özellikleri Çizelge 1'de; kompostun özellikleri Çizelge 2'de verilmiştir.

Araştırma, 1 Mayıs 2021 tarihinde başlamış olup 3 Kasım 2021 tarihinde sona ermiştir. Araştırmada, Olay F1 (AG Tohum, Antalya) hıyar çeşidi kullanılmıştır. Hıyar tohumları 150 gözlü viyollere, 10 Mart 2021 tarihinde ekilmiştir. Hıyar tohumları viyollere ekildikten sonra ortalama 4-5 gün karanlık ortamda bekletilerek tohumların şişmesi sağlanmış ve daha sonra viyoller fide sehpalarına dizilerek boom sistemi ile sulama ve gübreleme işlemi yapılmıştır. Sulama ve gübreleme, ilk hafta normal sulama daha sonraki haftalarda gübreli sulama şeklinde yapılmıştır. Denemede yaklaşık 40-45 gün içerisinde dikim büyüklüğüne gelen fideler (3 veya 4 gerçek yapraklı) 1 Mayıs 2021 tarihinde balkon saksılarına (6 ayaklı, yatay, 25 x 75 x 21 cm ebatlarında ve 24 L hacimli) aktarılmıştır. Her bir saksıda 3 bitki yetiştirilmiş ve her tekerrür için 2 saksı kullanılmıştır. Dolayısıyla her parselde 6 bitki yetiştirilmiştir. Gübreleme ve sulama sera içindeki otomasyon sistemi ile damla sulama şeklinde yapılmıştır. Bitkilerin beslenmesinde Hoagland & Arnon (1950) reçetesi kullanılmıştır. Fideler dikildikten yaklaşık 10 gün sonra normal sulama, 10 günden sonra N:P:K:Ca:Mg oranı 2:1:3:1.5:1 olacak şekilde hazırlanmış olan stok tanklardan çiçeklenme başlangıcına kadar 2.0 dS/m elektriksel iletkenlik (EC) ve çiçeklenmenin başlamasından sonra 2.2 dS/m EC şeklinde gübreleme yapılmıştır.

Çizelge 1. Hindistan cevizi lifi içeriği ve Perlitin kimyasal yapısı (Anonim, 2024ab)
Table 1. Content of cocopeat and chemical structure of Perlite (*Anonymus,* 2024ab)

Hindistan cevizi lifi - *Cocopeat*		Perlit-*Perlite*	
İçerik (*Contents*)	Oran (*Rate*)	İçerik (*Contents*)	Oran (*Rate*)
pH	5.7-6.5	pH	7
EC	2.5-3	Su %	3
Organik madde %	100	Si %	74
C/N	104:1	Al %	14
Azot (N) %	0.51	Na %	3
Fosfor (P) %	0.09	K %	5
Potasyum (K) %	1.87	Mg %	0.5
Kalsiyum (Ca) %	0.30	Ca %	0.5
Magnezyum (Mg) %	0.11	Fe %	1
Sülfür (S) %	0.10		
Demir (fe) ppm	289		
Bakır (Cu) ppm	17.3		
Mangan (Mn) ppm	18.4		
Çinko (Zn) ppm	17.9		
Bor (B) ppm	18.1		

Çizelge 2. Denemede kullanılan kompostların fiziksel ve kimyasal özellikleri (Anonim, 2022a)
Table 2. Physical and chemical properties of the composts used in the experiment (Anonymus, 2022a)

Kompost özellikleri (*Compost properties*)	Domates Kompostu *Tomato Compost*	Elma Kompostu *Apple Compost*	Üzüm Kompostu *Grape Compost*
Nem (%)	47.01	71.55	48.77
Organik Madde (%)	55.58	59.11	68.82
pH	8.68	7.84	8.52
C/N oranı	13.8	13.1	14.9
EC (dS/m)	5.84	6.1	2.51
Toplam Azot (N) (%)	3.06	4.09	2.71
Toplam Fosfor (P) (%)	0.68	0.88	0.58
Toplam Potasyum (K) (%)	1.38	2.85	3.26
Toplam Kalsiyum (Ca) (%)	15.8	8.91	6.54
Toplam Magnezyum (Mg) (%)	1.1	1.17	0.72
Toplam Demir (Fe) (%)	0.75	0.29	0.71
Toplam Çinko (Zn) (ppm)	170	200	250
Toplam Mangan (Mn) (ppm)	360	288	204.5
Toplam Bakır (Cu) (ppm)	180	200	210
Bakteri sayısı (kob g)	3.85×10^7	3.60×10^7	4.75×10^6

Çalışmada kompost, torf ve perlitin farklı kombinasyonları 9 farklı yetiştirme ortamı olarak kullanılmıştır (Çizelge 3).

Çizelge 3. Çalışmada kullanılan farklı yetiştirme ortam kombinasyonları
Table 3. Different growth media combinations used in the study

1. 2:1 oranında Hindistan cevizi lifi: Perlit (Kontrol)
2. 2:1 oranında Domates Kompostu: Perlit (DP)
3. 2:1 oranında Elma Kompostu: Perlit (EP)
4. 2:1 oranında Üzüm Kompostu: Perlit (ÜP)
5. 1:1:1:1 oranında Domates: Elma: Üzüm Kompostu: Perlit (DEÜP)
6. 1:1:1 oranında Domates Kompostu: Hindistan cevizi lifi: Perlit (DHP)
7. 1:1:1 oranında Elma Kompostu: Hindistan cevizi lifi: Perlit (EHP)
8. 1:1:1 oranında Üzüm Kompostu: Hindistan cevizi lifi: Perlit (ÜHP)
9. 1:1:1 oranında DEÜ Kompostu: Hindistan cevizi lifi: Perlit (DEÜHP)

Sulama aralıkları verilen suyun %20'si drene olacak şekilde haftalık olarak ayarlanmıştır. Dönemlere göre değişmekle birlikte günde 6 sulamaya kadar çıkılmış ve her sulama süresi 2 dakika olacak şekilde ayarlanmıştır. Besin çözeltisinin pH değeri 5.8'e ayarlanmıştır. Hıyar bitkilerinin drenaj çıkışlarında düzenli olarak EC ve pH ölçümleri yapılmıştır. Drenaj çözeltisinin EC değeri 3.0 dS/m'yi aştığı durumlarda, bir veya iki kez EC 1.0 dS/m çözeltisi ile sulama yapılmıştır. Benzer şekilde, drenaj çıkışlarında pH değeri 7'yi aştığında, bir veya iki EC 1.0 dS/m ve pH değeri 5.8 olan bir çözelti ile sulama yapılmıştır. Çalışmada, çoklu karışımlarda ve üzüm kompostu olan karışımlarda EC oranları yüksek çıkmamakla birlikte genellikle tekli karışımlarda (DP ve EP), EC oranları yüksek çıkmıştır. Bizde bu durumda haftalık uygulamalarla EC 1.0 dS/m çözeltisi ile sulama yapılarak EC istenen seviyelere düşürülmüştür ve ayrıca verim açısından bu durum uygulamalar arasında bir farklılığa sebep olmamıştır. Buna ek olarak, elma kompostu ve karışımları verim açısından bakıldığı zaman diğer uygulamalara göre daha iyi sonuç vermiştir.

Araştırma 03 Kasım itibari ile sonlandırılmış, uygulamaların hıyar bitkisi üzerindeki etkisini belirlemek için, birtakım gözlemler yapılmıştır. Bu çalışmada yapılan gözlemler; pazarlanabilir verim (ton/ha): Pazarlanabilir özellikte olmayan meyveler hasat döneminde toplanan meyvelerden ayrılmış ve kalan meyveler tartılmıştır. Toplam verim (ton/ha): Her bir parselden toplanan hıyar meyvelerinin ağırlıkları tartılmıştır. Pazarlanabilir meyve sayısı (adet/bitki): Her hasatta toplanan meyveler parsellere göre sayılmış ve parseldeki bitki sayısına bölünerek hesaplanmıştır. Iskarta meyve sayısı (adet/bitki): Hasat edilmiş meyveler arasından, pazarlanabilir değeri olmayan meyveler sayılmıştır. Pazarlanabilir meyve ağırlığı (g): Pazarlanabilir özellikteki meyvelerin ağırlığı meyve sayısına bölünerek ağırlıkları belirlenmiştir. Meyve suyunda pH tayini: Hıyar meyvesinin suyu çıkarılarak pH metre (Hanna HI-9812-5N, Amerika) ile ölçüm yapılmıştır. Suda çözünebilir kuru madde miktarı

tayini (SÇKM) (%): Hasatlar başladıktan sonra 4. ve 5. hasatlarda alınan meyvelerin katı meyve sıkacağı ile suları çıkarılmış ve filtre edildikten sonra zaman geçirilmeden dijital refraktometre ile ölçülmüştür. Titre edilebilir asit tayini (TA) (%): Cemeroğlu, (2010) tarafından önerilen metodolojiye uygun olarak % olarak hesaplanmıştır. Klorofil indeksi (SPAD): Bitkilerin üçüncü ve dördüncü yapraklarında (gelişimini tamamlamış yapraklar) klorofil metre (Minolta SPAD-502, Osaka, Japan) ile ölçüm yapılmıştır. C vitamini (mg/100g): C vitamini tayininde örneklerden elde edilen meyve suyundan 0.5 mL alınmış, üzerine %0,5'lik okzalik asit eklendikten sonra 5 mL'ye tamamlanmıştır. Ardından askorbik asit test kiti (Katalog no: 116981, Merck, Almanya) iki saniye süre ile çözeltiye daldırılıp, sekiz saniye dışarıda okside olması beklenmiş ve daha sonra onbeş saniyenin sonuna kadar Reflectoquant cihazı (Merck RQ flexplus 10, Türkiye) test adaptörü içerisine yerleştirilerek okuma yapılmıştır. Yaprak ve meyve kuru ağırlıkları (%): Hasat döneminin başlangıcında ve ortasında bitkilerden sağlıklı yapraklar ve meyveler alınmış ve 0.001 g hassasiyete sahip dijital terazide yaprak ve meyvelerin yaş ağırlıkları tartılmıştır. Yaş ağırlıkları alınan örnekler kese kağıtlarına konularak etüve yerleştirilmiş ve 80 °C'de kurumaya bırakılmıştır. Daha sonra kurumuş yaprak ve meyve örneklerinin yaş ve kuru ağırlıkları hassas terazi (0.01 g) ile belirlenmiştir. Yaprak besin elementi analizi**:** Bitkiler 150 ve 200 cm yüksekliğe ulaştıklarında büyüme ucunun altındaki 4. ve 5. yapraklar alınarak etüvde 65 °C'de kurutulmuştur. Kurutulan bitki örnekleri öğütülmüş ve Kjeldahl yöntemi kullanılarak (Chapman & Pratt, 1961) azot (N) tayini yapılmıştır. Öğütülen örnekler kuru yakma yöntemi ile yakılmış ve hazırlanan süzüklerde P, K, Fe, Cu, Zn ve Mn konsantrasyonları ICP-OES cihazında (Inductively Coupled Plasma) Halvin & Soltanpour (1980) yöntemi ile ölçülmüştür.

Araştırma 3 tekerrürlü olarak tesadüf parselleri deneme desenine göre yürütülmüştür. Verilerin analizinde SPSS 20.0 paket programı kullanılarak tek yönlü varyans analizi (ANOVA) yapılarak, ortalamaların karşılaştırılmasında Duncan's çoklu karşılaştırma testi %1- %5 düzeyinde kullanılmıştır.

BULGULAR ve TARTIŞMA

Kompost Uygulamalarının Hıyarda Verim Parametrelerine Etkisi

Kompost ve kompost karışımlarının yetiştirme ortamı olarak tek başına ve karışım halinde kullanılması hıyar veriminde, kontrole göre EP, EHP, DEÜP ve DEÜHP uygulamalarında önemli bir artış oluşturmuş, ancak bu artış diğer uygulamalarda (DP, ÜP,DHP,ÜHP) gözlenmemiştir. Pazarlanabilir verim 163 ton/ha ile 345 ton/ha arasında değişmiş ve en yüksek verim DEÜHP uygulamasında gözlenmiştir. Kontrol uygulamasında 216 ton/ha verim elde edilmiş olup, bu değer çalışmada kullanılan sekiz ortamın dördünde kontrolden daha yüksektir. Kontrol ile karşılaştırıldığında en yüksek verimin elde edildiği DEÜHP ortamında pazarlanabilir verim %62 oranında artmıştır. Toplam verim, pazarlanabilir verim değerleri ile benzerlik göstermiş ve toplam verim kontrole kıyasla %63 oranında artmıştır. Hem pazarlanabilir verim hem de toplam verim açısından uygulamalar arasındaki farklar $p \leq 0.001$ düzeyinde önemli bulunmuştur (Çizelge 4).

Çizelge 4. Kompost uygulamalarının hıyarda pazarlanabilir ve toplam verime etkisi
Table 4. Effect of compost applications on marketable and total yield of cucumber

Ortamlar (*Mediums*)	Pazarlanabilir Verim (ton/ha) *Marketable Yield (ton/ha)*		Toplam Verim (ton/ha) *Total Yield (ton/ha)*	
DP	163±39.90	d	173±44.28	d
EP	310± 9.48	ab	319±14.07	ab
ÜP	170±20.54	d	177±23.66	d
DHP	171±29.13	d	182±24.36	d
EHP	282±16.47	b	290±18.28	b
ÜHP	182±14.20	cd	191±14.33	cd
DEÜP	301±16.70	b	312±22.64	ab
DEÜHP	345±16.38	a	351±17.51	a
Kontrol	216± 8.59	c	224±08.84	c
P değeri	0.001		0.001	
Önem seviyesi	***		***	

*** : $p \leq 0.001$

Çalışmada kullanılan kompost ortamı kontrole kıyasla uygulamaların genelinde daha iyi sonuç verdiği gözlemlenmiştir. Bu bulgular literatürde bildirilenlerle uyumlu olduğu görülmektedir. Schroeder & Sell (2007) yılında yaptıkları çalışmada, ahır gübresinden elde edilen kompostu, hıyarda yetiştirme ortamı olarak torf ile karşılaştırmış, elde edilen sonuçlara göre pazarlanabilir verimin kompost ortamında 6.3 ile 7.96 kg/m^2 arasında, torf ortamında ise 5.2 ile 7.92 kg/m^2 arasında gerçekleştiğini ve kompost ortamından elde verimin torf ortamına

eşit olduğunu bildirmiştir. Pinamonti ve ark. (1997), hıyar yetiştiriciliğinde topraksız yetiştirme ortamı olarak torf ve kompostu karşılaştıran bir çalışmada, pazarlanabilir verim torfta 5.88 kg/bitki, kompostta ise 7.17 kg/bitki olmuş ve ikisi arasında önemli bir fark bulunmuştur. Basirat ve ark. (2022) palmiye atıklarından elde ettikleri kompostu hıyarda yetiştirme ortamı olarak kullandıkları çalışmada toplam verimin 1.91 kg/bitki olduğunu bildirmişlerdir. Buna karşılık, kontrol ortamı olarak kullanılan Hindistan cevizi lifinde toplam verim 1.97 kg/bitki olmuştur ve iki ortam arasındaki fark önemsiz olduğu bildirilmiştir. Araştırmalardan elde edilen farklı sonuçlar, kullanılan kompost türleri, yetiştirme koşulları ve diğer değişkenlerden kaynaklanıyor olabilir ve ayrıca, kompostun bileşimi, yetiştirme yöntemi veya yetiştirme ortamlarında bulunan materyalin farklı olması bu sonuçların farklılığını ortaya çıkarabilmektedir.

Araştırmada elde edilen bulgulara göre, tek başına kompost kullanımı veya kompost materyallerinin karışımı, bazı durumlarda pazarlanabilir meyve sayısı ve meyve ağırlığında artışa yol açmıştır. Çalışmada, kompost uygulamalarında pazarlanabilir meyve sayısı 73 adet/bitki ile 129 adet/bitki arasında değişirken, kontrol uygulamasında 84.61 adet/bitki olarak tespit edilmiştir. Ayrıca, denemede kullanılan 8 farklı kompost ortamından dördünde meyve ağırlığının kontrol grubundan yüksek olduğu gözlemlenmiş istatistiksel olarak $p \leq 0.001$ düzeyinde önemli bulunmuştur. Pazarlanabilir meyve ağırlıklarının da meyve sayısı ile uyumlu olduğu görülmüş, meyve sayısı bakımından kontrol grubuna göre daha yüksek sonuç veren uygulamalarda ortalama meyve ağırlığının da kontrol grubundan yüksek olduğu görülmektedir. Çalışmada, pazarlanabilir meyve ağırlığı kompost uygulamalarında 66.91 g ile 88.16 g arasında değişim gösterirken, kontrol uygulamasında bu değer 84.19 g olarak belirlenmiştir. Ortalama meyve ağırlığı bakımından uygulamalar arasındaki farklılıklar istatistiksel olarak önemli bulunmuş olmasına rağmen, kontrol ortamı ile kontrol grubundan daha yüksek ağırlığa sahip olan ortamlar arasında önemli bir fark görülmemiştir. Kompost uygulamaları arasında ıskarta meyve sayısı açısından küçük farklılıklar olsa da bu farklılıklar istatistiksel olarak önemsiz bulunmuştur (Çizelge 5).

Kompost materyallerinden bir kısmının pazarlanabilir ve toplam verimde artış sağlamasının yanında meyve sayısında da artış sağlaması yenilenebilir, dışa bağımlılığı olmayan ve çevre dostu bir materyalin ithal Hindistan cevizi lifi ortamı yerine kullanılabilecğini göstermektedir. Bu sonuçlar pek çok açıdan umut vericidir. Yapılan başka bir benzer çalışmada, Hurma atığı ve Hindistan cevizi lifinden elde edilen kompost arasında bir karşılaştırma yapılmış olup, meyve sayısı ve meyve ağırlığı açısından ikisi arasında önemli bir fark olmadığı bildirilmiştir (Basirat ve ark., 2022).

Çizelge 5. Kompost uygulamalarının hıyarda pazarlanabilir meyve sayısı, ıskarta meyve sayısı ve pazarlanabilir meyve ağırlığına etkileri

Table 5. Effects of compost applications on the number of marketable fruits, number of discarded fruits and marketable fruit weight in cucumber

Ortamlar (*Mediums*)	Pazarlanabilir Meyve Sayısı (adet/bitki) *Number of Marketable Fruits (piece/plant)*		Iskarta Meyve Sayısı (adet/bitki) *Number of Discarded Fruits (piece/plant)*	Pazarlanabilir Meyve Ağırlığı (g) *Marketable Fruit Weight (g)*	
DP	73±18.75	c	4.17±1.93	74.22±2.38	ab
EP	117± 3.28	ab	3.89±2.28	87.86±0.47	a
ÜP	80± 9.17	c	3.22±0.82	70.24±0.43	bc
DHP	84± 7.54	c	4.56±1.64	66.91±5.58	c
EHP	108± 7.34	b	3.94±1.93	86.34±1.75	a
ÜHP	85± 3.76	c	4.72±0.68	70.30±2.95	bc
DEÜP	116± 9.30	ab	4.44±2.08	85.87±2.23	a
DEÜHP	129± 6.84	a	3.00±0.58	88.16±1.78	a
Kontrol	85± 2.28	c	3.56±0.67	84.19±1.17	a
P değeri	0.001		0.662	0.001	
Önem seviyesi	***		n.s	***	

*** : $p \leq 0.001$ n.s : önemsiz

Kompost Uygulamalarının Hıyarda Kalite Özelliklerine Etkisi

Araştırmada, hıyar meyvelerinin pH ve TA üzerinde yetiştirme ortamlarının etkisi önemsiz bulunurken, SÇKM üzerine $p \leq 0.01$ düzeyinde önemli olduğu tespit edilmiştir. Kompost ortamlarında pH değerleri 6.00 (DP) ile 6.53 (EP) arasında değişirken, kontrolde 6.30 olduğu görülmüştür. Hıyar meyvelerinde TA miktarı, kompost uygulamalarında %0.217 (DHP) ile 0.260 (DEÜHP) arasında değişirken, kontrol grubunda %0.224 olarak saptanmıştır. SÇKM uygulamalarında ise bu miktar %2.83 (ÜHP) ile 3.73 (DEÜP) arasında değişirken, kontrolde %3.57 olarak belirlenmiştir. SÇKM içeriğinin en yüksek olduğu ortamlar ile kontrol grubu arasındaki fark önemli

bulunmamıştır (Çizelge 6).

Araştırmada kompostun yetiştirme ortamına ilave edilmesinin, meyve kuru ağırlığı, yaprak kuru ağırlığı ve yaprak klorofil indeksi (Spad) üzerindeki etkileri incelenmiş ve her üç özellik içinde kompost uygulamaları arasında ve kompost uygulamaları ile kontrol grubu arasında önemli farklılıklar tespit edilememiştir. Kompost uygulamaları arasında meyve kuru ağırlığı %3.87 (DHP) ile %4.82 (DEÜP) arasında değişirken, kontrol grubunda % 4.57 olarak belirlenmiştir (Çizelge 6). Yaprak kuru ağırlığı ise kompost uygulamalarında %10.08 (EHP) ile % 12.21 (DEÜP) arasında değişirken, kontrolde %10.63 olarak bulunmuştur. Hıyar yapraklarında klorofil indeksi, kompost uygulamalarına göre 58.53 (ÜHP) ile 75.00 (DP) arasında değişirken, en düşük değer kontrol grubunda 54.28 olarak tespit edilmiştir. Elde edilen sonuçlar, Du ve ark. (2015) tarafından yapılan çalışmada elde edilen sonuçlarla benzerlik göstermektedir. Araştırmacılar, üzüm posalarından elde ettikleri kompostla yetiştirdikleri hıyar meyvelerinde bitki boyu ve kuru ağırlığın, kontrol olarak kullandıkları torf + vermikulit ortamına göre daha yüksek olduğunu ve elde edilen bu farkın ise önemli olduğunu bildirmişlerdir.

Çizelge 6. Kompost uygulamalarının hıyarda pH, SÇKM, TA ve meyve kuru ağırlığı üzerine etkisi
Table 6. Effect of compost applications on pH, SÇKM, TA and fruit dry weight in cucumber.

Ortamlar (*Mediums*)	pH	SÇKM (%)		TA (%)	Meyve Kuru Ağırlığı (%) *Fruit Dry Weight (%)*
DP	6.00±0.10	3.00±0.10	de	0.237±0.046	4.80±0.23
EP	6.53±0.35	3.43±0.12	abc	0.233±0.021	4.41±0.51
ÜP	6.23±0.32	3.40±0.30	abc	0.233±0.035	4.45±0.49
DHP	6.27±0.15	3.10±0.10	cde	0.217±0.029	3.87±0.15
EHP	6.13±0.15	3.23±0.15	bcd	0.230±0.030	4.14±0.46
ÜHP	6.13±0.06	2.83±0.21	e	0.263±0.042	4.44±0.20
DEÜP	6.33±0.35	3.73±0.12	a	0.243±0.021	4.82±1.47
DEÜHP	6.27±0.15	3.23±0.32	bcd	0.260±0.046	4.55±0.10
Kontrol	6.30±0.10	3.57±0.21	ab	0.224±0.017	4.57±0.31
P değeri	0.510	0.009		0.935	0.756
Önem seviyesi	n.s	**		n.s	n.s

** : p≤0.01 n.s : önemsiz

Kompost Uygulamalarının Hıyarda Besin Elementi İçeriklerine Etkisi

Bu çalışmada, yapraktaki N içeriği, kompost uygulamalarına bağlı olarak %4.00 (ÜP) ile %4.34 (EHP) arasında değişkenlik gösterirken, kontrol grubunda %4.46 ile en yüksek seviyede tespit edilmiştir. Uygulamalara bağlı olarak P içeriği %0.54 (EHP) ile %0.63 (DHP) arasında değişkenlik gösterirken, kontrolde %0.50 ile en düşük seviyede bulunmuştur. K birikimi, N birikimiyle benzer sonuçlar elde edilmiş olup, kompost uygulamalarına bağlı olarak K %3.42 (DP) ile %3.56 (ÜP) arasında değişkenlik gösterirken, kontrol grubunda %3.59 ile en yüksek değere ulaşılmıştır. Kompost uygulamaları arasında Ca içeriği %2.13 (DP) ile 2.37 (ÜHP) arasında değişkenlik gösterirken, kontrol uygulamasında %2.26 olarak bulunmuştur (Çizelge 7).

Çizelge 7. Kompost uygulamalarının yaprakta makro besin elementi içeriğine etkisi
Table 7. Effect of compost applications on macronutrient content in leaves

Ortamlar (*Mediums*)	N (%)	P (%)	K (%)	Ca (%)
DP	4.11±0.11	0.57±0.04	3.42±0.09	2.13±0.05
EP	4.25±0.11	0.60±0.08	3.46±0.05	2.16±0.06
ÜP	4.00±0.23	0.56±0.09	3.56±0.05	2.17±0.12
DHP	4.19±0.18	0.63±0.03	3.50±0.13	2.23±0.09
EHP	4.34±0.19	0.54±0.03	3.51±0.06	2.34±0.18
ÜHP	4.20±0.06	0.56±0.09	3.50±0.09	2.37±0.07
DEÜP	4.33±0.22	0.57±0.04	3.47±0.06	2.29±0.04
DEÜHP	4.33±0.11	0.60±0.03	3.45±0.22	2.18±0.14
Kontrol	4.46±0.11	0.50±0.02	3.59±0.16	2.26±0.11
P değeri	0.937	0.963	0.952	0.913
Önem seviyesi	n.s	n.s	n.s	n.s

n.s : önemsiz

Çalışmada kompost uygulamalarının makro ve mikro besin element etkilerinde benzer sonuçlar elde edilmiştir. Hıyar yaprakları analiz sonuçlarına göre kompost ortamlarında Fe içeriği ÜHP-DEÜHP uygulamalarından sırasıyla 94.33-103.33 ppm sonuç elde edilirken, 106.00 ppm ile en yüksek sonuç kontrol uygulamasından elde edilmiştir. Uygulamalara bağlı olarak Zn içeriği en düşük 34.00 ppm DP uygulamasından en yüksek 39.00 ppm EP uygulamasından elde edilirken, kontrol grubunda 37.00 ppm olarak ölçülmüştür; sekiz kompost ortamından dördünde Zn içeriği en yüksek kontrol grubundan elde edilmiştir. Kompost uygulamalarında Cu konsantrasyonu en düşük 16.53 ppm ile DEÜP uygulaması, en yüksek DHP uygulaması 20.07 ppm arasında değişirken, kontrol grubunda 16.70 ppm olarak ölçülmüştür; kullanılan ortamlardan yedisi Cu konsantrasyonu açısından kontrol grubundan daha yüksek değer elde edilmiştir. Hıyar yapraklarında Mn konsantrasyonu kompost uygulamalarında en düşük 95.07 ile DP uygulamasından, en yüksek ise 105.33 ppm ile DHP uygulamasından elde edilmiş, kontrol grubunda 105.30 ppm olarak tespit edilmiştir. Mikro besin elementleri açısından kompost uygulamaları arasındaki farklılıklar istatistiksel olarak önemsiz olduğu görülmektedir (Çizelge 8).

Çizelge 8. Kompost uygulamalarının yaprakta mikro besin elementi içeriğine etkisi
Table 8. Effect of compost applications on micronutrient content in leaves

Ortamlar (*Mediums*)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
DP	94.67±10.07	34.00±1.00	19.13±1.99	95.07±07.00
EP	98.00±13.08	39.00±2.65	18.70±2.31	103.63±07.92
ÜP	99.67±13.20	38.00±1.85	18.83±0.50	97.93±10.46
DHP	96.67±10.26	38.67±2.52	20.07±2.31	105.33±07.07
EHP	102.67±08.08	36.67±5.13	18.30±0.60	101.17±10.09
ÜHP	94.33±09.07	37.33±6.66	18.37±0.86	96.73±05.17
DEÜP	97.67±08.50	35.00±4.58	16.53±0.71	101.50±03.22
DEÜHP	103.33±22.30	35.67±3.21	18.50±1.71	104.30±04.62
Kontrol	106.00±09.00	37.00±2.65	16.70±1.50	105.30±06.56
P değeri	0.662	0.714	0.486	0.542
Önem seviyesi	n.s	n.s	n.s	n.s

n.s : önemsiz

Çalışmada yetiştirme ortamının, hıyar yapraklarının makro ve mikro besin içerikleri üzerinde önemli bir etkiye sahip olmadığı hem makro hem de mikro besinler açısından ortamlar arasında önemli bir fark oluşmadığı görülmüştür. Ayrıca, kompost materyalleri ile kontrol ortamı arasında da önemli bir fark bulunmamıştır. Hıyar meyvelerinin besin içeriklerinin, özellikle besin alımının daha etkili olduğu ortamlarda, kontrole göre daha yüksek olması dikkat çekicidir. Yapılan çalışmalarda, kompostun hıyar yapraklarının makro ve mikro besin içerikleri üzerindeki etkisini inceleyen araştırmacılar da benzer sonuçlara ulaşmıştır. Schroeder & Sell (2007), hıyar yapraklarının N, P ve K içeriklerinin kompost ortamında sırasıyla %4.77-0.81 ve %5.41, torf ortamında ise %5.60-0.80 ve %4.96 olduğunu bildirmişlerdir. Pinamonti ve ark. (1997) N, P, K ve Ca içeriklerinin torfta %3.71, 0.39, 2.03 ve 5.83, kompostta ise 4.04-0.46-2.71 ve 5.20 olduğunu bildirmiştir. Sonuçlar, kompost ortamının dört besin maddesi açısından torftan daha etkili olduğunu göstermiştir.

Araştırmacılar, hıyar yapraklarında Fe ve Mn miktarının torf için sırasıyla 116 ve 118 ppm, kompost için ise 101 ve 94 ppm olduğunu rapor etmişlerdir. Hurma atıklarından elde edilen kompostun, Hindistan cevizi lifi + perlit karışımıyla karşılaştırıldığını belirten Basirat ve ark. (2022), hıyar yapraklarında N, P, K ve Ca içeriğinin kontrolde sırasıyla %5.16, 0.643, 4.16 ve 1.25 olduğunu, kompost ortamında ise sırasıyla %5.69, 0.727, 4.49 ve 1.89 olduğunu ve dört besin elementinin de kompost ortamında daha yüksek seviyelerde olduğunu bildirmişlerdir. Araştırmacılar, besin elementi birikimi konusundaki bulgularının, Mn dışında deneme sonuçlarıyla benzerlik gösterdiğini belirtmişlerdir.

SONUÇ

Topraksız tarımda, yetiştirme ortamı en önemli girdilerden biridir. Uygun yetiştirme ortamının seçimi ise verim ve kalite üzerinde belirgin bir etkiye sahiptir. Hem dünyada hem de Türkiye'de topraksız tarımda organik yetiştirme ortamı olarak torf ve Hindistan cevizi lifi kullanılmaktadır. Ancak torf ve Hindistan cevizi lifi dışa bağımlı materyallerdir. Özellikle Kuzey Avrupa ülkelerinde torf yataklarının azalması ve çevresel tahribatın zararlı etkileri sebebiyle torf materyalinin temin edilmesi giderek daha da zorlaşmaktadır. Ayrıca, materyallerin (torf-Hindistan cevizi lifi) taşınmasındaki ek zorluk, topraksız tarımda bu materyallere alternatif materyallerin araştırılması için bir fırsat oluşturmaktadır. Geliştirilecek alternatif ortamların steril olması son derece önemlidir.

Yerel kaynaklardan elde edilen kompostlar bu boşluğu doldurma potansiyeline sahiptir. Kompostun hem toprak uygulaması hem de topraksız bir ortam olarak bitkisel üretimdeki etkisi ve önemi üzerine çok sayıda çalışma yapılmıştır. Ancak son zamanlarda özellikle topraksız tarımda dışa bağımlılığın azalması, tarımsal atıkların tarımda yeniden kullanılması ve konvansiyonel tekniklerin insan ve çevre sağlığı açısından oluşturduğu riskler nedeniyle kompost kullanımının önemi artmıştır.

Yapılan çalışma sonucunda, domates-elma-üzüm kompostu: Hindistan cevizi lifi: perlit (DEÜHP), domates: elma: üzüm kompostu: perlit (DEÜP) ve elma kompostu: perlit (EP) kombinasyonlarının, topraksız tarımda hıyar yetiştiriciliğinde başarıyla kullanılabileceği ve ticari bir ortam olan Hindistan cevizi lifine benzer sonuçlar sergilediği ancak kompost uygulamalarının meyve kalite üzerinde herhangi bir etkisinin olmadığı görülmüştür. Bu çalışmada, ülkemizde dışa bağımlı olunan materyaller yerine yerli materyallerin alternatif olabileceği konusunda ümitvar sonuçlar elde edilmiştir.

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Genotypic Responses of Some Besni Pepper (*Capsicum annuum* L.) Genotypes to Anther Culture

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ABSTRACT

In this study, the response of Besni pepper (*Capsicum annuum* L), one of the local varieties of Turkey, to anther culture was determined. A total of 26 Besni pepper genotypes and 3 control cultivars were examined for their response to anther culture. One hundred and fifty anthers from each pepper genotype were cultured under *in vitro* conditions. A significant difference (0.0%-45.3%) was found among the genotypes in terms of response to anther culture. The highest embryo formation rate was found in genotype B11 with 45.3% (63 embryos) and the highest transformation rate to plant was found in genotype B15 with 30 plants (68%). Compared to control varieties, Besni pepper genotypes produced significantly more embryos. All genotypes except two out of 26 genotypes used produced more or less (1-68) embryos. B4, B10, B11, B12, B15, and G6 genotypes produced more than 25% of embryos and were separated from the control and other genotypes. The average embryo formation rate of the genotypes collected from Besni and Gölbaşı districts was 13% and 7%, respectively, while the embryo formation rate of the control varieties was only 0.2%. It was concluded that the Besni pepper population was highly responsive to androgenetic haploid. The highly responsive genotypes that form high embryos such as B4, B10, B11, B12, B15, and G6 have the potential to be used in developing new breeding lines and in studies investigating the genetics of anther culture.

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Bazı Besni Biberi (*Capsicum annuum* L.) Genotiplerinin Anter Kültürüne Karşı Tepkileri

ÖZET

Bu çalışmada, Türkiye'nin yerel çeşitlerinden biri olan Besni biberinin (*Capsium annuum* L.) anter kültürüne cevabı belirlenmiştir. Toplam 26 Besni biber genotipi ve 3 kontrol çeşidi anter kültürüne cevapları açısından incelenmiştir. Her biber genotipinden 150 anter *in vitro* koşullarda kültüre alınmıştır. Anter kültürüne cevap açısından genotipler arasında anlamlı bir fark (%0.0-%45.3) bulunmuştur. En yüksek embriyo oluşum oranı %45.3 (63 embriyo) ile B11 genotipinde, en yüksek bitkiye dönüşüm oranı ise 30 bitki (%68) ile B15 genotipinde bulunmuştur. Kontrol çeşitleriyle karşılaştırıldığında, Besni biber genotipleri anlamlı olarak daha fazla embriyo üretmiştir. Kullanılan 26 genotipten ikisi hariç tüm genotipler daha fazla veya daha az (1-68) embriyo üretmiştir. B4, B10, B11, B12, B15 ve G6 genotipleri %25'ten fazla embriyo üretmiş ve kontrol ve diğer genotiplerden ayrılmıştır. Besni ve Gölbaşı ilçelerinden toplanan genotiplerin ortalama embriyo oluşum oranı sırasıyla %13 ve %7 iken, kontrol çeşitlerinin embriyo oluşum oranı sadece %0.2'dir. Besni biber popülasyonunun androgenetik haploide oldukça duyarlı olduğu sonucuna varılmıştır. Yüksek embriyo oluşturan B4, B10, B11, B12, B15 ve G6 gibi oldukça duyarlı genotipler yeni ıslah hatlarının geliştirilmesinde ve

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Anahtar Kelimeler
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Genetik kaynak
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anter kültürünün genetiğinin araştırıldığı çalışmalarda kullanılma potansiyeline sahiptir.

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INTRODUCTION

Pepper (*Capsicum annuum* L.), a member of the *Solanaceae* family, is an important vegetable species that originated in South America (Vural et al., 2000). The economically important species of the genus *Capsium*, which includes 43 species (Barboza et al., 2019), are *C. annuum*, *C. frutescens*, *C. pendulum*, *C. baccaum,* and *C. pubescens* (Heiser and Smith, 1953; Samos and Kundt 1984). A diploid and self-fertile species with 2n=24 chromosomes, *C. annuum*, is the most cultivated and economically important species of *Capsicum* in both Türkiye and the world (Gyulai et al., 2000). Approximately 37 million tons of fresh (green and red) and 4.9 million tons of dried pepper are produced in the world and the world's top pepper-producing countries are China (16.81 million tons), Mexico (3.11 million tons), Indonesia (3.02 million tons) and Türkiye (3.01 million tons) (FAO, 2022). As in other plant species, the germplasm of pepper is faced with some threats arising from environmental conditions and cultivation activities. Hence, protecting pepper genetic resources and exploiting them in breeding programs is very important. During the cultivation adventure of pepper in Turkey, pepper genotypes known by the name of the regions have been selected and developed by farmers in different regions such as Demre, Uşak, Karaisalı, Gaziantep, Urfa, and Arapkir pepper. One of them is Besni Pepper, which is known as the Besni district of Adıyaman province (Şahin et al., 2022).

Turkiye is an important plant genetic resource due to its geographical location and different climate and soil conditions (Küçük, 2001). Local varieties (landraces) with high variation are very important for breeding studies due to their tolerance to biotic and abiotic stresses and their adaptation to different environmental and cultural conditions (Küçük et al., 2003). Breeding studies carried out to date for certain purposes (high yield etc.) have narrowed the genetic diversity of cultivated vegetable species. Therefore, it is important to include genetic resources that can expand the genetic infrastructure with high adaptability and exotic traits (taste, aroma, phytochemical content, etc.) in breeding programs (Xie et al., 2014).

Long periods are needed to meet the DUS (Distinct, Uniform, Stable) criteria in open-pollinated variety breeding and to produce pure parent lines in hybrid variety breeding with conventional breeding methods. Obtaining homozygous pure lines/varieties by using conventional methods requires a long time: 10-12 years/generation for open-pollinated and 6-7 years/generation for self-pollinated species (Keleş et al., 2015). The introduction of tissue culture and the ability to obtain new plants from different organs of plants with totipotency have provided plant breeders with new opportunities to accelerate new cultivar breeding (Heiser, 1976; Andrews, 1985; Hatipoğlu, 1997; Babaoğlu et al., 2001; Comlekcioglu and Ellialtioglu, 2018; Yılmaz and Güntay, 2023).

The ability to produce haploid plants *in vitro* conditions from cells with haploid chromosome numbers, such as pollen and egg cells, is very important as it shortens the cultivar breeding process. After Guha and Maheshwari (1964) demonstrated that haploid plants could be obtained by culturing anthers containing immature pollen grains of *Datura innoxia*, androgenic plant production techniques were developed very rapidly. Haploid plants can be obtained from anther culture and isolated microspore culture in many plant species. The advantageous method for producing haploid plants is the anther culture technique, which contains thousands of microspores and allows more than one haploid/double haploid plant to be formed from a single anther under suitable conditions (Gönülşen, 1987). The pioneering success of Wang et al. (1973) in China and George and Narayanaswamy (1973) in India in developing haploid embryos from *C. annuum* anthers led to significant advances in this field. Furthermore, the initiation of *in vitro* androgenesis studies on local pepper genotypes by Abak (1983) in Turkiye signifies the global importance and ongoing research of these techniques. Although genotypic selectivity exists, anther culture is the main method of producing haploid/double haploid plants in pepper. However, the complex process of haploid embryo induction and transformation to plant remains a significant challenge and hinders the efficient production of double haploid peppers (Seguí Simarro 2016). The complex interaction of various factors, including the genotype of the donor plant, growth conditions and physiological status of the donor plant, the developmental stage of microspores, anther pretreatments, optimum media combinations, and precise *in vitro* culture conditions play determining roles in achieving successful results (Çiner and Tipirdamaz, 2001; Nowaczyk and Kisiala, 2006). The natural variation of pepper species to androgenesis further emphasizes the importance of identifying and introducing high-frequency responsive genotypes for breeding to ensure the successful production of haploid embryos/plants. Therefore, it is very

important to identify genotypes with the ability to produce highly androgenic haploid plants in the germplasm and to transfer this trait to new lines (Ercan and Şensoy, 2011; Irikova et al., 2011a, b).

Several previous studies have reported that different genotypes responded differently to androgenesis in pepper. Keleş et al. (2015) used seven Charleston, six bell, eight capia, and seven green pepper genotypes and found significant differences in response to anther culture both among different pepper types and within pepper types. The highest embryo formation (20% to 5.7%) was obtained from the anthers of bell peppers, while the androgenic response of green peppers was the lowest (6.7–3.7%). Embryo formation was found to be up to 14% and 10% in capia and Charleston peppers, respectively. Ozsan and Onus (2017) reported that among four varieties, the most positive response was obtained from the capia pepper variety "Belissa" in different growing media. Ata et al. (2019) investigated the effects of different climatic conditions, different cultivation media, and genotypes on haploid embryo induction in pepper. The highest embryo formation was recorded in the İnan3363 variety with 22.14%, while the lowest was recorded in the 421 genotype with 1.40%. Shimira et al. (2019) investigated the responses of the Rwandan-origin Pili-Pili pepper variety (*C. chinense*) and Turkish pepper varieties A111, Kahramanmaraş, İnan3363 and Urfa (*C. annuum*) to anther culture in the Mediterranean Region conditions. It was reported that İnan3363 and A111 pepper varieties produced 19.4% and 4.46% haploid embryos, respectively, while the Rwandan-origin Pili-Pili variety did not produce embryos. In addition, it was determined that the appropriate anther development stage of the Pili-Pili variety, whose flower buds were examined, was not the same as the Turkish varieties, and the phase transition times of the flower buds were very short. In a study using 34 long green, 13 bell pepper, 13 Charleston, 6 California wonder, and 23 capia advanced breeding lines as plant material, it was emphasized that the response to androgenesis was dependent on the genotype and culture in medium containing activated charcoal for up to 35 days was recommended. It was also noted that the use of a medium without activated charcoal after 35 days was important to break the resistance and increase induction efficiency (Pınar et al., 2020).

Characterization of plant genetic resources and their use in plant breeding and production is very important. In species propagated from seeds such as pepper, whether open-pollinated or hybrid cultivar breeding, it is important to produce homozygous individuals. The production of pure lines that require long periods by the classical method (inbreeding) can be reduced to 1.5-2 generations by the haploidization method. For this reason, determining the response of genetic resources to haploidy applications is important. In this study, Besni pepper, one of the landraces that are a part of the genetic richness of Turkey, was used as plant material. For this purpose, a total of 26 genotypes were collected from different villages and different farmers of Besni and Gölbaşı districts. The response of these genotypes, which were morphologically characterized (Şahin et al., 2022), and three control varieties, namely Yalova Çorbacı, Sera Demre, and Cırgalan, to anther culture was investigated.

MATERIAL and METHOD

Experimental site and plant material

This study was carried out in the research greenhouses and laboratories of Erciyes University Faculty of Agriculture located at latitude 38° 42' 33" N and longitude 35° 32' 33". In the study, a total of 26 Besni pepper genotypes were collected from the villages of Besni district (20) and Gölbaşı district (6), and a total of 29 pepper genotypes, including Cırgalan, Yalova Çorbacı, and Sera Demre as control varieties, were used (Table 1). Pepper genotypes were collected by visiting villages and interviewing farmers. In terms of fruit shape, 29 genotypes include three groups: conical, bell, and elongated (Şahin et al., 2022).

Cultivation of donor plants

Seeds of the genotypes were sown in a 3:1 ratio peat-perlite mixture under unheated greenhouse conditions on 16.04.2021. The seedlings were fertilized twice with 15:15:15 (N:P: K) + microelement fertilizer until they reached planting size (3-4 true leaves). The electrical conductivity of the fertigation water was adjusted to 2 dS/m. When the seedlings reached the 3-4 true leaf stage, 3 plants from each genotype were planted in an unheated greenhouse at 80x30 cm distances on 25.05.2021. Drip irrigation was used as the irrigation system. Irrigation was done based on plant and soil observations. Fertilization was done depending on the plant development period by fertigation (Vural et al., 2000; Ifas, 2021). Black plastic mulch was used for weed control. Certified pesticides and fungicides for pepper were applied according to the disease and pest occurrence. Weeds between the rows were manually controlled.

Table 1. Pepper genotypes and sources used in the study
Çizelge 1. Çalışmada kullanılan biber genotipleri ve kaynakları

Genotypes	Sources	Fruit shape	Genotypes	Sources	Fruit shape
B1	Oyratlı Village/Besni	Cn	B16	Oyratlı Village/Besni	Cn
B2	Oyratlı Village/Besni	Cn	B17	Oyratlı Village/Besni	Cn
B3	Oyratlı Village/Besni	Cn	B18	Oyratlı Village/Besni	Cn
B4	Oyratlı Village/Besni	Cn	B19	Oyratlı Village/Besni	Cn
B5	Oyratlı Village/Besni	B	B20	Toklu Village/Besni	E
B6	Oyratlı Village/Besni	Cn	G1	Gölbaşı/City Center	E
B7	Oyratlı Village/Besni	E	G2	Gölbaşı/City Center	Cn
B8	Oyratlı Village/Besni	Cn	G3	Gölbaşı/City Center	Cn
B9	Oyratlı Village/Besni	Cn	G4	Maltepe Village/Gölbaşı	Cn
B10	Besni/City Center	Cn	G5	Maltepe Village/Gölbaşı	E
B11	Oyratlı Village/Besni	B	G6	Maltepe Village/Gölbaşı	Cn
B12	Oyratlı Village/Besni	Cn	C1 (Cırgalan)	ERÜ Agricultural Faculty	E
B13	Çamurcu Village/Besni	B	C2 (Yalova Çorbacı)	ERÜ Agricultural Faculty	E
B14	Çamurcu Village/Besni	Cn	C3 (Sera Demre)	ERÜ Agricultural Faculty	E
B15	Oyratlı Village/Besni	Cn			

B: Besni; G: Gölbaşı; C: Control; Cn: Conical; B: Bell; E: Elongate.

Anther culture

Plants were grown under the greenhouse conditions mentioned above, and flower buds on the 30th-40th day of flowering were used. The most suitable anther developmental stage for anther culture is the late-uninucleate or early-binucleate phase (beginning of the first mitotic division) in pepper. The length of the corolla should be equal to or slightly longer than the length of the calyx, and almost half of the anthers contain anthocyanin in this stage (shown with arrowheads in Figure 1) (Dumas de Vaulx et al., 1982; Bal et al., 2003; Büyükalaca et al., 2004; Mangal and Srivasatava, 2019). Flower buds used in anther culture were collected one day in advance at 17:00-18:00 and immediately transported to the laboratory in a cool and humid environment (ice box with ice pack) to preserve their viability. After the buds were washed with tap water to remove external contaminants and rinsed three times with pure water, they were subjected to low-temperature pre-treatment at 4 °C for 24 hours.

Figure 1. Pepper flower buds at the optimum stage for anther isolation (buds at the late-uninucleate or early-binucleate phase, as indicated by arrows in rows 1 and 5) (Büyükalaca et al., 2004).
Şekil 1. Anter izolasyonu için optimum aşamadaki biber çiçekleri (1. ve 5. sıralarda oklarla gösterildiği gibi, geç tek çekirdekli veya erken çift çekirdekli fazdaki tomurcuklar) (Büyükalaca ve ark., 2004).

For surface sterilization, the buds were kept in 80% ethyl alcohol for 60 seconds, then in 10% commercial sodium hypochlorite (4.5% sodium hypochlorite) for 13 minutes, and then washed with sterile distilled water three times and placed on sterile paper towels to remove excessive water in laminar air flow cabinet. After sterilization, the calyx, corolla, and filaments were removed from the buds without damaging the anthers, and the anthers were placed on a nutrient medium in 6 cm diameter glass Petri dishes using sterile forceps and scalpels (Figure 2 a, b). Isolated anthers were cultured in the induction medium whose content is given in Table 2. The culture medium was sterilized by autoclaving at 15 psi pressure at 121 °C for 30 min. The pH of the medium was adjusted to 5.8 using 1N HCl and NaOH solution before autoclaving. To prevent denaturation of the hormones, thermolabile hormones were first filter sterilized using 0.20 µm syringe filters and then added to warm (35–40 °C) autoclaved media before solidification. Anthers were planted in 6 cm diameter glass Petri dishes with their dorsal surfaces in contact with the medium (Table 2) using sterile forceps and scalpel. The planted petri dishes were labeled, wrapped with stretch film, and incubated in the dark at 35 °C for eight days for high-temperature pre-treatment. Then, the cultured anthers were incubated at 25±2 °C under 16 h light and 8 h dark conditions from the beginning of the culture in a climate cabinet. A total of 150 anthers were cultured from each genotype. The cultured anters and embryo formation were checked regularly and after 45 days, the anthers were transferred to the second hormone-free medium whose content is given in Table 3 (Figure 2 c. d). After the embryos observed in the cultured anthers germinated and reached a length of 0.3-0.5 cm, they were transferred to test tubes containing hormone-free medium (Table 3) (Figure 3 a, b). The plants that reached a certain size (2-3 true leaves) in the tubes were transferred from growth tubes to pots of 5 cm filled with sterile peat: perlite (2:1 v:v) mixture (Figure 3 c). To maintain the humidity around the plants, each pot was covered with polyethylene stretch film and 2-3 holes were opened in the stretch film for sufficient ventilation. These pots were placed in 50-liter transparent containers with lids and acclimatized at 25±2 °C and 16 hours/8 hours (light/dark) photoperiod for 5-7 days. As the plants developed, the lid and stretch film were gradually opened, and the plants were acclimated to external conditions (Figure 3 c; Figure 4). Embryo formation and transformation rates to plants were calculated according to the formulas below.

Embryo formation rate = (Number of embryos/Number of anthers cultured) x 100
Transformation rate to plant = (Number of plants/ Number of embryos) x 100

Tablo 2. Nutrient components used in anther induction medium
Çizelge 2. Anter uyartım ortamının bileşenleri

Chemicals	Concentration
MS	4.3 g
Sucrose	30 g L^{-1}
Activated charcoal	2.5 g L^{-1}
$AgNO_3$ (5 mg L^{-1})	10 mg L^{-1}
Agar	7 g L^{-1}
NAA (1 mg L^{-1})	4 mg L^{-1}
BAP	500 µl L^{-1}

Table 3. Components of hormone-free nutrient media used in anther culture
Çizelge 3. Anter kültüründe kullanılan hormonsuz besin ortamının bileşenleri

Chemicals	Concentration
MS	4.3 g L^{-1}
Sucrose	30 g L^{-1}
Silver nitrate (5 mg/L)	10 mg L^{-1}
Agar	7 g L^{-1}
pH	5.8

Correlation matrix heatmap

A heat map was created using GraphPad Software, version 10.3.1 (GraphPad Software Inc. La Jolla, CA) to visualize the correlation between morphological characters and anther culture results based on Pearson correlation. The correlation matrix heatmap displays values of the Pearson correlation coefficient, which is a measure of the strength of the linear relationship (positive/negative) between two variables. The correlation matrix heatmap shows the values of the Pearson correlation coefficient (Schober et al., 2018). A correlation matrix heat map was created between the morphological traits reported in Şahin et al. (2022) and the response parameters of genotypes to anther culture. The features with r>0.5 as a result of correlation analysis were evaluated in detail with one-way ANOVA and Cohen's (1988) eta squared and confidence intervals in the SPSS 22.0 statistical program.

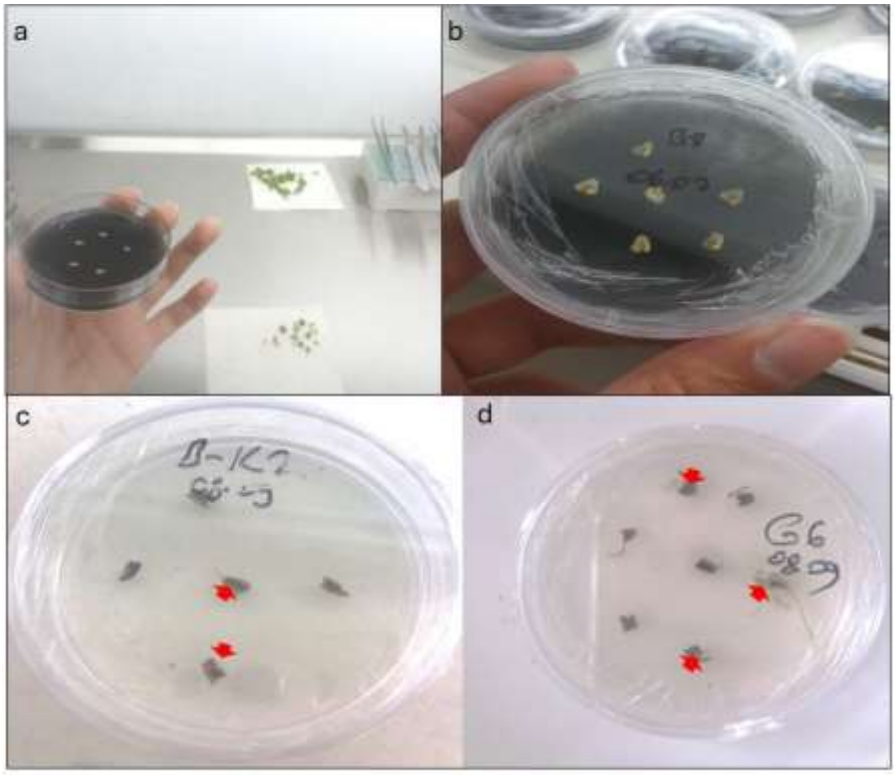

Figure 2. Stages of culturing anthers. a and b: Planting of anthers in the medium, callus (c), embryo, and root formation (d) from cultured anthers (shown with arrowheads).

Şekil 2. Anterlerin kültüre alınma aşamaları. a ve b: Anterlerin ortama ekilmesi, kültüre alınan anterlerden kallus (c), embriyo ve kök oluşumu (d) (ok uçlarıyla gösterilmiştir).

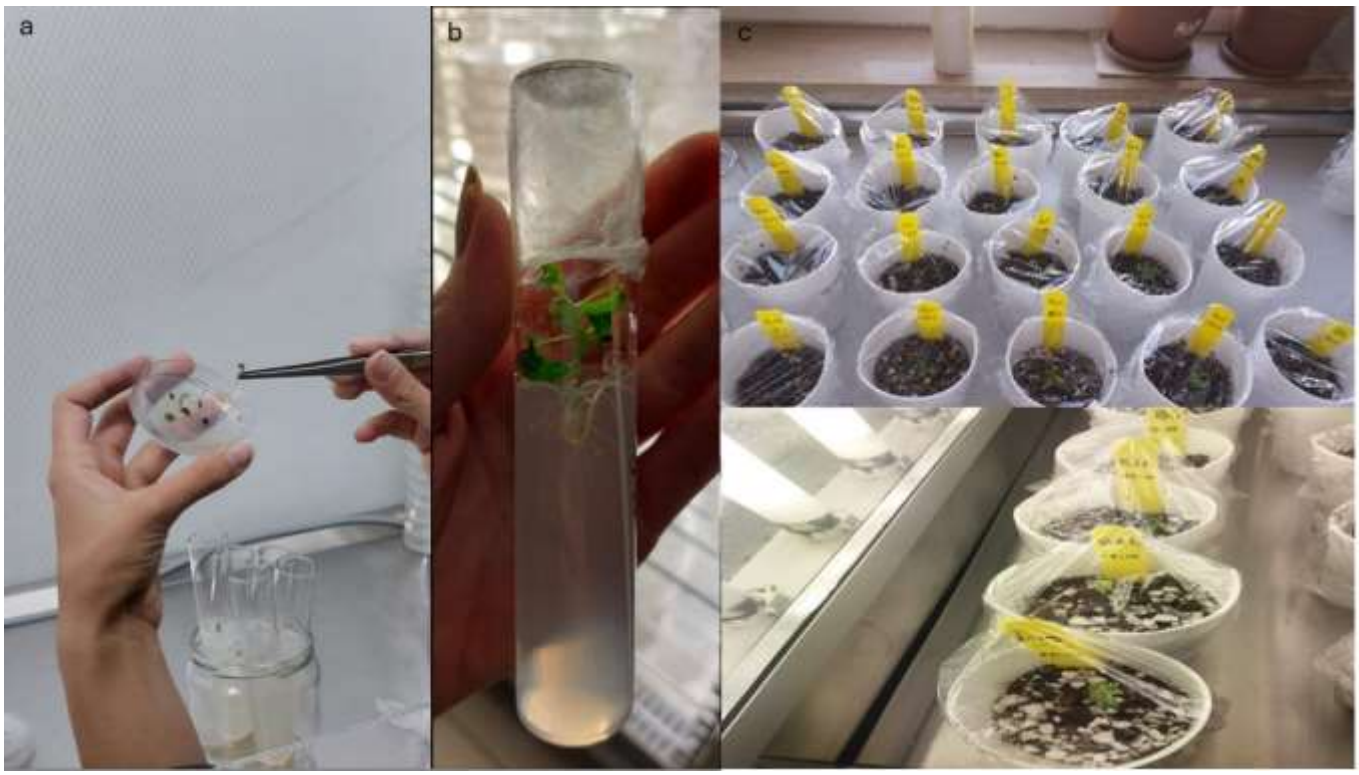

Figure 3. Transfer of developing plantlets to tubes (a and b) and plantlets transferred to sterile peat: perlite mixture and acclimatized to the outside environment (c).

Şekil 3. Gelişen bitkilerin tüplere transferi (a ve b) ve steril torf:perlit karışımına transfer edilen ve dış ortama alıştırılan bitkiler (c).

RESULTS and DISCUSSION

The responses of pepper genotypes used in the study to anther culture are presented in Table 4. One hundred and fifty anthers were cultured from each of the 29 pepper genotypes used. It was observed that there was a significant variation (0.0%-45.3%) in terms of embryo formation rate (Figure 2) among the genotypes used in the study. While the highest embryo formation rate was determined as 45.3% in genotype B11, no embryo formation was observed in genotypes B7, G5, C1, and C3. The B11 genotype had the highest embryo formation rate, followed by G6 with 31.3% and B15 with 29.3%. While the average embryo formation rate of the genotypes collected from Besni district was 13.1%, the embryo formation rate of the genotypes taken from Gölbaşı district was calculated as 7.08%. The number of haploid plants produced varied from 30 to 0 and haploid plants could not be obtained in 12 genotypes. The highest number of haploid plants was recorded in the B12 genotype with 30, while the lowest number of plants was determined in the B5, B8, and G2 genotypes with 1 plant. While the average haploid plant numbers formed by the genotypes collected from Besni and Gölbaşı were 7.8 and 1.3, respectively, haploid plant formation was not observed in the control plants. As in embryo formation and haploid plant number, significant differences were observed in the transformation rate to plant depending on the genotype. The transformation rate to plant varied between 88.9% and 0. While the highest transformation rate to plant was determined as 88.8% (30 plants) in genotype B1, no transformation was observed in 12 genotypes (B3, B7, B9, B16, B17, B20, G1, G4, G5, C1, C2, and C3). The B1 genotype was followed by B6 with 71.4% and B15 with 68%. The transformation rate of plant genotypes collected from Besni was found to be significantly higher than the genotypes collected from Gölbaşı and the control varieties. All genotypes produced a total of 491 embryos, of which 162 (33%) developed into plants. At the same time, embryo formation and plant formation were not observed in genotypes B7, G5, C1, and C3. Embryo formation was observed in genotypes B3, B9, B16, B17, B20, G1, G6, and C2, but transformation to plant was not observed. In the present study, genotypes that responded to anther culture at high levels (45%) (B11) were identified among Besni pepper genotypes. Significant differences were detected between the varieties collected from the two districts and the control varieties.

The success of anther culture is influenced by many factors, including plant and environmental (*in vivo* and *in vitro*). The plant factors include the genotype of the donor plant (Kim et al., 2004; Supena et al., 2006; Ari et al., 2016), the developmental stage of flower buds (microspore stage) (Parra-Vega et al., 2013; Mangal and Srivasatava, 2019), and the physiological state of the donor plant (Ercan et al., 2006; Ata et al., 2019), while the plant growth conditions (Ata et al., 2019), pretreatments (Dumas de Vaulx et al., 1981), nutrient medium (Supena et al., 2006; Irikova et al., 2011a, b; Bat et al., 2020), plant nutrient and carbon starvation, *in vitro* culture conditions (temperature, lighting and photoperiod), application of different additives and plant growth regulators (Niazian and Shariatpanahi, 2020) and growing season (Ercan et al., 2006; Rodeva and Cholakov, 2006) are the main environmental factors (Taskin et al., 2011).

The most important factor affecting the response to androgenesis is the genotype of the donor plants, and it varies considerably not only from species to species (inter-specific) but also within species (intra-specific). (Phippen and Ockendon, 1990; Taskin et al., 2011; Asif, 2013; Irikova et al., 2016). A variation between genotypes has been demonstrated for many field crops and horticultural crops, including the species representing *Capsicum*, *Solanum,* and *Brassica* genus (Rodeva et al., 2004, Nowaczyk et al., 2006, Lantos et al., 2012). Studies have shown that genetic differences in microspore embryogenesis can occur not only among various species, cultivars, and hybrid forms but also among individual plants of a single cultivar, depending on the plant's current physiological state (Kristiansen and Anders, 1993, Irikova and Rodeva, 2004, Nowaczyk et al., 2009). Genç (2023) reported that embryo formation induced by anther culture showed significant differences in different pepper types (fruit shape) and even in different genotypes. Atasoy et al. (2021) studied the anther culture response of a population consisting of 23 pepper genotypes of green pepper, capia, Charleston, and bell pepper. Significant differences were found among the genotypes and the embryo formation rate of the genotypes in anther culture varied between 22% and 74%. A significant portion of the genotypes used by Atasoy et al. (2021) had higher embryo formation rates (60-74%) than the genotypes we used. In a study investigating the effect of genotype on anther culture, it was reported that 11 different pepper species anther were cultured and embryos were obtained from all varieties except Kandil and Yalova Charleston (Ercan and Şensoy, 2011).

The pepper genotypes used in Ercan and Şensoy (2011) and this study were cultured under the same conditions using the same nutrient medium. However, Besni pepper genotypes formed more embryos and produced more plants. This once again demonstrated that the genotype effect is important in anther culture, as stated by Ercan and Şensoy (2011). Among the three genotypes tested by Niklas-Nowak et al. (2012), the embryo formation rate in F_2 population plants of *C. annuum* showed a significant difference (0-16%) among the genotypes. In agreement with the present study, the response to anther culture differed among the genotypes, but the genotypes used in this study produced significantly higher embryos (about three times more) compared to the cited study.

Following previous studies (Bajaj, 1980; Başay and Ellialtıoğlu, 2013; Ellialtıoğlu et al., 2015; İlhan and Kurtar, 2022), this study confirmed once again that genotype is one of the most important factors affecting the success of anther culture in pepper. Denli et al. (2022) reported at least two genes control the androgenic response in pepper and that it may be heritable. Conical and bell pepper genotypes had higher embryo formation rates.

Table 4. Responses of different Besni pepper genotypes to anther culture
Çizelge 4. Farklı Besni biber genotiplerinin anter kültürüne tepkileri

Genotypes	Number of anthers cultured	Number of embryos formed	Embryo formation rate (%)	Number of plants	Transformation rate into plant (%)
B1	150	9	6.0	8	88.9
B2	150	23	15.3	10	43.5
B3	150	15	10.0	0	0.0
B4	150	42	28.0	11	26.2
B5	150	6	4.0	1	16.7
B6	150	14	9.3	10	71.4
B7	150	0	0.0	0	0.0
B8	150	7	4.6	1	14.3
B9	150	4	0.26	0	0.0
B10	150	42	28.0	21	50.0
B11	150	68	45.3	20	29.4
B12	150	45	30.0	23	51.1
B13	150	21	14.0	3	14.3
B14	150	19	12.6	9	47.4
B15	150	44	29.3	30	68.2
B16	150	6	4.0	0	0.0
B17	150	8	5.3	0	0.0
B18	150	29	19.3	2	6.9
B19	150	10	6.6	6	60.0
B20	150	6	4.0	0	0
Besni Genotypes $\bar{X}$		20.9	13.1	7.8	37.3
G1	150	1	0.6	0	0
G2	150	5	3.3	1	20.0
G3	150	8	5.3	5	62.5
G4	150	11	7.3	2	18.2
G5	150	0	0.0	0	0
G6	150	47	31.3	0	0
Gölbaşı Genotypes $\bar{X}$		12	7.08	1.3	10.8
C1	150	0	0.0	0	0
C2	150	1	0.6	0	0
C3	150	0	0.0	0	0
Control Cultivars $\bar{X}$		0.3	0.3	0	0
Total	4350	491	11.3	163	33.2

Similarly, Grozeva et al. (2021) found a higher embryo formation rate in conical, bell, and pumpkin-shaped pepper genotypes than in long peppers, while Keleş et al. (2015) and Pınar et al. (2020) found a higher gynogenesis response in capia and bell peppers. On the other hand, Ercan and Falk (2011) reported a higher androgenic response in the long pepper variety Demre-8 compared to the other pepper varieties they used. This shows that the productivity of anther culture in pepper is affected by a series of factors such as genotype, culture conditions, pretreatments, and environmental conditions in which the plant is grown (Asif, 2013).

In this study, medium components that were found promising in previous studies were used. However, as reported in previous studies (Ercan and Şensoy, 2011; Ata et al., 2019; Genç, 2023), significant differences depending on the medium were reported in anther culture studies. Alremi et al. (2014) reported that different pepper genotypes (B, 151, 171, and Alfajer) responded differently to eight different nutrient medium combinations. It has been determined that B5 medium without silver nitrate gives a better response in embryo formation. In the current study, significant variations were observed in the rate of embryo formation and transformation to plant among pepper genotypes collected from a relatively narrow area (two districts of Adıyaman province). The results of this study also showed that anther culture was affected by the interaction of

many factors, especially genotype and culture medium. İlhan and Kurtar (2022) reported that the nutrient medium and genotype significantly affected embryo induction, formation, and plant transformation rate and that the B5 medium produced more successful results compared to the MS medium. Similarly, Özsoy (2019) reported that 209 embryos and 134 plants were obtained from the MS medium and 218 embryos and 100 plants were obtained from the B5 medium. In this study, MS was used as a basal medium, and approximately twice as many embryos were obtained compared to the results of Özsoy (2019). Obtaining different results in the same nutrient medium and culture conditions once again reveals the importance of the genetic structure and physiological status of the donor plant. The response of Elazığ pepper (Kofik), a local pepper variety, to anther culture was investigated using the same culture media and conditions used in this study and it was reported that 106 embryos were produced from a total of 1206 Petri dishes and 45 of these embryos developed into plants. It was also reported that embryo formation was not observed in 11 genotypes collected from Elazığ (Duruk, 2023). Approximately four times more embryos were obtained from the Besni pepper genotypes used in this study than from the Elazığ pepper genotypes. The conclusion from the studies and previous reports is that the effect of genotype and nutrient medium has a decisive role in the success of anther culture in pepper. The data of the study revealed that a single standard anther culture protocol does not produce the same productivity in different pepper genotypes and that appropriate protocols should be determined according to type/genotype. In addition, Pınar et al. (2020) reported that the response rate of genotypes in the culture medium may be different and emphasized that genotypes should be optimized according to time in order to obtain satisfactory results.

In this study, it was concluded that the differences detected between pepper genotypes may be the effect of a physiological variability characteristic for *in vitro* plant cultures. In addition, Şahin et al. (2022) reported that the pepper genotypes used in this study had significant morphological diversity, including flower bud size. In this study, the appropriate microspore stage study was not conducted on pepper genotypes, and since the flower size and morphology recommended in previous studies were used, the correct microspore stage for each genotype may not have been cultured. It was concluded that this also contributed to the difference between genotypes. By determining the correct flower bud stage for each genotype that did not respond to anther culture or gave a very low response (N7, G1, G5, C1, C2, and C3), and by using different media and additives, a satisfactory androgenic response can be obtained from these genotypes. Recently, many additives, including phytohormones (Khan et al., 2020; Hale et al., 2022), growth retardant hormones, stress hormones, compatible solutes, polyamines, histone deacetylase inhibitors, cellular antioxidants (enzymatic and non-enzymatic), and arabinogalactan proteins, which are endogenously produced organic compounds required to regulate plant growth and development, have been used to increase the efficiency of *in virto* haploid induction by enhancing tolerance to embryo-stimulating stresses (Niazian and Shariatpanahi, 2020; Hale et al., 2022). By using the additives listed above, it may be possible to produce androgenic haploid plants in genotypes with low or no response to anther culture in this study.

DNA methylation, histone methylation, and acetylation are important processes that control the functional state of chromatin and subsequently regulate gene expression during cell division, proliferation, and differentiation (Cedar and Begrman, 2009). One of the cellular processes that occurs during stress-induced embryogenesis is epigenetic reprogramming; essentially a general reduction in DNA methylation (Testillano, 2019). Low H3K9 methylation levels are positively correlated with microspore reprogramming from gametophytic to sporophytic development and the initiation of embryogenesis (Testillano et al., 2010; Testillano, 2019). In *Brassica napus*, high levels of acetylated histones H3Ac and H4Ac were reported in vacuolated microspores, a sign of reprogramming (Rodríguez-Sanz et al., 2014). Therefore, the addition of DNA demethylating agents and histone deacetylase inhibitors to the medium may increase the efficiency of androgenic haploid induction. Application of a DNA demethylating agent, 5-Azacytidine (AzaC) increased embryogenesis induction in isolated microspore culture of oilseed rape and *Hordeum vulgare* (í). In the same species, the application of BIX-01294, a small molecule that prevents H3K9 methylation, improved microspore reprogramming and embryogenesis (Berenguer et al., 2017).

Antioxidants, which can be enzymatic and non-enzymatic, are one of the most important components that provide ROS balance by scavenging cellular ROS accumulation (Chen et al., 2020; Hale et al., 2022). The positive effect of low-molecular-weight antioxidants glutathione and ascorbic asit on microspore embryogenesis and an increase in the number of embryo-like structures has been reported in isolated microspore cultures of triticale (Zur et al., 2019). Other materials with antioxidant characteristics, such as L-ascorbic acid, can increase the antioxidant enzyme activities and antioxidative response of treated cells (Chen et al., 2020). The ascorbic acid application under carbohydrate starvation and heat shock treatment (32 °C) caused a significant increase in the number of cotyledon embryos produced in isolated microspore culture (Heidari-Zefreh et al., 2018) and anther culture (Doğangüzel et al., 2021) in pepper. Confirming these two studies, Zeng et al. (2017) stated that the embryogenesis efficiency in isolated microspore culture of broccoli was increased by 1.2-fold and 2.5-fold with the

addition of reduced ascorbate and glutathione, respectively. In the microspore culture of flowering Chinese cabbage, a 10.33-fold increase in the frequency of embryogenesis was reported when L-ascorbic acid sodium salt was added to the NLN-13 medium (Niu et al., 2019). Methylene blue is another type of antioxidant that was reported to have a positive effect on the androgenic response of ornamental kale (Chen et al., 2019). Cell wall modification agents such as AGPs (Arabinogalactan protein) have been reported to be effective during both somatic embryogenesis (Pérez-Pérez et al., 2019) and microspore embryogenesis (Testillano, 2019; Camacho-Fernández et al., 2021). The addition of gum arabic as an AGPs carrier to the medium caused 2.8 times higher androgenesis in barley (Makowska et al., 2017), while in tomato it was found to be more effective than cold treatment and kinetin application in anther culture (Niazian et al., 2019). Different agents mentioned in this literature and whose positive effects on embryogenesis in different species have been reported can be used to obtain responses from pepper genotypes with low androgenic responses.

In the present study, the transformation rate of plantlets to mature plants was found to be 33%. It was concluded that the low transformation rate was due to inadequate laboratory/greenhouse conditions for the acclimation stage of the plantlets, acclimation being carried out on a single plant, and not all plants having the same physiological maturity. Although this rate is consistent with the results of many studies (Keleş et al., 2015; Pınar et al., 2020; Grozeva et al., 2021; Duruk, 2023; Shana et al., 2024) conducted on pepper, the loss of potential haploid plantlets produced with great effort was considered a bottleneck that needed to be overcome. This study has shown that it is risky to acclimate plants produced *in vitro* to the outside environment from a single plant without multiplying them. Instead of proceeding from a single plant, if multiple plantlets were produced to be transferred to the outdoor environment with several subcultures, the risk of a high loss rate could be overcome. Since not all plantlets formed after embryogenesis in anther culture are at the same physiological maturity (weak/strong), some weak plantlets may be lost during the acclimation phase. Plantlets with strong root and shoot development have a higher rate of forming mature plants that can be transferred successfully to the outdoor environment. In *in vitro* conditions, plants can be produced from very different tissues and organs due to the totipotency characteristics of plants, and incomplete and problematic embryos (aged, endospermless, and haploid) can be converted into mature plants (Chandra et al., 2010; Saskin et al., 2022). However, one of the disadvantages of *in vitro* plant propagation is that the survival rate of *in vitro* plants, which are produced with intensive labor and cost, is low when transferred to external conditions. One of the most important factors limiting the success of the *in vitro* plant production method is the process of acclimating the obtained plants to outdoor conditions. The success of transferring *in vitro* grown plants to external conditions is generally determined by the physiological state of the acclimatized plant (weak/strong) and the acclimatization conditions. Depending on the factors above, high losses occur during the acclimatization phase due to different factors such as light intensity, temperature, and water stress (Kumar and Rao, 2012). The main reasons for this are the inability of the plants to uptake sufficient water due to weak root development after transplanting, excessive water loss due to insufficient cuticle formation, transplanting shock, various pathogenic attacks, poor photosynthesis, and similar factors occurring in the post-transplanting period (Krishna et al., 2005; Kara et al., 2022). For any micropropagation protocol, successful rooting of plantlets is a prerequisite to facilitate acclimation to soil conditions. Only plantlets longer than 1.5 cm and with dense roots can be considered usable for acclimation (Khalafalla et al., 2011; Copetta et al., 2023). The low plant conversion rate observed in this study could be eliminated to a certain extent by increasing the number of plants to be acclimated to the external environment and by pre-transplanting applications increasing root quantity and quality. In many studies, basic media supplemented with only IBA and NAA or combinations of these hormones in the range of 0.2-2.0 ppm have been used successfully. While the addition of IBA to the medium increases primary/secondary root formation, NAA increases root hair formation. It has been reported that decreasing the concentration of inorganic salts is also beneficial in increasing root volume. Hardening of plants by exposing them to high light intensity, nutrient starvation, and low relative humidity before transplantation may contribute to reducing post-transplant mortality (Chacal and Gosal, 2002; Velasco and Watson, 2020).

Correlation analysis was performed between the morphological data produced in our previous study (Şahin et al., 2022) and the responses of genotypes to anther culture. A significant positive relationship was found between fruit shape (r=0.533; n=29), fruit cross-section shape (r= 0.519; n=29), and response to anther culture at 1% significance level (Figure 5).

The effects of fruit shape, fruit cross-sectional shape, and fruit neck formation on embryo number, embryo formation rate, plant number, and plant conversion rate parameters were evaluated in detail with eta squared and confidence intervals, where r>0.5 as a result of correlation analysis (Table 5 and 6). The eta squared value of fruit shape on embryo number is η^2=0.285, embryo formation rate is η^2=0.279, and plant formation rate is η^2=0.268. Fruit shape has a high correlation with embryo number, embryo, and plant formation rate (η^2>0.138). Eta squared value of fruit cross-section shape on embryo number is η^2=417, embryo formation rate is η^2=0.417,

and plant formation is η^2=0.240. As in fruit shape, fruit cross-section shape was found highly correlative with embryo number, embryo formation, and plant formation rate (η^2>0.138). Since the eta value of neck formation on fruit on all parameters (number of embryo, embryo, and plant formation rate) is greater than 0.138, the effect on the parameters is significant (Table 5).

Figure 4. Plants acclimatized to the outdoor environment and reached transplanting size (shown with green arrowheads), plantlets not grown (shown with red arrowheads).

Şekil 4. Dış ortama alıştırılmış ve dikim büyüklüğüne ulaşmış bitkiler (yeşil ok uçlarıyla gösterilmiştir), büyümemiş bitkicik (kırmızı ok ucuyla gösterilmiştir).

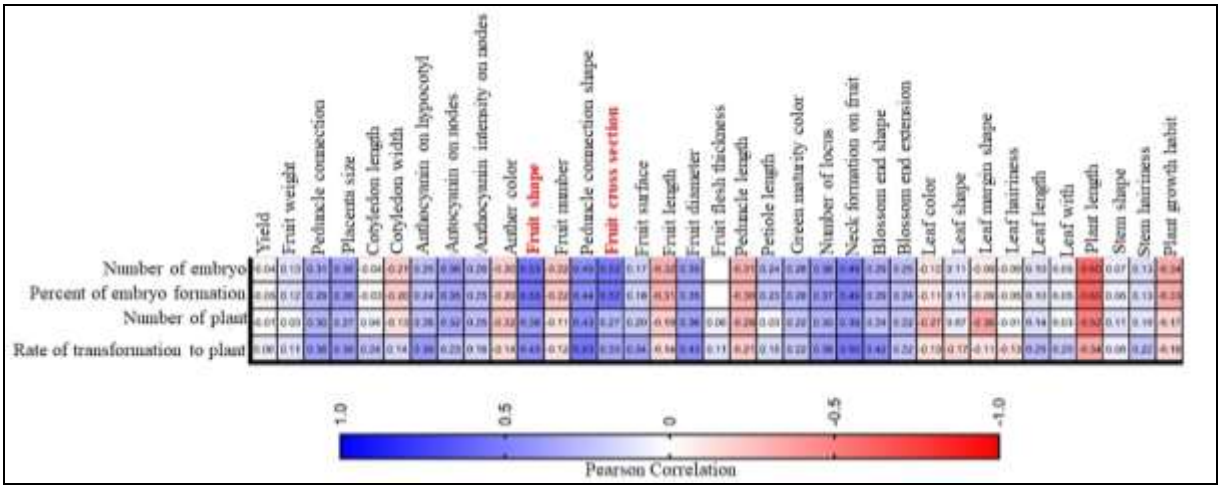

Figure 5. The correlation matrix heat map shows the values of the Pearson correlation coefficient between embryo number, embryo formation rate, plant number, and plant formation rate with plant morphological characteristics (Şahin et al., 2022), the positive values in blue and the negative in red. It ranges from -1 to 1, whereby -1 means a high negative linear relationship between variables, 1 indicates a high positive linear relationship between variables and 0 indicates that there is no relationship between studied variables.

Şekil 5. Korelasyon matrisi ısı haritası, embriyo sayısı, embriyo oluşum oranı, bitki sayısı ve bitki oluşum oranı ile bitki morfolojik özellikleri (Şahin et al., 2022) arasındaki Pearson korelasyon katsayısının değerlerini, pozitif değerleri mavi ve negatif değerleri kırmızı olarak gösterir. -1 ile 1 arasında değişir, burada -1 değişkenler arasında yüksek negatif doğrusal ilişki anlamına gelir, 1 değişkenler arasında yüksek pozitif doğrusal ilişki anlamına gelir ve 0 incelenen değişkenler arasında ilişki olmadığını gösterir.

Table 5. Eta square values for the effect of fruit shape, fruit cross-sectional shape, and fruit neck formation on embryo number, embryo formation percentage, plant number, and transformation rate (η^2:eta square)

Çizelge 5. Meyve şekli, meyve enine kesit şekli ve meyve boyun oluşumunun embriyo sayısı, embriyo oluşum yüzdesi, bitki sayısı ve bitkiye dönüşüm oranı üzerindeki etkisi için eta kare değerleri (η^2:eta kare)

Parameters	Fruit shape		Fruit cross-section shape		Neck formation on fruit	
	Sig.(p)	η^2	Sig.(p)	η^2	Sig.(p)	η^2
Number of embryo	0.013	0.285	0.001	0.417	0.008	0.236
Embryo formation rate	0.014	0.279	0.001	0.417	0.006	0.245
Number of plants	0.114	-	0.372	-	0.0357	0.153
Plant formation rate	0.017	0.268	0.028	0.240	0.006	0.247

η^2<0.01 small, η^2<0.059 medium, η^2>0.138 high, p>0.05, the eta squared of the parameters are insignificant.

When the confidence interval values of fruit shape on embryo number, embryo formation percentage, plant number, and transformation rate were examined, the confidence interval values of the genotypes with fruit shape 3 scale value did not contain zero value, so the reliability level was higher than the genotypes with the other two fruit shapes (1-4). When the confidence interval values of the genotypes with fruit cross-sectional shape 5 scale value did not include zero value, the level of reliability was higher than the genotypes with the other two fruit cross-sectional shapes (3-7). When the confidence interval values on fruit neck formation; embryo number, embryo formation percentage, plant number, and plant transformation rate were examined, the confidence interval values of the genotypes with fruit shape 1 scale value did not include zero value, so the level of reliability was higher than the genotypes with the other scale value (0) (Table 6).

CONCLUSIONS and RECOMMENDATIONS

In this study, 26 pepper genotypes of Besni pepper, a local pepper landrace, collected from Besni and Gölbaşı districts, and Yalova Çorbacı, Sera Demre, and Cırgalan pepper varieties as controls were used to investigate their responses to androgenic embryogenesis. As a result, Besni pepper responded positively (24 genotypes from 26 genotypes) to anther culture. While the embryo formation rate varied between 45.3 and 0.6, 11 of the pepper genotypes had embryo formation rates of 10% and above. This rate is higher than the rates reported in most previous studies. B11 (45.3%), B12 (30%), B10 (28%), B4 (28%) and G6 (31.3%) had significantly higher (>25%) embryo formation rates. The transformation rate of the embryos into plants varied between 0% and 89%. The highest rate was obtained in the B1 genotype, while no transformation into plants occurred in the 6 genotypes that formed embryos. It was concluded that it would be possible to produce pure lines from the Besni pepper population in a short time. As can be seen, there is a significant variation both in the rate of embryo formation and the rate of embryos turning into mature plants. The physiological and genetic basis of this difference can be investigated using genotypes with high androgenic response and genotypes with very low or no response. In addition, the effects of different flower bud sizes (microspore stage) and different culture media supplemented with promising additives (arabinagalactone, anti-oxidants, osmotic protectors, etc.) indicated in the discussion section on the success of anther culture in very low-reactive and non-reactive genotypes can be investigated. For this reason, the inheritance of this high androgenic plant formation potential detected in the Besni pepper population and the possibility of transferring it to lines with low androgenic response but high agronomic potential are among the important research topics. Of the 490 embryos formed, 133 developed into plants. The plant formation rate of the embryos was calculated as 33%. In other words, 67% could not be turned into plants. Therefore, studies should be conducted to develop protocols for transforming plantlets into mature plants (concentration and composition of nutrients and hormones used in vitro), hardening plantlets, and improving strong root and shoot development before acclimation to external conditions.

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Table 6. Effect of fruit shape, fruit cross-sectional shape, and fruit neck formation on embryo number, embryo and, plant transformation rate

Çizelge 6. Meyve şekli, meyve enine kesit şekli ve meyvede boynu oluşumunun embriyo sayısı, embriyo oluşum oranı, bitki sayısı ve bitkiye dönüşüm oranı üzerine etkisi

Parameters		Scale	n	Mean	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper mound		
Fruit shape	Number of embryo	1	7	1.14±0.83	-0.89	3.17	0.00	6.00
		3	19	20.42±3.62	12.81	28.03	4.00	47.00
		4	3	31.67±18.69	-48.69	112.02	6.00	68.00
		Total	29	16.93±3.37	10.02	23.84	0.00	68.00
	Embryo formation rate	1	7	0.74±0.55	-0.61	2.10	0.00	4.00
		3	19	13.46±2.45	8.31	18.61	0.26	31.30
		4	3	21.10±12.44	-32.42	74.62	4.00	45.30
		Total	29	11.18±2.26	6.55	15.81	0.00	45.30
	Number of plants	1	7	0.00±0.00	0.00	0.00	0.00	0.00
		3	19	7.32±2.02	3.08	11.55	0.00	30.00
		4	3	8.00±6.03	-17.94	33.94	1.00	20.00
		Total	29	5.62±1.53	2.49	8.75	0.00	30.00
	Plant formation arte	1	7	0.00±0.00	0.00	0.00	0.00	0.00
		3	19	33.08±6.68	19.04	47.12	0.00	88.89
		4	3	20.12±4.70	-0.08	40.33	14.29	29.41
		Total	29	23.75±5.09	13.32	34.19	0.00	88.89
Fruit Cross-Section Shape	Number of embryo	3	11	9.82±4.78	-1.03	20.66	0.00	42.00
		5	16	16.75±3.26	9.79	23.71	1.00	45.00
		7	2	57.50±10.50	-75.92	190.92	47.00	68.00
		Total	29	16.93±3.37	10.02	23.84	0.00	68.00
	Embryo formation rate	3	11	6.31±3.28	-0.99	13.62	0.00	28.00
		5	16	11.14±2.18	6.50	15.78	0.60	30.00
		7	2	38.30±7.00	-50.64	127.24	31.30	45.30
		Total	29	11.18±2.26	6.55	15.81	0.00	45.30
	Number of plants	3	11	3.00±2.05	-1.58	7.58	0.00	21.00
		5	16	6.88±2.14	2.32	11.43	0.00	30.00
		7	2	10.00±10.00	-117.06	137.06	0.00	20.00
		Total	29	5.62±1.53	2.49	8.75	0.00	30.00
	Plant formation rate	3	11	8.23±4.90	-2.70	19.15	0.00	50.00
		5	16	35.56±7.34	19.92	51.21	0.00	88.89
		7	2	14.71±14.71	-172.15	201.56	0.00	29.41
		Total	29	23.75±5.09	13.32	34.19	0.00	88.89
Neck formation on fruit	Number of embryo	0	7	1.57±0.92	-0.68	3.83	0.00	6.00
		1	22	21.82±3.90	13.71	29.92	1.00	68.00
		Total	29	16.93±3.37	10.02	23.84	0.00	68.00
	Embryo formation rate	0	7	0.69±056.	-0.67	2.06	0.00	4.00
		1	22	14.52±2.60	9.11	19.92	0.60	45.30
		Total	29	11.18±2.26	6.55	15.81	0.00	45.30
	Number of plants	0	7	0.00±0.00	0.00	0.00	0.00	0.00
		1	22	7.41±1.87	3.53	11.29	0.00	30.00
		Total	29	5.62±1.53	2.49	8.75	0.00	30.00
	Plant formation rate	0	7	0.00±0.00	0.00	0.00	0.00	0.00
		1	22	31.31±5.86	19.13	43.49	0.00	88.89
		Total	29	23.75±5.09	13.32	34.19	0.00	88.89

Fruit shape (1: Elongate; 3: Conical 4: Bell); Fruit cross-section shape (3: Slightly corrugated; 5: Intermediate; 7: Corrugated) and Neck formation on fruit (0: Absent; 1: Present).

Contribution of the Authors

The data of this study were collected by Prof. Dr. Halit Yetişir, Agricultural Engineer Mh. Miraç Şahin, Assoc. Prof. Dr. Hasan Pınar. Erciyes University, Scientific Research Project Coordination Office provided support for the conduct of the study. Laboratory analyses of the study were conducted by Agricultural Engineer Mh. Miraç Şahin and statistical analyses were conducted by Dr. Faculty member Alim Aydın. The text of the article was written by Agricultural Engineer Mh. Miraç Şahin under the supervision of Prof. Dr. Halit Yetişir.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Ichneumonidae (Hymenoptera) Biodiversity of Karlıova (Bingöl) in Türkiye

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ABSTRACT

This faunistic research was carried out to determine the species of Ichneumonidae (Hymenoptera) collected from Karlıova district of Bingöl province between 2022-2023. Adults of Ichneumonidae specimens collected from flowering plants and weeds in the deep passes and valleys of Karlıova district of Bingöl province samples were collected by entomological net (atrap) on vegetation period and between the altitudes of 1761-1963 m. As a results of this research, 10 different subfamilies (Anomaloninae Viereck, 1918; Banchinae Wesmael, 1845; Campopleginae Forster, 1869; Cremastinae Forster, 1869; Cryptinae Kirby, 1837; Cylloceriinae Wahl, 1990; Ichneumoninae Latreille, 1802; Ophioninae, Shuckard, 1840; Pimplinae Wesmael, 1845 and Tryphoninae Shuckard, 1840) belonging to 16 genera, 256 individuals were collected and 18 species were identified. Among these 18 identified species, 15 species, except three species, were determined as new records for Bingöl province, *Cremastus spectator* Gravenhorst, 1829 and *Aritranis longicauda* (Kriechbaumer, 1873) species were determined as new records for the Eastern Anatolia Region.

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ÖZET

Bu faunistik araştırma, Bingöl ili Karlıova ilçesinden 2022-2023 yılları arasında toplanan Ichneumonidae türlerinin belirlenmesi amacıyla yapılmıştır. Bingöl ili Karlıova ilçesi derin geçit ve vadilerindeki çiçekli bitki ve yabancı otlardan toplanan Ichneumonidae örneklerinin erginleri, vejetasyon döneminde ve 1761-1963 m rakımlar arasında entomolojik ağ (atrap) ile toplanmıştır. Bu araştırma sonucunda 10 farklı alt familya (Anomaloninae Viereck, 1918; Banchinae Wesmael, 1845; Campopleginae Forster, 1869; Cremastinae Forster, 1869; Cryptinae Kirby, 1837; Cylloceriinae Wahl, 1990; Ichneumoninae Latreille, 1802; Ophioninae, Shuckard, 1840; Pimplinae Wesmael, 1845 and Tryphoninae Shuckard, 1840)'ya bağlı 16 cinse ait 256 birey toplanmış ve 18 tür tespit edilmiştir. Tespit edilen 18 türden 3 tür hariç 15 tür Bingöl ili için yeni kayıt, *Cremastus specator* Gravenhorst, 1829 ve *Aritranis longicauda* (Kriechbaumer, 1873) türü ise Doğu Anadolu Bölgesi için yeni kayıt olarak belirlenmiştir.

Bitki Koruma

Araştırma Makalesi

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Geliş Tarihi : 04.11.2024
Kabul Tarihi : 05.01.2025

Anahtar Kelimeler
Ichneumonidae
Biyoçeşitlilik
Yeni kayıtlar
Bingöl
Türkiye

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INTRODUCTION

The Ichneumonidae, known as "ichneumon wasps", "ihneumonids", "Darwin wasps" are a family of parasitoid wasps of the insect of Hymenoptera.

They are one of the most diverse groups within Hymenoptera with roughly 25,000 species described (Yu et al., 2016).

They fulfill an important role as regulators of insect populations, in natural and semi-natural systems, making them promising agents for biological control (Klopfstein et al., 2019).

The Ichneumonidae constitute one of the largest families in the animal kingdom. This family is important because

their larvae can be either endo or ectoparasitoids of larvae or pupae of holometabolous insects and Chelicerata (Fernandes et al., 2019).

In recent studies in Türkiye, the number of Ichneumonidae species was updated to 1451. (Barik, 2022; Birol, 2022; Çoruh, 2002; Doğru, 2022; Ataş & Çoruh, 2022; Çoruh, & Riedel, 2022; İneciklioğlu, 2022; Kolarov & Çoruh, 2022; Korkmaz & Çoruh, 2022; Teymuroğlu & Çoruh, 2022; Çoruh, Kolarov & Ercelep, 2022a; Çoruh, Tezcan & Gülperçin 2022b; Kaplan, 2023; Ataş & Çoruh, 2023; Barik & Çoruh, 2023a, Barik & Çoruh, 2023 b; Narmanlıoğlu & Çoruh, 2023; Kaplan, 2024; Ayhan & Çoruh, 2024; Çoruh & Kolarov, 2024; Korkmaz & Çoruh, 2024).

The study is conducted to identify Ichneumonidae (Hymenoptera) species in Karlıova district of Bingöl province and to contribute to Ichneumonidae biodiversity.

MATERIALS and METHODS

Data sampling

Adults of Ichneumonidae specimens collected from flowering plants and weeds in the deep passes and valleys of Karlıova district of Bingöl province (Figures 1, 2) constitute the material of the study. Adult samples - were collected by entomological net (atrap) in the years 2022-2023 vegetation period and between the altitudes of 1761-1963 m (Table 1).

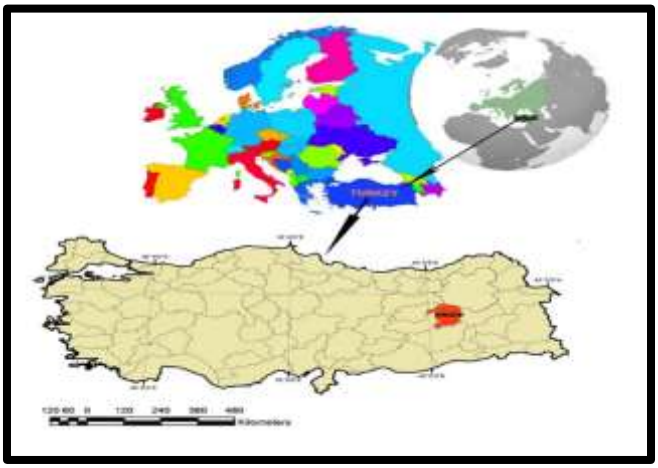


Figure 1. Map of study area.
Şekil 1. Araştırma alanın haritası.

Figure 2. Research localities.
Şekil 2. Çalışma alanından lokaliteler.

Study area

Survey studies were carried out in the Karlova district of Bingöl province. The research area's unique characteristics—deep gorges and valleys, location between mountains and small lakes at different altitudes, and outflow of water resources—were effective in choosing it as the study area.

Karlıova district is located in the Upper Euphrates Section of the Eastern Anatolia Region. The settlement, located in the northeast of the Central district of Bingöl province, is located in the middle part of the mountains, which extend roughly in the east-west direction and are mostly 3000 m high (Karagöl Mountains 3057 m, Bingöl Mountain 3193 m, Satan Mountains 2,839 m, Şerafettin Mountains 2388 m). The region, whose altitude exceeds 1900 meters and where high flat areas cover a large area, also has the feature of being the hydrographic border where several rivers (Peri Stream, Göynük Stream, Murat River) receive their sources. The district's surface area is 1,311 km². Its ratio to the provincial surface area is 16.60%. The district's altitude above sea level is 1,940 meters. Its distance from the city center is 70 km. Sunrise can be watched within the borders of this district (Anonymous, 2024 a,b).

The study material was collected from 10 different localities (Karlıova center, Çukurtepe, Hacılar, Göynük, Yoncalık, Halifan, Hasanova, Kargapazarı, Viranşehir and Ortaköy) in the Karlıova district of Bingöl province (Table 1).

Laboratory studies

All the materials that make up the study were collected and photographed by the first author. Ichneumonid adults transported to the laboratory were prepared for identification, subfamily density composition was made according to the discriminatory taxonomic characters and preserved. After the field studies were completely completed, genus and species information was obtained, identified samples were used in the identification of the samples, some of the identifications were made in the Hikmet Özbek Taxonomy Laboratory by second author, while the unidentified samples were identified by Dr. J. KOLAROV (Bulgaria).

After the species were identified, the appearance of each species was monitored using the digital shooting unit (Canon EOS 1100D camera, Canon EF 100 mm, f/2.8L Macro lens, Kaiser digital shooting unit) installed in the Ataturk University Biodiversity Application and Research Center and the Lenovo Research brand camera Helicon focus 6.7.1. program. The names of species and their distribution in world and associated plants were used in a limited number from Yu et al. (2016) catalog.

Table 1. Localities speceies are collected
Çizelge 1. Türlerin toplandığı lokaliteler

Locality	Altitude (m)	Cordinates	
ÇUKURTEPE	1881	39°24'14.76"K	41° 2'5.64"D
ÇUKURTEPE	1874	39°24'13.68"K	41° 2'10.68"D
ÇUKURTEPE	1919	39°25'6.96"K	41° 2'17.16"D
ÇUKURTEPE	1920	39°25'7.68"K	41° 2'17.16"D
ÇUKURTEPE	1941	39°25'11.28"K	41° 2'29.04"D
HACILAR	1444	39° 5'47.40"K	40°48'52.92"D
GÖYNÜK	1761	39° 9'2.52"K	40°53'54.96"D
YONCALIK	1936	39°20'8.16"K	41° 4'39.72"D
YONCALIK	1937	39°20'2.40"K	41° 4'46.92"D
YONCALIK	1938	39°20'4.20"K	41° 4'49.08"D
YONCALIK	1936	39°20'6.36"K	41° 4'45.48"D
HALİFAN	1694	39° 8'37.32"K	40°52'1.20"D
HALİFAN	1684	39° 8'31.20"K	40°51'55.80"D
HALİFAN	1728	39° 8'45.24"K	40°52'26.40"D
HALİFAN	1727	39° 8'44.88"K	40°52'26.04"D
HASANOVA	1961	39°10'19.92"K	41° 2'16.80"D
KARGAPAZARI	1963	39°18'46.08"K	41° 8'22.20"D
KARGAPAZAR)	1956	39°18'47.88"K	41° 8'18.96"D
KARGAPAZARI	1816	39°18'44.99"K	41° 5'52.61"D
KARGAPAZARI	1826	39°18'44.74"K	41° 5'56.26"D
KARGAPAZARI	1803	39°17'33.36"K	41° 4'27.48"D
KARGAPAZARI	1802	39°17'41.28"K	41° 4'23.16"D
VİRANŞEHİR	1897	39°23'15.72"K	40°58'19.92"D
VİRANŞEHİR	1894	39°23'15.00"K	40°58'19.56"D
KARLIOVA	1866	39°17'43.44"K	40°59'48.12"D
KARLIOVA	1857	39°17'43.08"K	40°59'43.08"D
KARLIOVA	1861	39°17'33.00"K	40°59'45.24"D
KARLIOVA	1863	39°17'33.72"K	40°59'48.48"D
KARLIOVA	1785	39°18'8.28"K	41° 1'32.16"D
KARLIOVA	1786 m	39°18'10.44"K	41° 1'32.52"D
KARLIOVA	1788 m	39°18'6.48"K	41° 1'33.60"D
KARLIOVA	1793 m	39°18'2.16"K	41° 1'20.64"D
ORTAKÖY	1967 m	39°24'6.84"K	40°53'16.44"D
ORTAKÖY	1981 m	39°24'7.56"K	40°53'15.72"D
ORTAKÖY	1955 m	39°23'50.28"K	40°53'32.64"D
ORTAKÖY	1909 m	39°23'47.76"K	40°53'39.12"D
ORTAKÖY	1931 m	39°23'52.44"K	40°53'35.16"D
ORTAKÖY	1925 m	39°23'52.80"K	40°53'33.72"D
ORTAKÖY	1929 m	39°23'54.96"K	40°53'33.72"D
ORTAKÖY	1973 m	39°24'19.80"K	40°53'27.60"D
ORTAKÖY	1971 m	39°24'21.24"K	40°53'25.80"D
ORTAKÖY	1784 m	39°23'32.28"K	40°53'13.20"D

RESULTS

During field studies, 256 specimens belonging to 16 genera belonging to subfamilies Anomaloninae Viereck, 1918; Banchinae Wesmael, 1845; Campopleginae Forster, 1869; Cremastinae Forster, 1869; Cryptinae Kirby, 1837; Cylloceriinae Wahl, 1990; Ichneumoninae Latreille, 1802; Ophioninae Shuckard, 1840; Pimplinae Wesmael, 1845 and Tryphoninae Shuckard, 1840 were collected and 18 species were identified. Of these, *Cremastus spectator* Gravenhorst, 1829 and *Aritranis longicauda* (Kriechbaumer, 1873) were determined to be new East Anatolia Region. The species are listed below:

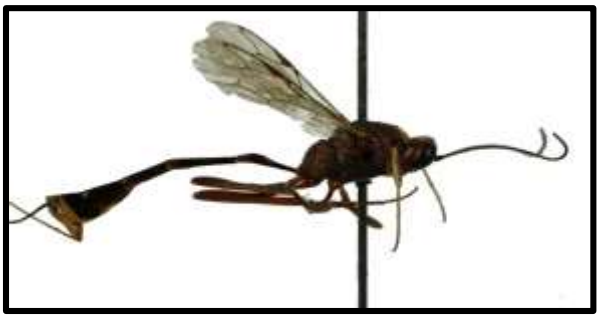
Anomaloninae Viereck, 1918

Anomalon cruentatum (Geoffroy, 1785) (Figure 3-1a).

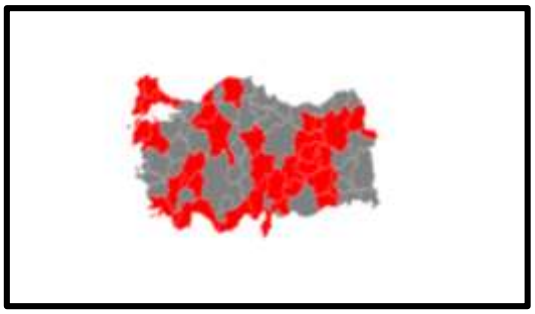
Material examined: Göynük: 39° 9' 2.52" N, 40° 53' 54.96" E, 1761 m, 20.X.2022, 5 ♀♀. Çukurtepe: 39° 25' 11.28" N, 41° 2' 29.04" E, 1941 m, 06.XI.2022, 4 ♀♀. Halifan: 39° 8' 31.20" N, 40° 51' 55.80" E, 1684 m, 20.XI.2022, ♂, ♀; 39° 8' 37.32" N, 40° 52' 1.20" E, 1694 m, 20.XI.2022, 2 ♀♀; 39° 8' 44.88" N, 40° 52' 26.04" E, 1727 m, 20.XI.2022, 3 ♂♂; 39° 8' 45.24" N, 40° 52' 26.40" E, 1728 m, 20.XI.2022, 2 ♂♂, ♀. Viranşehir: 39° 23' 15.00" N, 40° 58' 19.56" E, 1894 m, 02.VII.2023, 5 ♂♂, 2 ♀♀. Ortaköy: 39° 23' 47.76" N, 40° 53' 39.12" E, 1909 m, 26.VII.2023, 2 ♀♀; 39° 23' 52.80" N, 40° 53' 33.72" E, 1925 m, 26.VIII.2023, 5 ♂♂; 39° 23' 54.96" N, 40° 53' 33.72" E, 1929 m, 26.VIII.2023, 5 ♂♂. Karlıova: 39° 18' 2.16" N, 41° 1' 20.64" E, 1793 m, 23.IX.2023, 3 ♂♂, 5 ♀♀.

Distribution: Oriental and Palaearctic, known from Türkiye (Figure 3-1b,Table 2).

Associate plants: *Anthriscus sylvestris* (L.), *Peucedanum oreoselinum* (L.) (Yu et al., 2016).



1a



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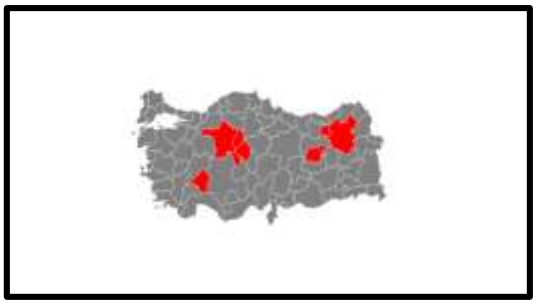
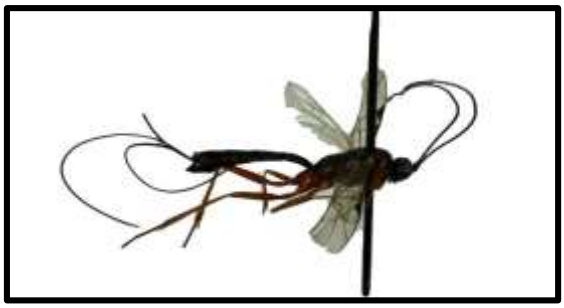
2a



2b

3a

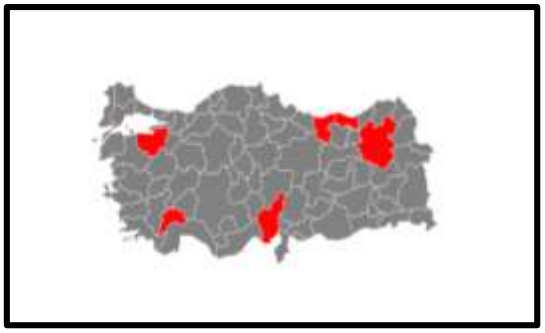
3b



4a

4b

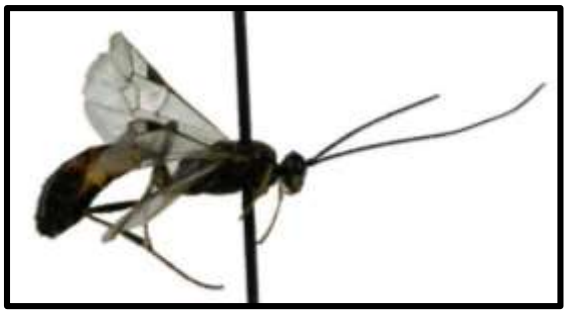
5a



5b

6a

6b

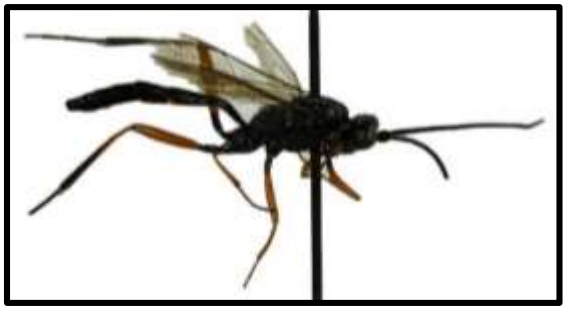


7a

7b

8a

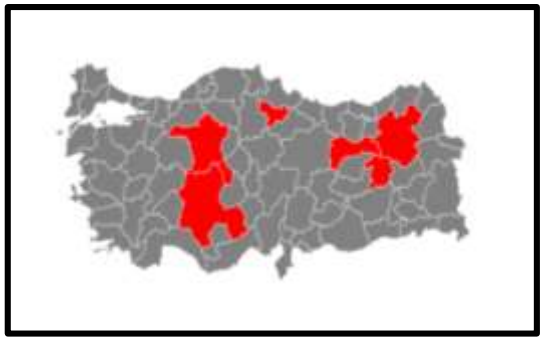
8b



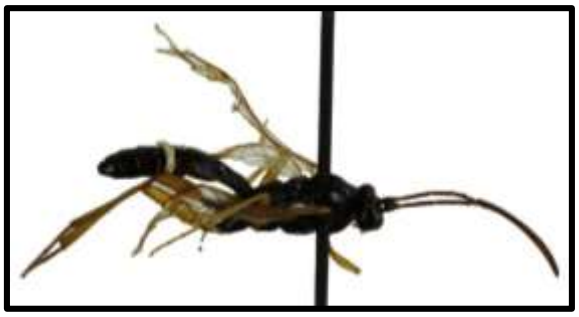
9a

9b

10a

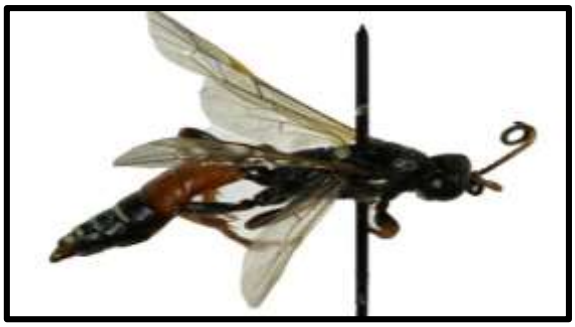


10b

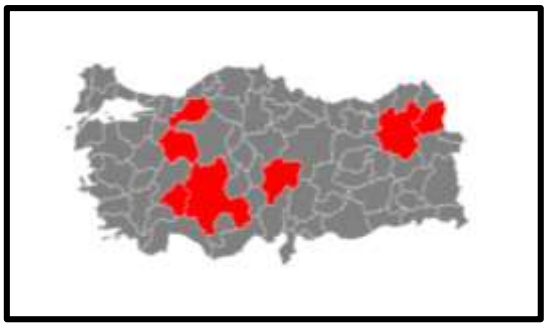


11a

11b



12a



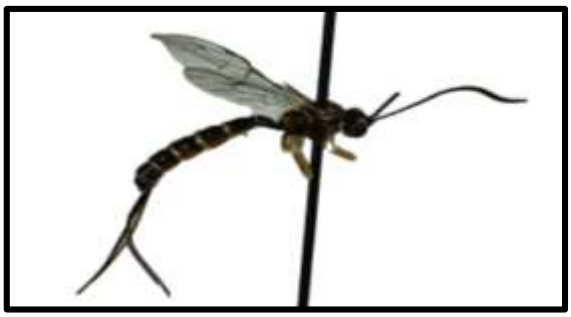
12b

13a

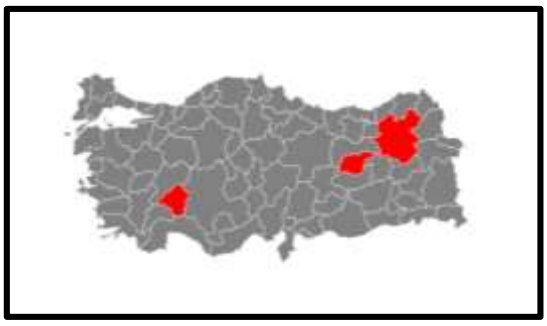
13b

14a

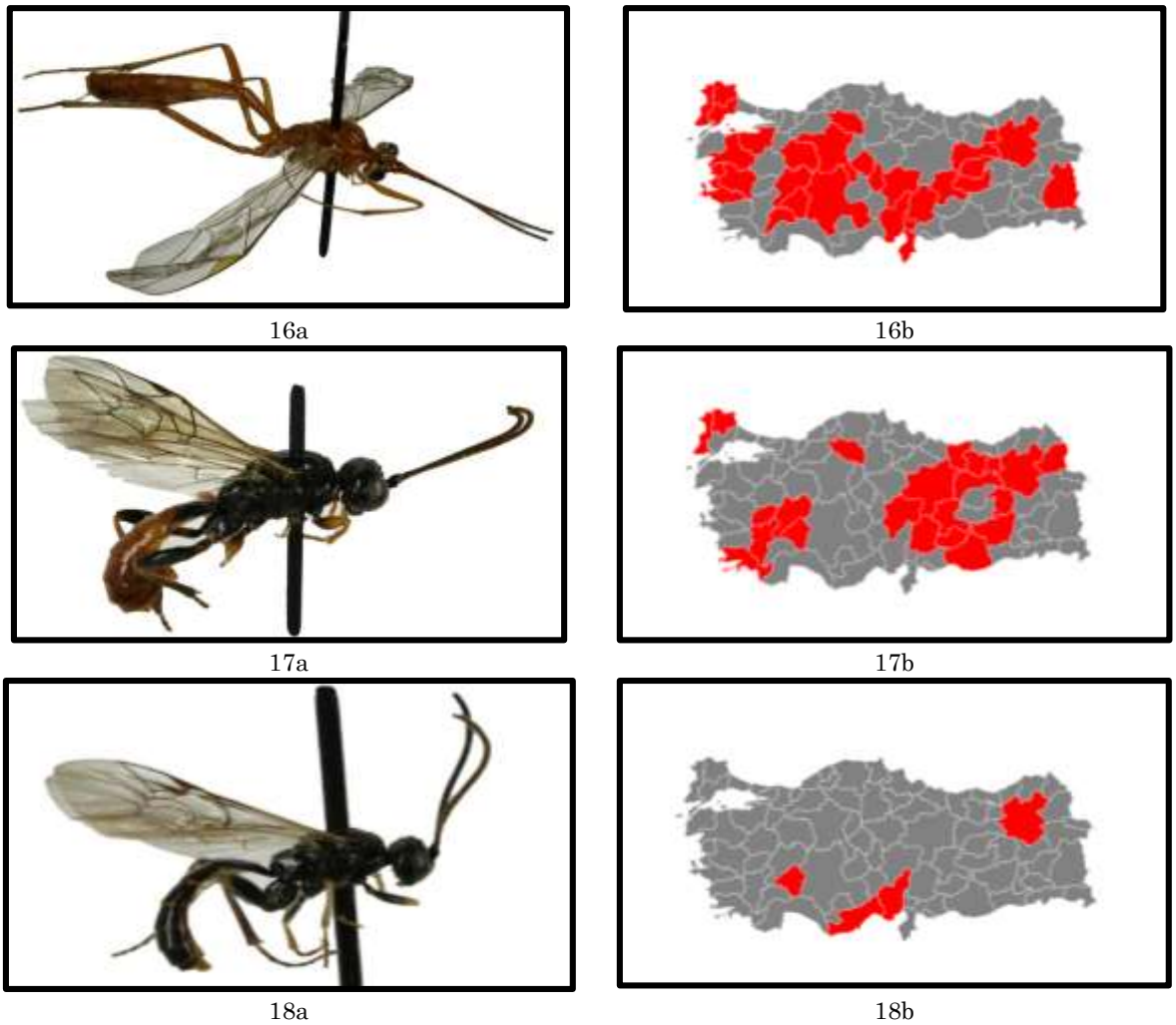
14b



15a



15b



16a 16b

17a 17b

18a 18b

Figure 3. a) Habitus, b) Distribution of Türkiye: 1) *Anomalon cruentatum* (Geoffroy, 1785); 2) *Exetastes adpressorius* (Thunberg, 1822); 3) *Lissonota (Loxonota) lineata* Gravenhorst, 1829; 4) *Lissonota* (*Lissonota*) *pleuralis* Brischke, 1880; 5) *Campoletis crassicornis* (Tschek, 1871); 6) *Cremastus geminus* Gravenhorst, 1829; 7) *C. spectator* Gravenhorst, 1829; 8) *Aritranis longicauda* (Kriechbaumer, 1873); 9) *Cryptus viduatorius* Fabricius, 1804; 10) *Latibulus argiolus* (Rossi, 1790); 11) *Cylloceria melancholica* (Gravenhorst, 1820); 12) *Spilichneumon occisorius* (Fabricius, 1793); 13) *Virgichneumon maculicauda* (Perkins, 1953); 14) *Ophion mocsaryi* Brauns, 1889; 15) *Perithous septemcinctorius* (Thunberg, 822); 16) *Netelia fuscicornis* (Holmgren, 1860); 17) *Tryphon thomsoni* Roman, 1939; 18) *T. psilosagator* Aubert, 1966.

Şekil 3. a) Habitus, b) Türkiye dağılışları: 1) Anomalon cruentatum (Geoffroy, 1785); 2) Exetastes adpressorius (Thunberg, 1822); 3) Lissonota (Loxonota) lineata Gravenhorst, 1829; 4) Lissonota (Lissonota) pleuralis Brischke, 1880; 5) Campoletis crassicornis (Tschek, 1871); 6) Cremastus geminus Gravenhorst, 1829; 7) C. spectator Gravenhorst, 1829; 8) Aritranis longicauda (Kriechbaumer, 1873); 9) Cryptus viduatorius Fabricius, 1804; 10) Latibulus argiolus (Rossi, 1790); 11) Cylloceria melancholica (Gravenhorst, 1820); 12) Spilichneumon occisorius (Fabricius, 1793); 13) Virgichneumon maculicauda (Perkins, 1953); 14) Ophion mocsaryi Brauns, 1889; 15) Perithous septemcinctorius (Thunberg, 822); 16) Netelia fuscicornis (Holmgren, 1860); 17) Tryphon thomsoni Roman, 1939; 18) T. psilosagator Aubert, 1966

Banchinae Wesmael, 1845

Exetastes adpressorius (Thunberg, 1822) (Figure. 3-2a)

Material examined: Çukurtepe: 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 2 ♂♂, 2 ♀♀. Göynük: 39° 9' 2.52" N, 40° 53' 54.96" E, 1761 m, 20.X.2022, 2 ♀♀. Hacılar: 39° 5' 47.40" N, 40° 48' 52.92" E, 1444 m, 20.X.X.2022, 3 ♂♂. Karlıova: 39° 17' 43.08" N, 40° 59' 43.08" E, 1857 m, 02.VII.2023, ♂, 39° 17' 43.44" N, 40° 59' 48.12" E, 1866, m, 02.VII.2023, 4 ♂♂, 2 ♀♀.

Distribution: Nearctik and Palaearctic, known from Türkiye (Figure 3-2b,Table 2).

Table 2. Provinces and references of collected species in Türkiye
Çizelge 2. Türkiye'deki türlerin dağılış gösterdiği iller ve ilgili referanslar

Taxa name	Provinces	References
ANOMALONINAE VIERECK, 1918		
Genus *Anomalon* Panzer, 1804		
Anomalon cruentatum	Adana, Adıyaman, Afyon, Ankara, Antalya, Balıkesir, Batman, Bayburt, Bingöl, Bolu, Çanakklae, Denizli, Diyarbakır, Edirne, Elazığ, Erzincan, Erzurum, Gaziantep, Gümüşhane, Hatay, Iğdır, Isparta, İstanbul, Kahramanmaraş, Kars, Kastamonu, Kayseri, Kırklareli, Malatya Mardin, Mersin, Muğla, Tekirdağ, Tunceli, Yozgat Zonguldak.	Kohl, 1905; Sedivy, 1959; Kolarov, 1989; Kılınçer, 1990; Öncüer, 1991; Yurtcan, Beyaslan & Kolarov, 1994; Kolarov, 1995, Kolarov, Beyarlan & Yurtcan, 1997a; Kolarov, Yurtcan & Beyaslan, 2002; Gürbüz, 2004; Çoruh, Özbek & Kolarov, 2004; Akkaya, 2005; Kolarov & Gürbüz, 2006; Beyarslan, Yurtcan, Erdoğan & Aydoğdu, 2006; Okyar & Yurtcan, 2007; Bolu, Özdemir & Özgen, 2007; Buncukçu, 2008; Kırtay, 2008; Gürbüz, Ljubomirov, Kolarov, Yurtcan, Tabur, Çoruh & Buncukçu, 2008; Gürbüz, Aksoylar & Buncukcu, 2009a; Gürbüz, Kırtay & Birol, 2009b; Özdemir & Güler, 2009; Hepdurgun, Turanlı & Kaplan, 2009, Çıkman, Beyaslan & Yurtcan, 2009; Birol, 2010; Gürbüz, Kolarov, Özdan & Tabur, 2011; Çoruh, Kolarov & Özbek, 2014b; Kolarov, Yıldırım, Çoruh & Yüksel, 2014; Kolarov, Çoruh & Çoruh, 2016; Çoruh & Kolarov, 2016, Özdan & Gürbüz, 2016; Kolarov, Çoruh & Çoruh, 2017; Özek & Avcı, 2017; Sarı, 2017; Sarı & Çoruh, 2018; Özdan & Gürbüz, 2019; Kıraç & Gürbüz, 2020; Barik, 2022; Kaplan & Riedel, 2022; Doğru, 2022; Çoruh, Tezcan & Gülperçin, 2022b..
BANCHINAE WESMAEL, 1845		
Genus *Exetastes* Gravenhorst, 1829		
Exetastes adpressorius	Anadolu, Ankara, Bayburt, Erzurum, Isparta, Kırıkkale, Kırşehir, Tunceli, Isparta.	Fahringer, 1921; Aubert, 1978; Kolarov, 1995; Kolarov & Beyarslan, 1994b; Özdemir, 1996; Pekel, 1999; Çoruh et al., 2014b, Kolarov et al., 2014, Çoruh & Çalmaşur, 2016; Çoruh, Kolarov & Çoruh, 2018; Özdan & Gürbüz, 2019; Doğru, 2022; Çoruh & Riedel, 2022; Birol, 2022.
Genus *Lissonota* (*Loxonota*) Aubert, 1993		
Lissonota (Loxonota) lineata	Anadolu, Diyarbakır, Erzurum, Erzincan, Hatay, Osmaniye.	Öncüer, 1991; Kolarov, 1995; Akkaya, 2005; Gürbüz et al., 2011; Çoruh & Çoruh, 2012; Kolarov et al., 2017; Çoruh & Riedel, 2022.
Genus *Lissonota* (*Lissonota*) Gravenhorst, 1829		
Lissonota (*Lissonota*) *pleuralis*	Bursa, Çanakkale, Erzincan, Erzurum, Giresun.	Kolarov et al., 1997a, Kolarov et al., 1997b, Kolarov et al., 2017, Çoruh & Riedel, 2022.
CAMPOPLEGINAE FORSTER, 1869		
Genus *Campoletis* Förster 1869		
Campoletis crassicornis	Adana, Burdur, Bursa, Erzurum, Giresun, Trabzon.	Kolarov & Beyarslan, 1995; Çoruh, Gürbüz, Kolarov, Yurtcan & Buncukçu Özdan, 2013, Çoruh et al., 2018; Çaylak, 2019, Çaylak & Çoruh, 2020b; Kolarov, Çoruh & Ercelep, 2021.
CREMASTINAE FORSTER, 1869		
Genus *Cremastus* Gravenhorst, 1829		
Cremastus geminus	Anadolu, Erzurum, Kırklareli	Kolarov, 1997; Kolarov & Beyarslan, 1999; İnecikliоğlu, 2022; Pekel & Özbek, 2000; Çoruh et al., 2014b.
Cremastus spectator	Isparta, Tekirdağ	Kolarov, 1997; Gürbüz, 2005; İnecikliоğlu, 2022.
CRYPTINAE KIRBY, 1837		
Genus *Aritranis* Förster 1869		
Aritranis longicauda	Isparta	Gürbüz & Kolarov, 2008; Gürbüz et al, 2009a,b; Özdan, 2014; Çoruh, 2019.
Genus *Cryptus* Fabricius 1804		
Cryptus viduatorius	Bayburt, Bilecik, Bursa, Diyarbakır, Erzurum, İçel, Isparta, İstanbul, Kırklareli, Rize	Kolarov, 1987; Öncüer, 1991; Beyarslan & Kolarov, 1994; Kolarov, 1995, Kolarov, 1987; Öncüer, 1991; Beyarslan & Kolarov, 1994; Kolarov, 1995, Kolarov et al., 1997a, Gürbüz & Kolarov, 2008; Çoruh & Çoruh, 2008, Gürbüz et al., 2009a, Çoruh & Çoruh, 2012; Özdan, 2014; Çoruh et al., 2014a,b Çoruh et al., 2016, Kolarov et al., 2016, Sarı & Çoruh, 2018; Çoruh et al., 2018; Çoruh, 2019; Yılmaz, 2020; Kaplan & Riedel, 2022; Barik & Çoruh, 2023a; Birol, 2022.
Genus *Latibulus* Gistel 1848		
Latibulus argiolus	Amasya, Ankara, Bingöl, Erzurum, Erzincan, Konya	Fahringer, 1922; Horstmann, 1986; Kolarov, 1995; Kolarov et al., 2014, Çoruh et al., 2014b, Kolarov & Yurtcan, 2008, Kolarov & Çalmaşur, 2011; Çoruh, 2019; Kaplan & Riedel, 2022.
CYLLOCERIINAE WAHL, 1990		
Genus *Cylloceria* Schiødte, 1838		
Cylloceria melancholica	Ardahan	Çoruh, Özbek & Kolarov, 2002; Çoruh et al., 2014b.
SUBFAMILY ICHNEUMONINAE LATREILLE, 1802		
Genus *Spilichneumon* Thomson, 1894		
Spilichneumon occisorius	Bolu, Eskişehir, Erzurum, Isparta, Kars, Kayseri, Konya.	Özdemir, 1996; Özbek, Çoruh & Kolarov, 2003; Özdan, 2014; Riedel, Çoruh & Özbek, 2010; Çoruh, 2017; Riedel, Diller & Çoruh, 2018; Birol, 2022.
Genus *Virgichneumon* Heinrich, 1977		
Virgichneumon maculicauda	Bayburt, Erzurum	Riedel et al., 2010; Çoruh et al., 2011; Çoruh et al., 2014b, Çoruh, 2017
OPHIONINAE SHUCKARD, 1840		
Genus *Ophion* Fabricius, 1798		
Ophion mocsaryi	Adana, Bayburt, Edirne, Erzurum, Isparta, Mersin	Kolarov, 1989; Kolarov et al., 2000; Kolarov & Gürbüz, 2006; Çoruh & Çoruh, 2008; Gürbüz et al., 2009a, Altıparmak, 2010; Gürbüz et al., 2011; Çoruh et al., 2014b, İnecikliоğlu, 2022.
PIMPLINAE WESMAEL, 1845		
Genus Cins *Perithous* Holmgren, 1859		
Perithous septemcinctorius	Erzurum, Isparta, Tunceli	Kolarov & Gürbüz, 2004; Çoruh & Kolarov, 2010; Kolarov et al., 2014;

		Çoruh, 2016.
TRYPHONINAE SHUCKARD, 1840		
Genus *Netelia* Gray 1860		
Netelia fuscicornis	Afyon, Adana, Ankara, Balıkesir, Bayburt, Burdur, Bursa, Çankırı, Edirne, Elazığ, Erzurum, Erzincan, Eskişehir, İzmir, Isparta, Hatay, Kahramanmaraş, Kayseri, Kırklareli, Kırşehir, Konya, Malatya, Manisa, Nevşehir, Osmaniye, Tekirdağ, Tunceli, Van.	Tolkanitz, 1981; Kohl, 1905; Delrio, 1975; Kolarov, 1987; Kolarov, 1994; Öncüer, 1991; Kolarov & Beyaslan, 1994b; Kolarov, 1995; Kolarov et al., 1997a; Kolarov, Özbek & Yıldırım, 1999; Özdemir, 2001; Yurtcan et al., 2002; Yurtcan et al., 2006, Gürbüz & Kolarov, 2006; Beyarslan et al., 2006; Kırtay, 2008; Eroğlu et al., 2011; Yaman, 2014; Çoruh & Çalmaşur, 2016; Çoruh, 2019; Yurtcan, Çoruh, Kolarov, Özdan, Gürbüz & Erkaya, 2021.
Genus *Tryphon* Fallén, 1813		
Tryphon thomsoni	Adıyaman, Afyon, Bayburt, Bingöl, Çankırı, Denizli, Diyarbakır, Edirne, Erzincan, Erzurum, Giresu, Gümüşhane, Isparta, Kahramanmaraş, Kars, Kayseri, Kırklareli, Malatya, Muğla, Sivas, Şanlıurfa Uşak.	Kolarov & Beyarslan, 1994a; Kolarov et al., 1999; Çoruh, Özbek & Kolarov, 2005, Yurtcan & Beyarslan, 2006; Gürbüz & Kolarov, 2006; Gürbüz et al., 2009a,b; Kolarov & Çoruh, 2012; Yaman, 2014; Çoruh et al., 2014a,b, Kolarov et al., 2016; Çoruh, 2019; İnecikliоğlu, 2022.
Tryphon psilosagator	Adana, Erzurum, Isparta, İçel .	Kolarov & Beyarslan 1994a; Kolarov et al., 1999; Gürbüz & Kolarov, 2006; Çoruh et al., 2014b; Çoruh, 2019.

Associate plants: *Angelica sylvestris* L., *Chaerophyllum aromaticum* L., *C. bulbosum* L., *Chrysothamnus nauseosus speciosus* (Nutt.) H. M. Hall & Clem., *Corylus avellana* L., *Daphne gnidium* L., *Daucus carota* L., *Euphorbia segueriana* Wall Art., *Euphorbia virgata* Waldst. & Kit., *Ferula communis* L., *Fraxinus excelsior* L., *Heracleum sphondylium* (Eltrot), *Juniperus communis* L., *Pastinaca sativa L.*, *Peucedanum oreoselinum* (L.), *Phacelia* sp., *Prunus cerasifera* Ehrh., *Quercus sessiliflora* Salisb., *Reseda lutea* L., *Rubus idaeus* L., *Salsola pestifer* A.Nelson, *Thapsia villosa* L. (Yu et al., 2016).

Lissonota (Loxonota) lineata Gravenhorst, 1829 (Figure 3-3a).

Material examined: Çukurtepe: 39° 24' 13.68" N, 41° 2' 10.68" E, 1874 m, 15.VII.2022, 2 ♂♂. Göynük: 39° 9' 2.52" N, 40° 53' 54.96" E, 1761 m, 20.X.2022, 2 ♂♂. Yoncalık: 39° 20' 4.20" N, 41° 4' 49.08" E, 1938 m, 15.XI.2022, 4 ♂♂, 2 ♀♀. Ortaköy: 39° 24' 7.56" N, 40° 53' 15.72" E, 1981 m, 02.VII.2023, 3 ♂♂, ♀. Viranşehir: 39° 23' 15.72" N, 40° 58' 19.92" E, 1897 m, 02.VII.2023, 3 ♂♂.

Distribution: Palaearctic, known from Türkiye (Figure 3-3b, Table 2).

Lissonota (*Lissonota*) *pleuralis* Brischke, 1880 (Figure 3-4a).

Material examined: Çukurtepe: 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 4 ♂♂. Halifan: 39° 8' 31.20" N, 40° 51' 55.80" E, 1684 m, 20.XI.2022, 2 ♂♂, 2 ♀♀. Kargapazarı: 39° 18' 47.88" N, 41° 8' 18.96" E, 1956 m, 20.XI.2022, ♀. Ortaköy: 39° 24' 21.24" N, 40° 53' 25.80" E, 1971 m, 26.VIII.2023, 3 ♂♂, 2 ♀♀.

Distribution: Palaearctic, known from Türkiye (Figure 3-4b, Table 2).

Associate plants: *Anethum graveolens* (Dill.), *Chaerophyllum bulbosum* L., *Cirsium lanceolatum* (L.), *Daucus carota sativus* L., *Epilobium angustifolium* L., *Fraxinus excelsior* L., *Heracleum sphondylium* (Eltrot), *Pastinaca graveolens* (L.), *Peucedanum oreoselinum* (L.), *Quercus sessiliflora* Salisb. (Yu et al., 2016).

Campopleginae Förster, 1869

Campoletis crassicornis (Tschek, 1871) (Figure 3-5a).

Material examined: Çukurtepe: 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 3 ♀♀. Halifan: 39° 8' 44.88" N, 40° 52' 26.04" E, 1727 m, 20.XI.2022, 2 ♀♀.

Distribution: Europea and Palaearctic, known from Türkiye (Figure 3-5b, Table 2).

Associate plant: *Peucedanum oreoselinum* (L.) (Yu et al., 2016).

Cremastinae Forster, 1869

Cremastus geminus Gravenhorst, 1829 (Figure 3-6a).

Material examined: Halifan: 39° 8' 31.20" N, 40° 51' 55.80" E, 1684 m, 20.XI.2022, 2 ♀♀.

Distribution: Palaearctic, known from Türkiye (Figure 3-6b, Table 2).

Associate plant: *Peucedanum oreoselinum* (L.) (Yu et al., 2016).

Cremastus spectator Gravenhorst, 1829 (Figure 3-7a)

Material examined: Hasanova: 39° 10' 19.92" N, 41° 2' 16.80" E, 1961 m, 20.XI.2022, 2 ♀♀. Ortaköy: 39° 24' 6.84" N, 40° 53' 16.44" E, 1967 m, 02.VII.2023, 4 ♂♂, 2 ♀♀; 39° 24' 7.56" N, 40° 53' 15.72" E, 1981 m, 02.VII.2023, 2 ♀♀; 39° 23' 32.28" N, 40° 53' 13.20" E, 1784 m, 26.VIII.2023, 3 ♂♂.
Distribution: Palaearctic, known from Türkiye (Figure 3-7b, Table 2).

Associate plants: *Heracleum sphondylium* (Eltrot), *Inonotus hispidus* (Bull.) P. Karst. (Yu et al., 2016).

Remarks: *Cremastus spectator* is new East Anatolia.

Cryptinae Kirby, 1837

Aritranis longicauda (Kriechbaumer, 1873) (Figure 3-8a)

Material examined: Hacılar: 39° 5' 47.40" N, 40° 48' 52.92" E, 1444 m, 20.X.2022, 3 ♂♂, ♀. Hasanova: 39° 10' 19.92" N, 41° 2' 16.80" E, 1961 m, 20.XI.2022, ♀. Yoncalık: 39° 20' 8.16" N, 41° 4' 39.72" E, 1936 m, 15.XI.2022, ♂, 4 ♀♀. Karlıova: 39° 18' 6.48" N, 41° 1' 33.60" E, 1788 m, 02.VII.2023, ♂, 3 ♀♀. Ortaköy: 39° 24' 6.84" N, 40° 53' 16.44" E, 1967 m, 02.VII.2023, ♂, ♀.

Distribution: Europea and Palaearctic, known from Türkiye (Figure 3-8b, Table 2).

Associate plants: *Elymus sabulosus* M. Bieb., *Euphorbia segueriana* Necker (Yu et al., 2016).

Remarks: *Aritranis longicauda* is new East Anatolia.

Cryptus viduatorius Fabricius, 1804 (Figure 3-9a)

Material examined: Göynük: 39° 9' 2.52" N,40° 53' 54.96" E, 1761 m, 20.X.2022, 5 ♂♂. Çukurtepe: 39° 25' 6.96" N,41° 2' 17.16" E, 1919 m, 06.XI.2022, 4 ♀♀. Halifan: 39° 8' 44.88" N,40° 52' 26.04" E, 1727 m, 20.XI.2022, ♂, 2 ♀♀. Center: 39° 18' 8.28" N,41° 1' 32.16" E, 1785 m, 02.VII.2023, 5 ♂♂, 3 ♀♀. Ortaköy: 39° 23' 47.76" N,40° 53' 39.12" E, 1909 m, 26.VIII.2023, 5 ♂♂; 39° 23' 50.28" N,40° 53' 32.64" E, 1955 m, 26.VIII.2023, ♀; 39° 24' 19.80" K, 40° 53' 27.60" E, 1973 m, 26.VIII.2023, ♂, ♀.

Distribution: Palaearctic, known from Türkiye (Figure 3-9b, Table 2).

Associate plants: *Anethum graveolens* (Dill.), *Angelica sylvestris* L., *Daucus carota* L., *Daucus carota sativus* L., *Euphorbia nicaeensis* All., *Euphorbia virgata* Waldst. & Kit., *Ferula communis* L., *Heracleum sphondylium* (Eltrot), *Medicago sativa* L. *Peucedanum oreoselinum* (L.) (Yu et al., 2012).

Latibulus argiolus (Rossi, 1790) (Figure 3-10a)

Material examined: Çukurtepe: 39° 24' 13.68" K, 41° 2' 10.68" E, 1874 m, 15.VII.2022, ♂. Karlıova: 39° 18' 2.16" K, 41° 1' 20.64" D, 1793 m, 23.IX.2023, 3 ♂♂.

Distribution: Palaearctic, known from Türkiye (Figure 3-10b, Table 2).

Cylloceriinae Wahl, 1990

Cylloceria melancholica (Gravenhorst, 1820) (Figure 3-11a)

Material examined: Çukurtepe: 39° 24' 13.68" N, 41° 2' 10.68" E, 1874 m, 15.VII.2022, ♂. Karlıova: 39° 17' 33.00" N, 40° 59' 45.24" E, 1861 m, 02.VII.2023, 2 ♀♀; 39° 17' 33.72" N, 40° 59' 48.48" E, 1863 m, 02.VII.2023, 2 ♂♂.

Distribution: Nearctic and Palaearctic, known from Türkiye (Figure 3-11b, Table 2).

Associate plants: *Chaerophyllum aromaticum* L., *Heracleum sphondylium* (Eltrot), *Rubus idaeus* L. (Yu et al., 2016).

Remarks: Bingöl is second locality for *Cylloceria melancholica.*

Ichneumoninae Latreille, 1802

Spilichneumon occisorius (Fabricius, 1793) (Figure 3-12a.)

Material examined: Yoncalık: 39° 20' 2.40" N, 41° 4' 46.92" E, 1937 m, 15.XI.2022, 2 ♀♀; 39° 20' 4.20" N, 41° 4' 49.08" E, 1938 m, ♀. Ortaköy: 39° 23' 52.44" N, 40° 53' 35.16" E, 1931 m, 26.VII.2023, 2 ♂♂; 39° 24' 21.24" N, 40° 53' 25.80" E, 1971 m, 26.VIII.2023, 2 ♂♂, ♀.

Distribution: Palaearctic, known from Türkiye (Figure 3-12b, Table 2).

Associate plant: *Daucus carota* L., *Deschampsia cespitosa* (L.) P. Beauv., *Euphorbia virgata* Waldst. & Kit., *Heracleum sphondylium* (Eltrot), *Poa pratensis* L. (Yu et al., 2016).

Virgichneumon maculicauda (Perkins, 1953) (Figure 3-13a)

Material examined: Kargapazarı: 39° 17' 33.36" N, 41° 4' 27.48" E, 1803 m, 20.XI.2022, 3 ♂♂; 39° 18' 44.99" N, 41° 5' 52.61" E, 1816 m, ♂; 39° 18' 44.74" N, 41° 5' 56.26" E, 1826 m, 3 ♀♀. Viranşehir: 39° 23' 15.72" N, 40° 58' 19.92" E, 1897 m, 02.VII.2023, 4 ♀♀.

Distribution: Palaearctic, known from Türkiye (Figure 3-13b, Table 2).

Remarks: Bingöl province is third locality for *Virgichneumon maculicauda.*

Ophioninae Shuckard, 1840

Ophion mocsaryi Brauns, 1889 (Figure 3-14a)
Material examined: Çukurtepe: 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 2 ♀♀; 39° 24' 13.68" N, 41°

2' 10.68" E, 1874 m, 15.VII.2022, 3 ♀♀. Kargapazarı: 39° 18' 46.08" N, 41° 8' 22.20" E, 1963 m, 20.XI.2023, 3 ♂♂.

Distribution: Palaearctic, known from Türkiye (Figure 3-14b, Table 2).

Associate plants: *Carum carvi* L., *Seseli libanotis* (L.) (Yu et al., 2016).

Pimplinae Wesmael, 1845

Perithous septemcinctorius (Thunberg, 1822) (Figure 3-15a)

Material examined: Kargapazarı: 39° 17' 33.36" N, 41° 4' 27.48" E, 1803 m, 20.XI.2022, ♂; 39° 18' 44.99" N, 41° 5' 52.61" E, 1816 m, 20.XI.2022, 3 ♀♀. Karlıova:39° 17' 33.00" N, 40° 59 '45.24" E, 1861 m, 02.VII.2023, ♀; 39° 17' 33.72" N, 40° 59' 48.48" E, 02.VII.2023, 1863 m, ♀; 39° 18' 8.28" N, 41° 1' 32.16" E, 1785, 02.VII.2023, 2 ♂♂; 39° 18' 10.44" N, 41° 1' 32.52" E, 1786 m, 02.VII.2023, 2 ♀♀.

Distribution: Nearctic and Palaearctic, known from Türkiye (Figure 3-15 b,Table 2).

Associate plants: *Ampelopsis hederacea* DC., *Carpinus* sp., *Chaerophyllum bulbosum* L., *Prunus domestica* L., *Prunus domestica insititia* (L.), *Pyrus communis* L. (Yu et al., 2016).

Tryphoninae Shuckard, 1840

Netelia fuscicornis (Holmgren, 1860) (Figure 3-16a)

Material examined: Yoncalık: 39° 20' 8.16" N, 41° 4' 39.72" E, 1936 m, 15.XI.2022, 6 ♂♂, 2 ♀♀; 39° 20' 2.40" N, 41° 4' 46.92" E, 1937 m, 15.XI2022, 5 ♂♂, 2 ♀♀; 39° 20' 4.20" N, 41° 4' 49.08" E, 1938 m, 15.XI.2022, 3 ♀♀. Karlıova: 39° 17' 43.08" N, 40° 59' 43.08" E, 1857 m, 02.XII.2023, 4 ♀♀; 39° 17' 33.00" N, 40° 59 '45.24" E, 1861 m, 02.VII.2023, 4 ♂♂; 39° 17' 33.72" N, 40° 59' 48.48" E, 1863 m, 02.VII.2023, 3 ♂♂; 39° 17' 43.44" N, 40° 59' 48.12" E, 1866 m, 02.VII.2023, ♀.

Distribution: Oriental and Palaearctic, known from Türkiye (Figure 3-16b, Table 2).

Tryphon thomsoni Roman, 1939 (Figure 3-17a)

Material examined: Çukurtepe: 39° 25' 11.28" N, 41° 2'2 9.04" E, 1941 m, 06.XI.2022, 2 ♀♀; 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 3 ♂♂; 39° 24' 13.68" N, 41° 2' 10.68" E, 1874 m, 15.VII.2022, 2 ♀♀. Ortaköy: 39° 24' 6.84" N, 40° 53' 16.44" E, 1967 m, 02.VII.2023, 2 ♀♀; 39° 23' 47.76" N, 40° 53' 39.12" E, 1909 m, 26.VIII.2023, 2 ♀♀, 39° 23'52. 44" N, 40° 53' 35.16" E, 1931 m, 26.VIII.2023, 4 ♂♂.

Distribution: Palaearctic, known from Türkiye (Figure 3-17b, Table 2).

Associate plant: *Peucedanum oreoselinum* (L.) (Yu et al., 2016).

Tryphon psilosagator Aubert, 1966 (Figure 3-18a)

Material examined: Çukurtepe: 39° 24' 14.76" N, 41° 2' 5.64" E, 1881 m, 10.VII.2022, 2 ♀♀; 39° 25' 11.28" N, 41° 2' 29.04" E, 1941, 06.XI.2022, 2 ♀♀; Hacılar: 39° 5' 47.40" N, 40° 48' 52.92" E, 1444 m, 20.X.2022, ♂. Halifan: 39° 8' 45.24" N, 40° 52' 26.40" E, 1728 m, 20.XI.2022, ♂; Ortaköy: 39° 23' 47.76" N, 40° 53' 39.12" E, 1909 m, 26.VIII.2023, 2 ♀♀;

Distribution: Palaearctic, known from Türkiye (Figure 3-18b, Table 2).

DISCUSSION

The Ichneumonidae within the Hymenoptera order holds an important place in terms of species diversity. The main reason for this importance is that many species are evaluated as biological control agencies. In the "Turkish Ichneumonidae Catalogue," where Ichneumonids have been evaluated over many years, 393 species are listed under 19 subfamilies with their initial details. (Kolarov, 1995).

The work carried out over the past 29 years initially gained momentum in the Thrace, Eastern Anatolia, and Mediterranean regions, and later spread throughout Türkiye. Today, it has been found that the number of Ichneumonidae species is approximately 1,500 (Barik & Çoruh, 2023a).

548 ichneumonid species belonging to 158 genera from 20 subfamilies have been recorded so far from the study area (Figure 4), which includes the region where most of the studies in Türkiye have been carried out and where the study carried out , while 316 species have been considered as new records for in country (Barik, 2022).

The study aimed to detect the Ichneumonidae fauna of Karlıova district of Bingöl province. Field studies were carried out especially between 2022 and 2023. Karlıova district was determined as the sample locality area of the study, and samples were collected from varying altitudes of the district in different months. A total of 256 samples from 16 genus belonging to different subfamilies were obtained, and their identification results were determined to belong to 18 species (Table 3).

Table 3. Data of collected species

Çizelge 3. Toplanan türlere ait veriler

Data of collected species: Individual numbers (IN), vertical distribution (VD), seasonal dynamics (SD), geographical regions (GR), zoogeographical regions (ZR), first record of Türkiye (FTR)

Vertical distribution (VD) (meter): A: 1251-1500 , B: 1501-1750 , C: 1751-2000 . Seasonal dynamics (SD):, Jl: July, Aug: August, S: September, O: October, N: November. Geographical regions (GR): AR: Aegean Region, BSR: Black Sea Region, CAR: Central Anatolia Region, EAR: Eastern Anatolia Region, MR: Marmara Region, MtR: Mediterranean Region, SAR: Southeastern Anatolia. Zoogeographical regions (ZR):

Taxa name	IN	VD	SD	GR	ZR	FRT
FAMILY ICHNEUMONIDAE LATREILLE, 1802						
ANOMALONINAE VIERECK, 1918						
Genus *Anomalon* Panzer, 1804						
Anomalon cruentatum	46	B,C	Jl, Aug, S, O, N	AR, BSR, CAR, EAR, MR, MtR, SAR	ORR, P	Kohl, 1905
BANCHINAE WESMAEL, 1845						
Genus *Exetastes* Gravenhorst, 1829						
Exetastes adpressorius	16	A,C	Jl, O	BSR, CAR, EAR, MR, MtR	HOL	Fahringer, 1921
Genus *Lissonota* (*Loxonota*) Aubert, 1993						
Lissonota (*Loxonota*) *lineata*	17	C	Jl, O, N	EAR, MtR, SAR	P	Öncüer, 1991
Genus *Lissonota (Lissonota))* Gravenhorst 1829						
Lissonota (*Lissonota*) *pleuralis*	14	B, C	Jl, Aug, N	BSR, EAR, MR	P	Kolarov et al. 1997a
CAMPOPLEGINAE FORSTER, 1869						
Genus *Campoletis* Förster, 1869						
Campoletis crassicornis	5	B, C	Jl, N	BSR, EAR, MtR, MR	E, P	Kolarov & Beyarslan, 1995
CREMASTINAE FORSTER, 1869						
Genus *Cremastus* Gravenhorst, 1829						
Cremastus geminus	2	B	N	EAR, MR	P	Kolarov, 1997
Cremastus spectator	13	C	Jl, Aug, N	MR, MtR	P	Kolarov, 1997
CRYPTINAE KIRBY, 1837						
Genus *Aritranis* Förster, 1869						
Aritranis longicauda	17	A, C	Jl, O, N	MtR	E, P	Gürbüz & Kolarov, 2008
Genus *Cryptus* Fabricius 1804						
Cryptus viduatorius	28	C	Jl, Aug, O, N	BSR, EAR, MR, MtR	P	Kolarov, 1987
Genus: *Latibulus* Gistel, 1848						
Latibulus argiolus	3	C	Jl, O	CAR, EAR	P	Fahringer, 1922
CYLLOCERIINAE WAHL, 1990						
Genus *Cylloceria* Schiødte, 1838						
Cylloceria melancholica	5	C	Jl	EAR	HOL	Çoruh et al., 2002
ICHNEUMONINAE LATREILLE, 1802						
Genus *Spilichneumon* Thomson, 1894						
Spilichneumon occisorius	8	C	Aug, N	BSR ,CAR, EAR, MtR	P	Özdemir, 1996
Genus *Virgichneumon* Heinrich, 1977						
Virgichneumon maculicauda	11	C	Jl, N	EAR	P	Riedel et al., 2010
OPHIONINAE SHUCKARD, 1840						
Genus *Ophion* Fabricius 1798						
Ophion mocsaryi	8	C	Jl, N	BSR, EAR, MR, MtR	P	Kolarov, 1989
PIMPLINAE WESMAEL, 1845						
Genus *Perithous* Holmgren, 1859						
Perithous septemcinctorius	10	C	Jl, N	EAR, MtR	HOL	Kolarov & Gürbüz, 2004
TRYPHONINAE SHUCKARD, 1840						
Genus *Netelia* Gray 1860						
Netelia fuscicornis	30	A, B, C	Jl, N	AR, BSR, CAR, EAR, MR, MtR	ORR, P	Tolkanitz, 1981
Genus *Tryphon* Fallén, 1813						
Tryphon thomsoni	15	C	Jl, Aug, N	BSR, EAR, MR, MtR, SAR	P	Kolarov & Beyarslan, 1994
Tryphon psilosagator	8	A, C	Au, O, N	AR, EAR, MtR	P	Kolarov & Beyarslan, 1994

E: Europe, HOL: Holarctic, ORR: Oriental, P: Palearctic.

When Table 2 is evaluated, it is understood that out of 10 different subfamilies, 46 samples from Anomaloninae (1 species), 47 samples from Banchinae (3 species), 5 samples from Campopleginae (1 species), 15 samples from Cremastinae (2 species), 48 samples from Cryptinae (3 species), 5 samples from Cylloceriinae (1 species), 19 samples from Ichneumoninae (2 species), 8 samples from Ophioninae (1 species), 10 samples from Pimplinae (1 species) and 53 samples from Tryphoninae (3 species) are available (Figure 5).

Figure 4. Map of region
Şekil 4. Bölge haritası

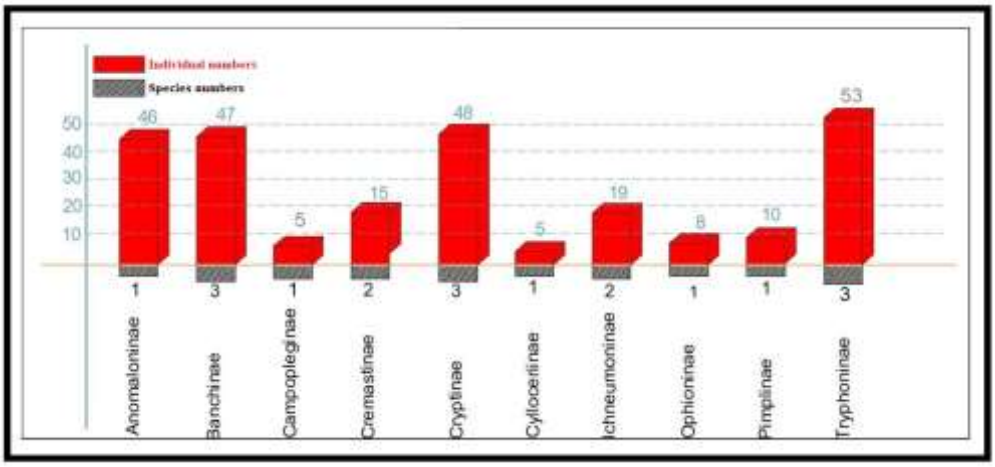


Figure 5. Distribution of species according to subfamilies.
Şekil 5. Altfamilyalara göre türlerin dağılım.

Faunistic and systematic studies on Ichneumonidae in Bingöl province are limited. A thesis study included the province of Bingöl, and while three species were recorded from this province (Çoruh and Özbek, 2008). While Kaplan and Riedel (2022) recorded 35 ichneumonidae species from this province, in another study, nine species were reported from Bingöl (Kaplan, 2023). Along with the number of species, a large number of individuals were also collected in these studies.

When we look at the density of the samples obtained, we see that Tryphoninae makes up 20.7% of the total number of samples with 53 individuals, Cryptinae makes up 18.5% with 48 individuals, Banchinae makes up 18.1% with 47 individuals, and Anomaloninae makes up 17% with 46 individuals (Figure 6).

When the collected samples are evaluated in terms of the number of individuals, *Anomalon cruentatum* (46 individuals), *Netelia fuscicornis* (30 individuals), and *Cryptus viduatorius* (28 individuals) are common in the region, while *Cremastus geminus* (two individuals) was the least common species.

The samples were collected from distances between 1250 m and 2000 m. There are four species in the altitude range of 1250-1500 m, five species in the altitude range of 1501-1750 m, and 17 species in the altitude range of 1751-2000 m. Most of the collected samples were taken from an altitude of 1751-2000 meters, while the fewest samples were taken from an altitude of 1250-1500 meters (Figure 7a). This situation has resulted in an outcome parallel to the frequency of visits to the examined areas.

While the samples that made up the study were collected mainly in July, August, September, October and November, it was considered interesting that so many samples were collected in November. While this situation is directly proportional to the preferred months of visit, the most samples were collected in November and the fewest samples were collected in September (Figure 7b, 8).

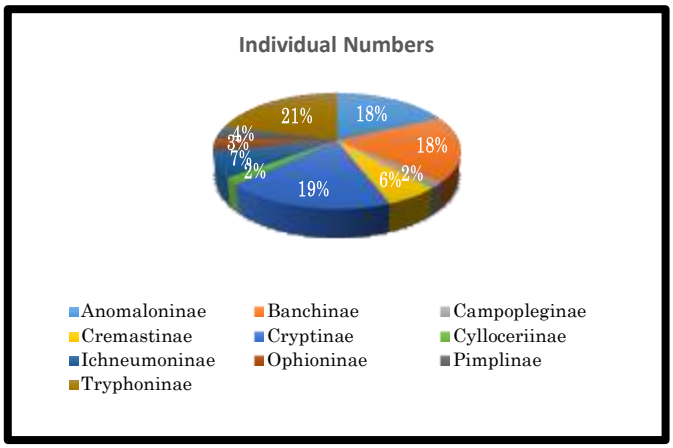
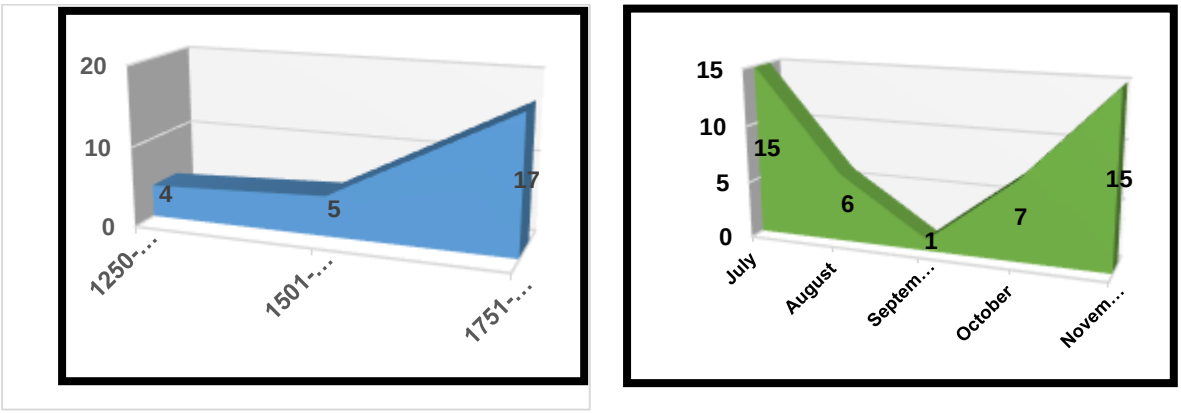


Figure 6. Distribution on subfamily according to individual numbers.
Şekil 6. Birey sayılarına göre altfamilya dağılımı.



a b

Figure 7. Distribution of species: a) according to altitude, b) according to months.
Şekil 7. Türlerin dağılışı: a) rakıma göre, b) aylara göre.

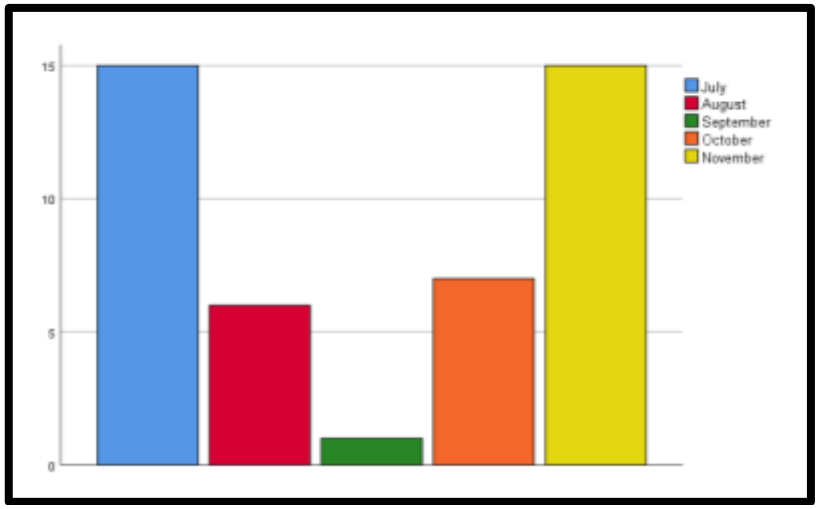


Figure 8. Difference between altitudes according to the chi-square test.
Şekil 8. Chi-square testine göre rakımlar arasındaki fark.

	Observed N	Expected N	Residual
July	15	8,8	6,2
August	6	8,8	-2,8
September	1	8,8	-7,8
October	7	8,8	-1,8
November	15	8,8	6,2
Total	44		

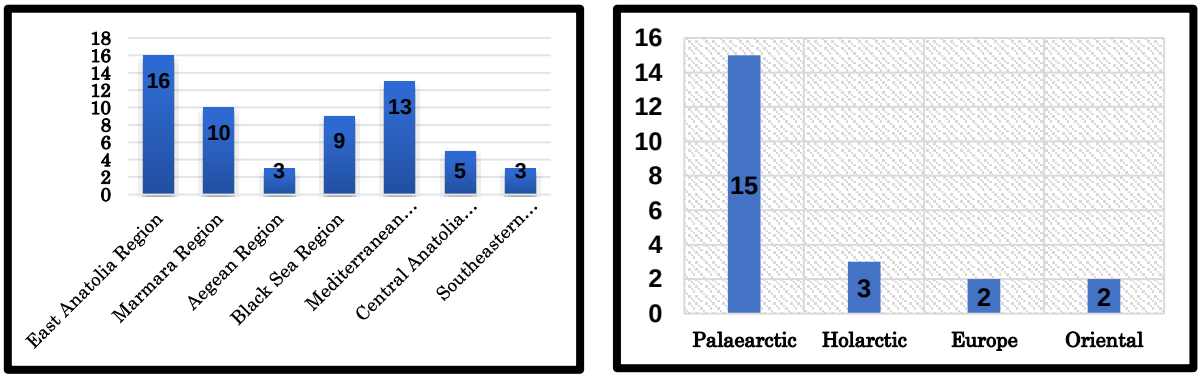
Test Statistics

	Months
Chi-Square	16,909[a]
df	4
Asymp. Sig.	0,002

a. 0 cells (0,0%) have expected frequencies less than 5. The minimum expected cell frequency is 8,8.

The localities where the collected samples were previously collected in Türkiye were determined. Accordingly; while 16 of the species that constitute the studies were previously recorded from Eastern Anatolia Region, 13 were collected from Mediterranean Region and 10 from Marmara Region. This situation is directly proportional to the density of the regions studied. The regions where the species were least distributed were Southeastern Anatolia Region and Aegean Region, with three species each (Figure 9a). When examined on a provincial basis, it was also analyzed that the samples were previously obtained from 58 different provinces, and that Erzurum, Isparta, Ankara and Adana were the provinces where the most samples were obtained.

The zoogeographic distribution of the species constituting the study was also attempted to be analysed. Fifteen species in Palaerctic Region and three in Holarctic Region. It is also determined that the European and Oriental Regions host only two species (Figure 9b).



a b

Figure 9. Distribution of species: a) according to geographic regions, b) according to zoogeographical regions.
Şekil 9. Türlerin dağılış: a) coğrafik bölgelere göre, b) zoocoğrafik bölgelere göre.

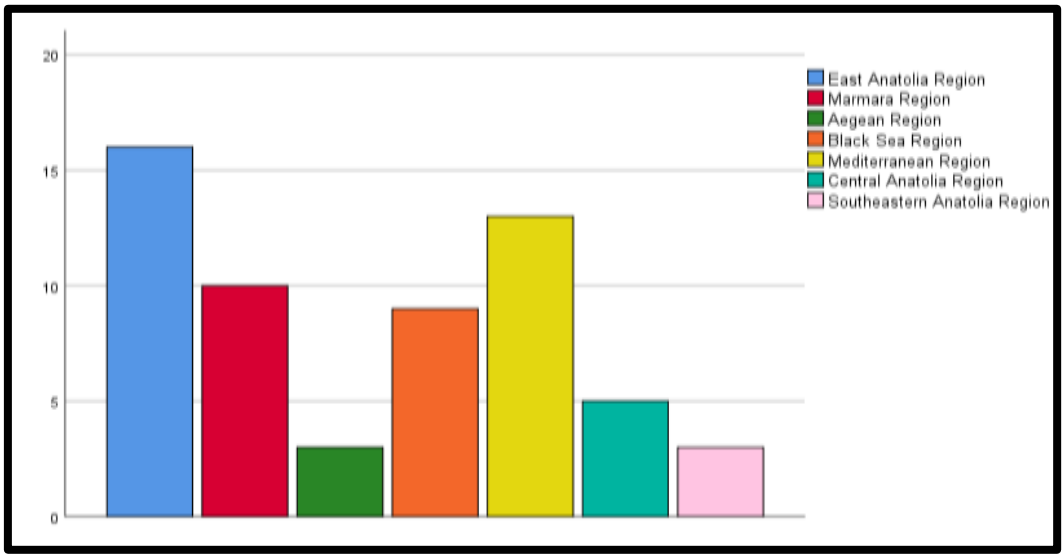


Figure 10. Difference between geographic regions to the chi-square test.
Şekil 10. Chi-square testine göre coografik bölgeler arasındaki fark

	Observed N	Expected N	Residual
East Anatolia Region	16	8,4	7,6
Marmara Region	10	8,4	1,6
Aegean Region	3	8,4	-5,4
Black Sea Region	9	8,4	0,6
Mediterranean Region	13	8,4	4,6
Central Anatolia Region	5	8,4	-3,4
Southeastern Anatolia Region	3	8,4	-5,4
Total	59		

Test Statistics

	Geographic-Regions
Chi-Square	18,000[a]
df	6
Asymp. Sig.	0,006

a. 0 cells (0,0%) have expected frequencies less than 5. The minimum expected cell frequency is 8,4.

Cremastus geminus was last recorded in 2000 (Pekel & Özbek, 2000), *C. melancholica* was last recorded in 2002 (Çoruh et al., 2002); *V. maculicauda* was last recorded in 2011 (Çoruh et al., 2011), *Ophion mocsaryi* was last recorded in 2011 (Gürbüz et al., 2011). These species were not encountered in subsequent studies, but were detected again in this study.

Considered as a whole, out of the 18 existing species, 15 species, except three, is new records for Bingöl province, *Cremastus spectator* and *Aritranis longicauda* species is new records for the Eastern Anatolia Region.

The new additional records obtained in this study will provide a good basis for future studies.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Conflict of Interest Statement

There is no conflict of interest between the authors.

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Transfer Öğrenme Temelli Bitki Yaprak Hastalıklarının Tespiti İçin Karşılaştırmalı Bir Çalışma

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ÖZET

Bitkilerin sağlıklı bir şekilde yetiştirilmesi ve verimli ürün alınması

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi :
Kabul Tarihi :

Anahtar Kelimeler
Bitki Yaprak Hastalığı
Derin Öğrenme
Transfer Öğrenme
Evrişimli Sinir Ağı
Görüntü Sınıflandırma

Transfer Öğrenme Temelli Bitki Yaprak Hastalıklarının Tespiti İçin Karşılaştırmalı Bir Çalışma

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ÖZET

Bitkilerin sağlıklı bir şekilde yetiştirilmesi ve verimli ürün alınması için hastalıkların erken teşhisi kritik öneme sahiptir. Bitki hastalıklarının bir çiftçi tarafından görsel olarak tanımlanması genellikle zordur. Ancak, makine öğrenmesi yöntemleri kullanılarak, bitki hastalıkları tespiti sürecini daha hızlı ve hassas bir şekilde gerçekleştirilebilir. Bu sayede, ürün kayıplarını azaltarak, maliyetlerinin düşürülmesi ve tarımsal üretkenliğin artırılmasıyla genel ekonomik verimliliği yükseltebilmek mümkündür. Bu çalışmada, 12 farklı sağlıklı bitki ve 30 farklı hastalıkla bulaşık bitki yaprağı görüntüleri kullanılarak bitki hastalıklarının yapay zeka ile sınıflandırması amaçlanmıştır. Geliştirilen sistemde yapay zeka modeli olarak VGG16, VGG19, AlexNet, MobileNetV1 ve MobileNetV2 olmak üzere 5 farklı Evrişimli sinir ağı modeli kullanılmıştır. Tüm modeller eğitilmiş ve doğruluk değerleri üzerinden karşılaştırılmıştır. MobileNetV1 üzerinden %99,20 ile en yüksek doğruluk değeri elde edilmiştir. Önerilen yöntem, çeşitli performans analizlerinden geçirilerek doğrulanmıştır. Yapay zeka tabanlı bir web uygulama da son kullanıcı için geliştirilmiştir.

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A Comparative Study for Detection of Plant Leaf Diseases Based on Transfer Learning

ABSTRACT

Early diagnosis of diseases is critical for growing plants in a healthy manner and obtaining productive products. Plant diseases are generally difficult to visually identify by a farmer. However, by using machine learning methods, the process of detecting plant diseases can be realized more quickly and precisely. Hence, it can reduce product losses, reduce costs, and increase overall economic efficiency by increasing agricultural productivity. In this study, classifying plant diseases with artificial intelligence has been aimed by using images obtained from 12 different images of healthy plants and plant leaves infected with 30 different diseases. In the developed system, 5 different Convolutional neural networks (CNN) models including VGG16, VGG19, AlexNet, MobileNetV1, and MobileNetV2, have been used as artificial intelligence models. All models have been trained and compared based on their accuracies. The highest accuracy value of 99.20% has been obtained by The MobileNetV1. The proposed method has been validated through various performance analyses. An artificial intelligence-based web-based application has also been developed for the end-user.

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A Comparative Study for Detection of Plant Leaf Diseases Based on Transfer Learning

ABSTRACT

Early diagnosis of diseases is critical for growing plants in a healthy manner and obtaining productive products. Plant diseases are generally difficult to visually identify by a farmer. However, by using machine learning methods, the process of detecting plant diseases can be realized more quickly and precisely. Hence, it can reduce product losses, reduce costs, and increase overall economic efficiency by increasing agricultural productivity. In this study, classifying plant diseases with artificial intelligence has been aimed by using images obtained from 12 different images of healthy plants and plant leaves infected with 30 different diseases. In the developed system, 5 different Convolutional neural networks (CNN) models including VGG16, VGG19, AlexNet, MobileNetV1, and MobileNetV2, have been used as artificial intelligence models. All models have been trained and compared based on their accuracies. The highest accuracy value of 99.20% has been obtained by The MobileNetV1. The proposed method has been validated through various performance analyses. An artificial intelligence-based web-based application has also been developed for the end-user.

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GİRİŞ

Bitki hastalıkları, dünya çapında küresel gıda güvenliği ve çevresel sürdürülebilirlik açısından önemli riskler oluşturmakta ve etkilenen bölgelerin çevresel ve sosyo-ekonomik koşullarını olumsuz etkileyen doğrudan verimlilik ve biyolojik çeşitlilik kaybına yol açmaktadır (Ristaino ve ark., 2021). Bitkilerde hastalığa sebep olan canlı (biyotik): virüs, viroid, bakteri, molliküt (fitoplazma ve spiroplazma) ve fungus gibi patojenik mikroorganizmalar ile parazitik bitkiler ve cansız (abiyotik): sıcaklık, nem, ışık, besin elementi eksikliği veya fazlalığı, toprak yapısı ve pH gibi etkenler bulunmaktadır. Abiyotik faktörler geniş alanlarda bitkilere zarar verebilmelerine rağmen, patojenlerden farklıdırlar. Çünkü çoğalmazlar ve bitkiden bitkiye yayılmazlar. Kültürü yapılan bitki türleri arasında ortaya çıkan bulaşıcı hastalıklar gıda sürdürülebilirliği için ciddi bir tehdit oluşturmaktadır.

Patojenler konukçu bitkilerinden ihtiyaç duydukları besinleri absorbe ederek, enzim toksin, büyüme düzenleyicileri gibi biyokimyasallar salgılayarak fizyolojik olaylarında önemli değişikliklere sebep olurlar. Fizyolojik olaylar arasında, fotosentez, yaprak hastalıklarından birincil olarak etkilenir (Bastiaans , 1991; Lopes

& Berger, 2001). Örneğin, yaprakların bir kısmını enfekte eden ve öldüren veya klorofilini yok eden bir patojen, bitkinin fotosentezinin, büyümesinin ve veriminin azalmasına vb. yol açar. Enfeksiyonun sonucu olarak da veriminin azalması, ürün kalitesinin düşmesi, hasat sonrası ürün bozulması, çok yıllık ürün plantasyonlarının yok olması ve bazı durumlarda diğer biyotik veya abiyotik faktörlere (örn. don) karşı duyarlılığın artması söz konusudur. Bu nedenle, bitki hastalıklarının doğru ve zamanında tespit edilmesi, hastalıkla mücadelenin erken dönemde başlanması ve böylece hastalığın yayılmasının önlenmesi açısından oldukça önemlidir. Enfekteli bitki, patojen-konukçu kombinasyonuna bağlı olarak çeşitli simptomlar (hastalık belirtileri) gösterir. Bu simptomlar bitkinin farklı organlarında görülebileceği gibi bitkinin tümünde de görülebilmektedir. Arazi koşullarında hastalıkların tespit edilmesi ve mücadelesinin başlanması, genellikle üretici ve uzmanların bilgi ve tecrübeleri doğrultusunda çıplak gözle yapılmaktadır. Çoğu zaman bu hastalıkların doğru zamanda tespit edilememesi mücadeleye geç başlanmasına neden olmaktadır. Bu durum gereksiz tarım ilacı (pestisit) kullanımı ile üretim maliyetinin artmasının yanı sıra çevresel kirlenmeye zemin hazırlamaktadır (Erdoğan, 2024). Ayrıca, insan sağlığına olumsuz etkiye neden olmaktadır. Bu hastalıkların kontrol altına alınması, sağlıklı bir tarım sistemi için kritik önem taşır. Dolayısıyla, hastalıkların etkili bir şekilde yönetilmesi, tarımsal sürdürülebilirliğin sağlanması ve gıda güvenliğinin korunması için önemli bir gerekliliktir. Çiftçiler veya uzmanlar, bitki yaprak hastalıklarını genel bir şekilde tanıyabilir ve teşhis edebilir. Bununla birlikte, bu yaklaşım zaman alıcı, maliyetli ve her zaman güvenilir değildir (Luckey, 2012). Makine öğrenimi ve derin öğrenmedeki son gelişmeler, bitki hastalıklarının tespit edilmesini kolaylaştırmıştır (Heltin Genitha ve ark., 2019; Harakannanavar ve ark., 2022). Hastalıkların üretim alanındaki diğer bitkilere yayılmadan önce erken tespit edip sınıflandırarak ürün kaybının önlenmesi sağlar (Rajasekaran ve ark., 2020). Bu sayede hastalıkların erken teşhisi, çiftçilerin zamandan tasarruf etmesine ve bitki büyümesini teşvik etmesine yardımcı olabilir. Bitki yapraklarından hastalık tanımlaması ve sınıflandırması için görüntü işleme yöntemlerinin benimsenmesi üzerine gerçekleştirilen anket sonuçları, görüntü işleme tekniklerinin bitki büyümesini, verimliliği, kaliteyi ve ekonomik değeri artırdığını göstermiştir (Chouhan ve ark., 2019).

Derin öğrenme, son yıllarda, özellikle bitki yaprak hastalığı görüntülerini hassas ve hızlı bir şekilde tespit etmek ve sınıflandırmak için görüntü sınıflandırmasında önemli ölçüde ilerlemiştir (Abade ve ark., 2021). Transfer öğrenme (Zhao ve ark., 2022; Xu ve ark., 2022; Nigam ve ark., 2023; Ibarra-Pérez ve ark., 2024) ise, derin öğrenme modellerinin çeşitli dezavantajlarını giderir. Transfer öğrenme, daha önce öğrenilen bir görevden elde edilen bilgileri kullanarak öğrenmeyi geliştiren güçlü bir derin öğrenme tekniğidir (Mohanty ve ark., 2016). Transfer öğrenme modellerinin sınıflandırma birimi probleme özgü olarak yeniden eğitildiği için hesaplama maliyeti oldukça azalmaktadır. Bu sayede eğitim süresi ve hesaplama için donanım maliyetler azalır (Yosinski ve ark., 2014). Transfer öğrenme hesaplama karmaşıklığı ve kontrol parametreleri, kullanılan modelin karmaşıklık seviyesi ve katman sayısına göre belirlenir (Picon ve ark., 2019). Böylece transfer öğrenme, daha önce edinilmiş bilgileri etkili ve hızlı bir şekilde kullanarak nesne tespiti ve görüntü sınıflandırmasında önemli ilerlemelere yol açmaktadır (Wasswa ve ark., 2024).

Bu çalışmanın amacı, bitki hastalıklarının tespitinde beş farklı transfer öğrenme tekniğinin VGG16, VGG19 (Simonyan & Zisserman, 2014), AlexNet (Krizhevsky ve ark., 2012), MobileNetV1 (Howard ve ark., 2017) ve MobileNetV2 (Sandler ve ark., 2018) başarımını karşılaştırmaktır. En iyi sonucu veren model, geleneksel yöntemlere kıyasla daha yüksek doğruluk ve verimlilik sağlayarak tarımsal üretimde verimliliği ve sürdürülebilirliği artırmayı hedeflemektedir.

Bu çalışmada sırasıyla literatür özeti, materyal ve metot, bulgular ve tartışma bölümler sunulmaktadır. En son çıkarımlar ve gelecek çalışmalar ile sonlandırılmaktadır.

LİTERATÜR ÖZETİ

Literatürde, bitki hastalıklarının tespiti alanında bugüne kadar önemli araştırmalar yapılmıştır. Literatür taramasında transfer öğrenme ele alınan problem üzerinde iyi sonuçlar verdiği görülmektedir (Yang ve ark., 2019; Espejo-Garcia ve ark., 2021)

Nachtigall ve ark. (2016) tarafından yapılan çalışmada elma ağaçlarındaki hastalıkları tespit etmek ve sınıflandırmak için AlexNet mimarisi kullanmışlardır. Sonuçlar, CNN tarafından elde edilen %97,3'lük bir doğruluk göstermiştir. Wang ve ark. (2017) elma hastalıklarının sınıflandırılması için en iyi model olarak transfer öğrenimi ile eğitilen VGG16 olduğunu ve %90,84 doğruluk verdiğini belirtmişlerdir. Wallelign ve ark. (2018) soya fasulyesindeki hastalıkları sınıflandırmak için CNN sınıflandırıcısını kullanarak bir model tasarlamıştır. Modellerinin %99,32 doğruluk elde ettiklerini bildirmişlerdir. Dawei ve ark. (2019) bahçecilikte zararlıları tespit etmek için transfer öğrenimini kullanarak toplam 10 sınıfı tahmin edebilen model ile %93,84 doğruluğa ulaşmıştır. Ferentinos (2018) 25 farklı bitki hastalığının tanılanması için derin öğrenme sınıflandırma tekniğini uygulayarak, %99,53 doğruluk elde etmişlerdir. Chen ve ark. (2020) bitki hastalıklarını tespit etmek için transfer öğrenimini

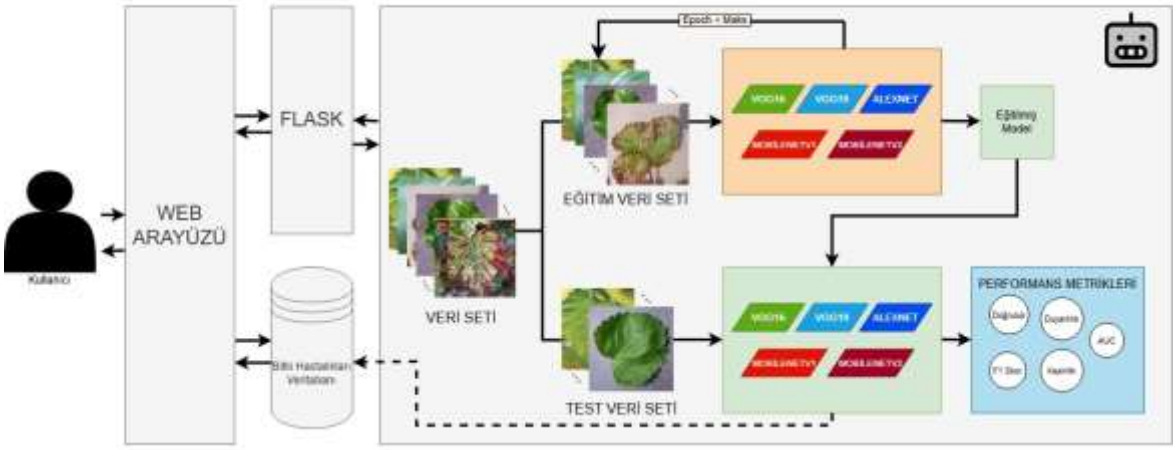
incelemişlerdir. VGGNet-19 modelini seçilmesiyle önerilen yaklaşım, %92'lik doğruluk sağlamıştır. Cruz ve ark. (2017) *Xylella fastidiosa* etmeninin zeytinde neden olduğu Xylella yaprak yanıklığı hastalığının tespitini transfer öğrenme ile gerçekleştirmiştir. Yapılan analiz sonucunda, simptom taşıyan zeytin yapraklarının kullanılmasıyla hastalığın tespit edilmesindeki başarı oranının yaklaşık %98,60 olduğu görülmüştür. Marzougui ve ark. (2020) tarafından gerçekleştirilen çalışmada CNN ile bitki hastalıklarını tespit etmek için ResNet modelini kullanılmıştır. Çalışmada, 500 bitki görüntüsünden oluşan özelleştirilmiş bir veri seti kullanılmıştır. Tasarlanan model ile görüntüleri sağlıklı ve hastalıklı olmak üzere iki kategoriye ayırmakta olup, geliştirilen sistem ile son teknolojide önerilenlerden daha iyi tespit performansları elde ettiklerini belirtmişlerdir. Sibiya ve Sumbwanyambe (2019) yılında mısır bitkisinde kuzey mısır yaprak yanıklığı, gri yaprak lekesi ve mısır pası gibi hastalıklarını tespit etmek için CNN kullanmıştır ve doğruluk oranı %92,85 olarak bulunmuştur. Shrivastava ve ark. (2019) pirinç hastalığının sınıflandırılması için derin CNN'nin transfer öğrenimini kullanmışlardır ve hastalığı %91,37 doğrulukla tanımlamışlardır. Jiang ve ark. (2020) transfer öğrenimini, bitki hastalığı tanıma modeli oluşturmak için ResNet üzerine uygulayarak, transfer öğrenme modelinin hastalık tanımlama doğruluğunun %83,75 olduğunu ve bunun geleneksel ResNet-101 modelinden çok daha yüksek olduğunu ortaya koymuşlardır. Çalışma verilerine dayanarak, transfer öğrenme algoritmasına dayalı bitki hastalığı tanıma modelini son derece uygulanabilir bir çözüm olarak sunmuşlardır. Xie ve ark. (2021) çatlak, şekil bozukluğu, çatallı, kırık olan havuçları satıştan önce tespit etmek için derin öğrenme ve transfer öğrenmeye dayalı yöntemi önermiştir. Kusurlu havuçların tanınması için beş klasik CNN (Densenet-121, ResNet-50, Inception-V3, VGG-16 ve VGG-19) uygulanmıştır. Bu topluluk modellerinde ResNet-50 sabit bir model olarak seçilmiş ve diğer dört modelden herhangi ikisiyle ortalama alma yöntemiyle birleştirilmiştir. Sonuçlar ResNet-50, Densenet-121 ve VGG-16'dan (R-D-V16) oluşan topluluk modelinin sırasıyla %97,34, %99,53, %94,62, %99,62, %97,01 ve görüntü başına 0,09s doğruluk, kesinlik, duyarlılık, özgüllük, F1-skoru ve tespit hızı ile en iyi performansı gösterdiği ortaya konulmuştur.

Mehedi ve ark. (2022) çeşitli bitki hastalıklarını tespit etmek için transfer öğrenme yaklaşımını, önceden eğitilmiş modeller olan EfficientNetV2L, MobileNetV2 ve ResNet152V2 ile kullanmışlardır. Çalışmada 14 farklı bitki türünde 38 çeşit yaprak hastalığını tespit etmek için, önceden eğitilmiş üç modeli çeşitli niceliksel performans değerlendirme parametrelerine göre karşılaştırarak, EfficientNetV2L modelinin %99,63 doğrulukla en iyi performansı sergilediğini belirtmişlerdir. Shahoveisi ve ark. (2023), dört evrişimli sinir ağı modelinin üç ticari açıdan önemli tarla ürününde pas hastalığının tespitindeki potansiyelini değerlendirmiş ve EfficientNetB4 modelinin doğruluk oranını ortalama %94,29 olarak bulmuşlar. Vallabhajosyula ve ark. (2024) Vision Transformer ve ResNet9 modellerini kullanan yeni bir hiyerarşik kalıntı vizyon dönüştürücüsü (Residual Vision Transformer) ile yaprak hastalıklarının erken tespitine yardımcı olan bir model ile 13, 38 ve 51 farklı yaprak hastalığı sınıfına sahip Local Crop veri seti, Plant Village veri seti ve Extended Plant Village veri seti üzerinde değerlendirilmiştir. Performans değerlendirmesi, veri setlerinde geniş kapsamda yapılmış ve sonuçlar, önerilen modelin InceptionV3, MobileNetV2 ve ResNet50 gibi diğer modellere göre daha iyi performans gösterdiğini ortaya koymuştur.

Literatürdeki bu çalışmalar göz önüne alındığında, bitki hastalıklarının sınıflandırılmasında transfer öğrenme yöntemlerinin etkin sonuçlar verdiği görülmektedir. Fakat, bitki yapraklarından hastalık sınıflandırılmasında transfer öğrenme yöntemlerinin karşılaştırmalı olarak sunulmasında sınırlılık gözlenmiştir.

MATERYAL ve METOD

Bu çalışmada, bitki yaprak hastalıklarının tanımlanması ve sınıflandırılması için 5 farklı transfer öğrenme modelleri kullanılmıştır. Amaç, VGG16, VGG19, AlexNet, MobileNetV1 ve MobileNetV2 modellerini kullanarak en iyi sonuçları veren algoritmayı belirlemek ve bu modellerin performansını artırmaktır. Çalışmanın genel akış diyagramı Şekil 1'de verilmiştir.



Şekil 1. Bitki yaprak hastalıklarının tespiti için Genel Akış Diyagramı
Figure 1. The diagram of general flow for detection of plant leaf diseases

Veri Seti

Çalışmada kullanılan ana veri setinde (Kaggle, 2020) sağlıklı ve hastalıklı bitki yapraklarının 87.867 adet görüntüsü bulunmaktadır. Fakat, veri setinde yaban mersini, ahududu ve soya fasulyesi bitkilerine ait sadece sağlıklı bitkilerin görüntüleri bulunmaktadır. Dolayısıyla veri setinde hastalıklı durumlarına ait herhangi bir görüntü bulunmadığı için veri setinden çıkarılmıştır. Ayrıca, asma siyah çürüklüğü hastalığının yaygın bulunmaması ve akarın hastalık etmeni olmaması sebebiyle domatesteki akar zararlısına ait görüntüler veri setinden kaldırılmıştır. Veri seti analizinde, uzman görüşü ile elma memeli pası hastalığına ait görüntüler ise problemi temsil kabiliyetinin sınırlı bulunması nedeniyle veri setinden çıkarılarak, yerel görüntüler ile bu sınıfa yeni görüntüler eklenmiştir. Bu işlemler sonucunda veri setinden toplam 13761 adet görüntü azalmıştır. Çalışmada bitki yaprak hastalıklarının kapsamının genişletilebilmesi için bağ antraknozu, bağ mildiyösü, bağ küllemesi, şeftali yaprak kıvırcıklığı, domates lekeli solgunluk virüsü, ceviz antraknozu ve elma memeli pası hastalıklarına ait görüntüler ile birlikte, sağlıklı portakal, kabak ve ceviz bitkilerine ait görüntülerin derlenmesi sonucunda 11.520 adet yeni görüntü ile veri seti genişletilmiştir (Hastalıklı ve sağlıklı bitki görüntüleri Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümünden temin edilmiştir). Eklenen yeni görüntünler ile Çalışmada kullanılan veri setinde toplam 85.626 RGB görüntüsü bulunmaktadır. Şekil 2'de veri setinden örnek görüntüler sunulmuştur.

Veri setindeki sağlıklı bitkiler;

- Elma, kiraz, mısır, asma, portakal, şeftali, biber, patates, kabak, çilek, domates, ceviz'dir.

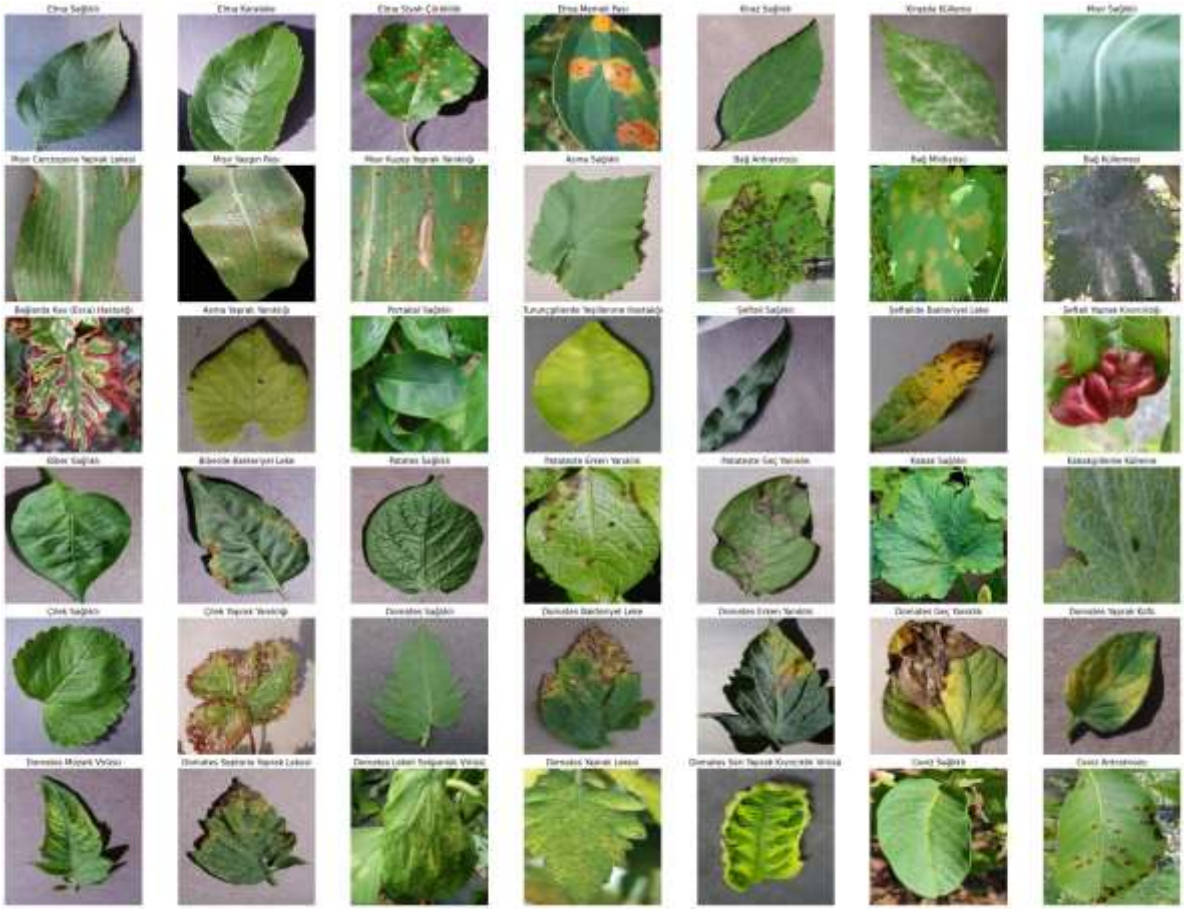
Veri setindeki hastalıklar;

- Elma karaleke, elma siyah çürüklük, elma memeli pası, kirazda külleme, mısır cercospora yaprak lekesi, mısır yaygın pası, mısır kuzey yaprak yanıklığı, bağ antraknozu, bağ mildiyösü, bağ küllemesi, bağlarda kav (esca) hastalığı, asma yaprak yanıklığı, turunçgillerde yeşillenme hastalığı, şeftalide bakteriyel leke, şeftali yaprak kıvırcıklığı, biberde bakteriyel leke, patateste erken yanıklık, patateste geç yanıklık, kabakgillerde külleme, çilek yaprak yanıklığı, domates bakteriyel leke, domates erken yanıklık, domates geç yanıklık, domates yaprak küfü, domates mozaik virüsü, domates septoria yaprak lekesi, domates lekeli solgunluk virüsü, domates yaprak lekesi, domates sarı yaprak kıvırcıklık virüsü, ceviz antraknozu'dur.

Toplam veri seti, dizin yapısı korunarak 80/20 oranında eğitim ve test setine bölünmüştür. Eğitim setinde 68.501, test setinde 17.125 olmak üzere toplam 85.626 görüntü bulunmaktadır. Veri seti genel olarak sınıflar açısından dengeli bir veri setidir. Bitkilere göre görüntü sayıları Çizelge 1'de rapor edilmiştir.

Veri Arttırma

Veri artırımı, modelin genelleme yeteneğini artırabilmekte ve aşırı öğrenmeyi önleyebilmek için kullanılmaktadır (Wang, 2017). Bu nedenle, modelleme süreçlerinde yaygın olarak kullanılan etkili bir yaklaşımdır (Simonyan & Zisserman 2014). CNN yöntemlerini eğitmeye başlamadan önce veri setini çeşitlendirmek amacıyla Çalışmada veri artırımı gerçekleştirildi. Bu çalışmada kullanılan veri arttırma işlemi şunlardır;



Şekil 2. Veri Setinden örnek bir görüntü kümesi
Figure 2. A set sample images from the used dataset

(a) Yatay ve Dikey Çevirme (Yatay): Giriş verilerinin yatay veya dikey olarak çevrilmesini içeren sıklıkla kullanılan veri büyütme işlemleridir. Bu, modelin eğitim veri kümesindeki nesnelerin farklı yönelimlerini öğrenmesine yardımcı olur. Ayrıca yeni verilere genelleme yeteneğini geliştirebilir.

(b) Genişlik Kaydırma Aralığı (0,2): Görüntüleri yatay yönde kaydırmak için kullanılan bir aralığı belirtir. Bu değer, orijinal görüntü genişliğinin bir yüzdesi olarak ifade edilir.

(c) Yakınlaştırma Aralığı (0,2): Görüntülerin yakınlaştırma veya uzaklaştırma derecesini belirler.

(d) Dolgu Modu (En yakın): Görüntülerin yeniden boyutlandırılması, döndürülmesi ya da kırpılması gibi işlemler sırasında oluşabilecek boşlukları nasıl doldurulacağını belirtir. "En yakın" parametresi ise bu boşlukları en yakın piksel ile doldurulmasını sağlar.

(e) Doğrulama Ayrımı (0,2): Verilerin eğitim ve doğrulama setlerine nasıl bölüneceğini belirten bir parametredir. Bu parametre, verilerin belirli bir oranının doğrulama seti olarak ayrılacağını belirtir.

(f) Yeniden Ölçekleme (1/255): Görüntülerin yeniden ölçeklendirilmesi işlemi sırasında kullanılır. Bu parametre, görüntüdeki piksel değerlerini yeniden ölçeklendirerek 0 ile 1 arasında düzenlenmesini sağlar. İşlemin amacı, modelin daha iyi öğrenebilmesi için veri setini uygun bir ölçeğe getirmektir.

(e) Rastgele Rotasyon (90^0): Giriş verilerinin rastgele bir açıyla döndürülmesini içeren yaygın bir veri büyütme işlemidir. Bu, modelin eğitim veri kümesindeki nesnelerin farklı yönelimlerini öğrenmesine yardımcı olur.

VGG16

VGG16 (Karen ve ark., 2014), genel olarak görsel sınıflandırma görevlerinde kullanılmak üzere tasarlanmış derin bir evrişimli sinir ağıdır. VGG16'nın önemli özelliklerinden biri, basit ve tutarlı bir mimariye sahip olmasıdır. Model, ardışık bir dizi evrişim katmanından oluşur. Bu katmanlar, görsel özellikleri çıkarmak için filtreleri kullanır. Her evrişim katmanının çıkışında genellikle ReLU gibi doğrusal olmayan aktivasyon fonksiyonları uygulanır. Evrişim katmanlarından sonra havuzlama katmanları gelir. Bu katmanlar, özellik haritasını küçültmek ve öğrenilen özellikleri özetlemek amacıyla kullanılır. Modelin çıkışında genellikle softmax aktivasyon fonksiyonu kullanılır.

Bu projede VGG16 modeli farklı batch boyutu ve epoch değerleri üzerinde eğitilmiş ve başarı oranı ortalama olarak %90 elde edilmiştir. Modelleme de elde edilen doğruluk ve kayıp grafiği Çizelge 2'de sunulmuştur.

Çizelge 1. Veri Seti Dağılımı
Table 1. Data Set Distribution

Sınıf İsimleri	Sayı
Elma Sağlıklı	2510
Elma Karaleke	2520
Elma Siyah Çürüklük	2483
Elma Memeli Pası	1020
Kiraz Sağlıklı	2282
Kirazda Külleme	2104
Mısır Sağlıklı	2324
Mısır Cercospora Yaprak Lekesi	2052
Mısır Yaygın Pası	2384
Mısır Kuzey Yaprak Yanıklığı	2385
Asma Sağlıklı	2115
Bağ Antraknozu	980
Bağ Mildiyösü	960
Bağ Küllemesi	1020
Bağlarda Kav (Esca) Hastalığı	2400
Asma Yaprak Yanıklığı	2152
Portakal Sağlıklı	1300
Turunçgillerde Yeşillenme Hastalığı	2513
Şeftali Sağlıklı	2160
Şeftalide Bakteriyel Leke	2297
Şeftali Yaprak Kıvırcıklığı	1100
Biber Sağlıklı	2485
Domates Lekeli Solgunluk Virüsü	1740
Patates Sağlıklı	2280
Patateste Erken Yanıklık	2424
Patateste Geç Yanıklık	2424
Kabak Sağlıklı	1060
Kabakgillerde Külleme	2170
Çilek Sağlıklı	2280
Çilek Yaprak Yanıklığı	2218
Domates Sağlıklı	2407
Domates Bakteriyel Leke	2127
Domates Erken Yanıklık	2400
Domates Geç Yanıklık	2314
Domates Yaprak Küfü	2352
Domates Mozaik Virüsü	2238
Domates Septoria Yaprak Lekesi	2181
Domates Yaprak Lekesi	2284
Domates Sarı Yaprak Kıvırcıklık Virüsü	2450
Ceviz Sağlıklı	1260
Ceviz Antraknozu	1080

Çizelge 2. Eğitim Modellerinde Kullanılan Batch Boyutu ve Epoch Sayıları
Table 2. Batch Sizes and Number of Epochs Used in Training Models

Model	Batch Boyutu	Epoch
M1	32	10
M2	20	10
M3	20	40

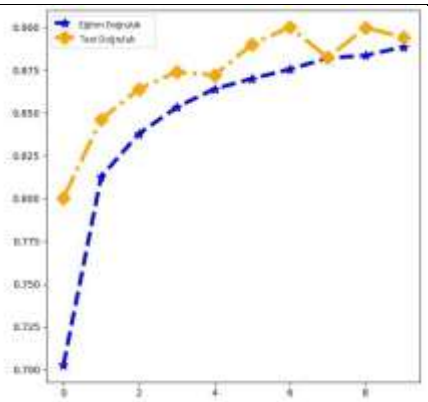
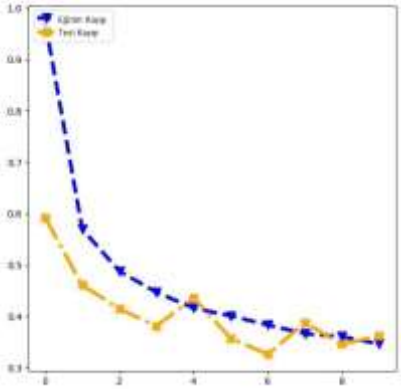
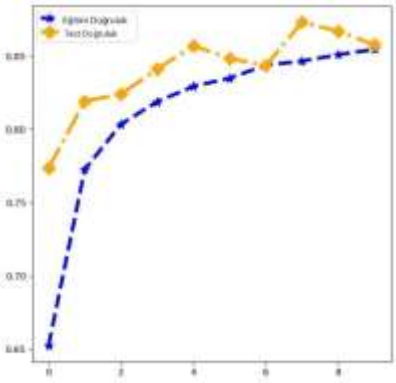
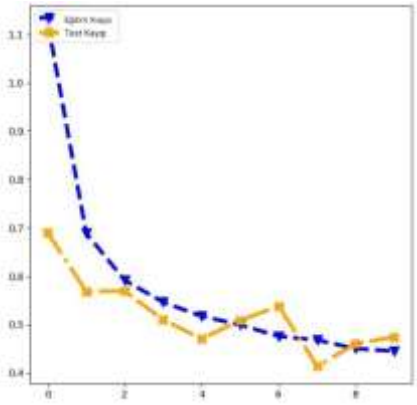
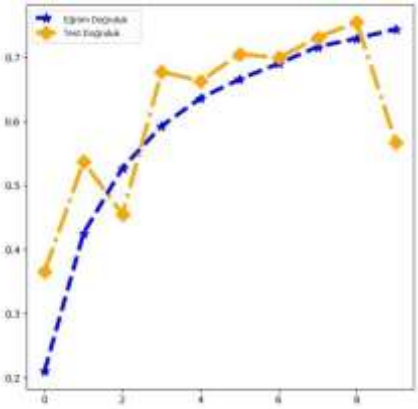
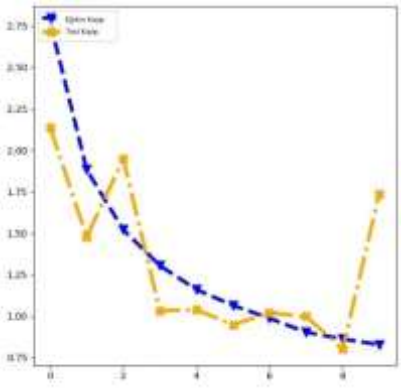
VGG19

VGG19 (Karen ve ark., 2014), Oxford Üniversitesi'nde yer alan Visual Geometry Group (VGG) tarafından geliştirilmiş bir evrişimli sinir ağı mimarisidir. VGG16'nın genişletilmiş bir sürümüdür. Daha derin bir yapıya

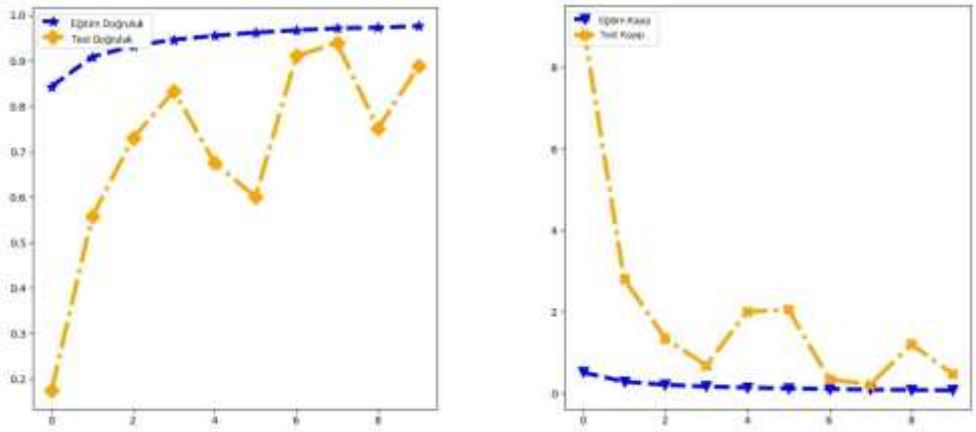
sahiptir. VGG modelleri, büyük veri setlerinde öğrenme kapasitesi ve genelleme yeteneğiyle tanınan derin öğrenme modelleridir. VGG19, VGG16'nın daha kompleks bir versiyonudur. Bu nedenle daha fazla parametreye sahiptir. Bu sayede daha karmaşık görevlerde daha iyi performans elde etme potansiyeli sağlamaktadır. Ancak daha fazla hesaplama gücü ve veri seti gerektirebilir.

Çalışmada VGG19 modeli farklı batch boyutu ve epoch değerleri üzerinde eğitilmiş ve başarı oranı ortalama %86 olarak elde edilmiştir. Modelleme de elde edilen doğruluk ve kayıp grafiği Çizelge 3'de sunulmuştur.

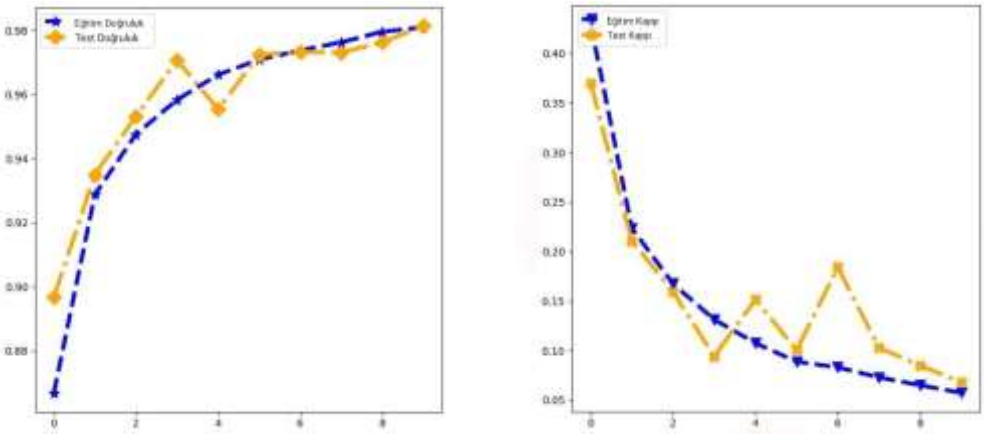
Çizelge 3. Transfer Öğrenme yöntemlerinin doğruluk ve kayıp grafikleri
Table 3. The Accuracy and loss graphs of transfer learning methods

Yöntem	Doğruluk Grafiği	Kayıp Grafiği
VGG16		
VGG19		
AlexNet		

MobileNetV2



MobileNetV1



AlexNet

AlexNet (Krizhevsky, 2012), 2012 yılında ImageNet Large Scale Visual Recognition Challenge yarışmasında büyük bir başarı elde ederek derin öğrenme alanında öne çıkmıştır. Modelin önemli özelliklerinden biri, o döneme kadar yapılmış en başarılı derin sinir ağı olmasıdır. AlexNet 8 evrişim ve 3 tam bağlantılı katman içermektedir. Evrişim katmanları, giriş görüntülerindeki özellikleri öğrenmek için ReLU aktivasyon fonksiyonlarıyla birlikte kullanılmaktadır. Ardından, özellik haritalarını küçültmek ve özetlemek için maksimum havuzlama katmanları gelmektedir. Ayrıca, aşırı öğrenmeyi azaltmak için dropout ve veri artırımı gibi teknikler uygulanmaktadır.

Bu çalışmada AlexNet modeli farklı batch boyutu ve epoch değerleri üzerinde eğitilmiş ve diğer modellere göre daha düşük başarı oranı vermiştir. Başarı oranı ortalama olarak %73 elde edilmiştir. Modelleme de elde edilen doğruluk ve kayıp grafiği Çizelge 3'de sunulmuştur.

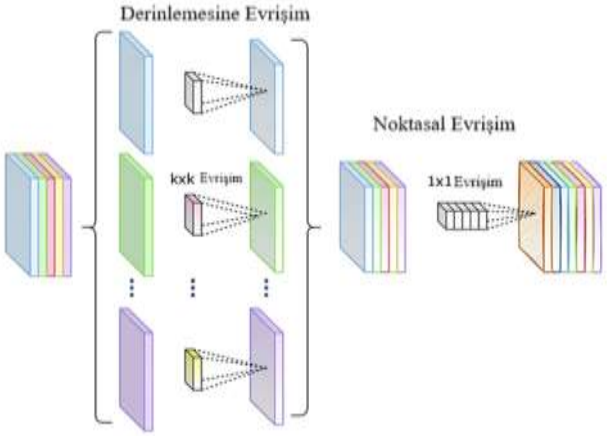
MobileNetV2

MobileNetV2 (Sandler ve ark., 2018), hesaplama maliyeti düşük ve etkili bir evrişimli sinir ağı (CNN) mimarisidir. Genellikle mobil cihazlarda veya kaynak sınırlı ortamlarda kullanılmak üzere tasarlanmıştır. Bu mimari, önceki MobileNet modellerinin geliştirilmiş bir versiyonudur. Çeşitli uygulamalarda düşük hesaplama maliyeti ile iyi performans sağlamayı amaçlar. MobileNetV2'de temel yapı Ters Çevrilmiş Artık Blok (Inverted Residual Block) olarak adlandırılır. Bu çalışmada MobileNetV2 modeli farklı batch boyutu ve epoch değerleri üzerinde eğitilmiş ve başarı oranı ortalama olarak %93 elde edilmiştir. Modelleme de elde edilen doğruluk ve kayıp grafiği Çizelge 3'de sunulmuştur.

MobileNetV1

MobileNetV1 (Howard ve ark., 2017), Google tarafından geliştirilen ve özellikle düşük güç tüketimli derin öğrenme modeli mimarisidir. MobileNetV1'in mimarisi Şekil 3'de sunulmuştur. Bu mimari, bilgisayar görüşü görevlerini yerine getirmek üzere tasarlanmış olup, mobil uygulamalarda gerçek zamanlı olarak çalışabilecek hızda ve etkili bir şekilde performans göstermeyi hedefler. MobileNetV1'in en belirgin özelliği, geleneksel evrişim katmanlarını daha verimli hale getirmek için Derinlemesine Ayrılabilir Evrişim (Depthwise Separable Convolution) tekniğini kullanmasıdır.

Bu çalışmada MobileNetV1 modeli farklı batch boyutu ve epoch değerleri üzerinde eğitilmiş ve başarı oranı diğer modellere göre daha yüksek bir sonuç vermiştir. Başarı oranı ortalama olarak %97 elde edilmiştir. Modelleme de elde edilen doğruluk ve kayıp grafiği Çizelge 3'de sunulmuştur.



Şekil 3. MobileNetV1 Mimarisi
Figure 3. MobileNetV1 Architectural

Web Entegrasyonu

MobileNetV1 modeli, bitki hastalıkları tespiti için geliştirilen web sitesi uygulamasında kullanılmak üzere .h5 uzantılı dosya formatında kaydedildi. Modelin .h5 formatında kaydedilmesi, web sitesi uygulamasına entegrasyon sürecini kolaylaştırmaktadır. Bu entegrasyon, hızlı ve etkili web uygulamaları geliştirmek için ideal bir framework olan Flask (Vangala ve ark., 2019) ile Visual Studio (Microsoft, 2024) kullanılarak gerçekleştirilmiştir.

Web uygulaması, kullanıcıların bitki yaprağı görüntülerini yükleyebileceği ve bu görüntülerin işlenerek hastalık tespitinin yapılacağı bir platform sağlar. Kullanıcılar, web sitesinde bulunan dosya yükleme formunu kullanarak bitki yaprağı görüntülerini yükleyebilirler. Bu form, kullanıcıların sunucuya dosya yüklemesini sağlar. Form etiketi içindeki method="post" ve action="/file-upload" özellikleri, formun HTTP POST yöntemiyle gönderileceğini ve gönderilen verilerin /file-upload adresine yönlendirileceğini belirtir. Bu işlem, @app.route('/file-upload', methods=['POST']) rotası ile yönetilir. Bu rota, kullanıcıların yüklediği görüntülerin sunucuya gönderilmesini ve işlenmesini sağlar.

HTTP isteği ile yüklenen bitki yaprağı dosyası alınır ve Pillow kütüphanesi kullanılarak bir görüntüye dönüştürülür. Pillow (Clark ve ark), güçlü bir görüntü işleme kütüphanesidir. Python'da görüntülerle çalışmayı kolaylaştırmaktadır. Görüntü, modelin gereksinimlerine uygun olarak 224x224 piksel boyutlarına yeniden boyutlandırılır. Bu, çoğu derin öğrenme modelinin girdi olarak beklediği boyuttur.

Daha sonra, görüntü verisi normalleştirilir. Normalleştirme, piksel değerlerini [0, 1] aralığına ölçeklendirerek görüntü işleme işlemini standardize edilmektedir. Bu işlem modelin daha tutarlı sonuçlar üretmesine yardımcı olabilir. Normalleştirilmiş görüntü verisi, modelin işleyebileceği bir diziye dönüştürülür. Genellikle, modelin beklediği formata uygun hale getirmek için görüntüye bir dizi ek boyut eklenir.

Model, `model.predict(img array)` komutu ile görüntüyü sınıflandırır ve sonuçlar, sınıflandırma doğruluğu açısından en yüksek olasılığa sahip sınıf belirlenerek elde edilir. Bu sonuç, ilgili hastalık veya sağlıklı durum ile eşleştirilir.

BULGULAR ve TARTIŞMA

Eğitim sürecinde optimize edilen parametreler, öğrenme oranı, epoch sayısı ve batch boyutu, model performansını artırmada etkili olmuştur. Modellerin performansını değerlendirebilmek için farklı epoch ve batch boyutları için eğitim işlemi gerçekleştirilmiştir. Tanımlanan eğitim modelleri Çizelge 2'de sunulmuştur.

M1 modeli üzerinden elden edilen deneysel sonuçlar Çizelge 4'de, M2 modeli üzerinden elde edilen sonuçlar Çizelge 5'de ve M3 modeli üzerinden elde edilen sonuçlar ise Çizelge 6'de rapor edilmiştir. Transfer öğrenme yöntemleri bu parametre değerleri kullanılarak model doğrulukları karşılaştırılmıştır. Çizelge 4, Çizelge 5 ve Çizelge 6 incelendiğinde, M3 modeli MobileNetV1 algoritması bitki hastalıklarını tespit etmede %99,20 başarı ile en iyi sonuç veren algoritma olduğu sonucuna ulaşılmıştır.

Transfer öğrenme yöntemlerinin modelleme süreçlerinde ki performanslarının değerlendirilebilmesi için M2 modeli üzerinden doğruluk ve kayıp grafikleri Çizelge 3'de sunulmuştur. Modelleme grafikleri incelendiğinde aşırı

öğrenme problemi ile karşılaşılmadığı görülmektedir. Bu bulgu, deneysel sonuçlar üzerinden gerçek-zamanlı uygulamada arzu edilen sonuçların elde edilebileceğini göstermektedir.

Çizelge 4. M1 modeli üzerinden elde edilen sonuçlar
Table 4. Obtained results on the M1 model

	Kayıp	Doğruluk	Doğrulama Kaybı	Doğrulama Doğruluğu
VGG16	0,2989	0,9021	0,3155	0,9081
VGG19	0,3796	0,8753	0,4579	0,8671
AlexNet	0,6202	0,8045	0,8411	0,7651
MobileNetV1	0,0464	**0,9850**	0,1108	0,9679
MobileNetV2	0,0617	0,9800	0,1808	0,9484

Çizelge 5. M2 modeli üzerinden elde edilen sonuçlar
Table 5. Obtained results on the M2 model

	Kayıp	Doğruluk	Doğrulama Kaybı	Doğrulama Doğruluğu
VGG16	0,3410	0,8895	0,3619	0,8941
VGG19	0,4411	0,8555	0,4745	0,8575
AlexNet	0,8224	0,7437	1,7370	0,5664
MobileNetV1	0,0593	**0,9801**	0,0679	0,9814
MobileNetV2	0,0789	0,9750	0,4788	0,8888

Çizelge 6. M3 modeli üzerinden elde edilen sonuçlar
Table 6. Obtained results on the M3

	Kayıp	Doğruluk	Doğrulama Kaybı	Doğrulama Doğruluğu
VGG16	0,2626	0,9183	0,4072	0,9036
VGG19	0,3612	0,8877	0,4193	0,8862
AlexNet	0,5372	0,8434	0,5127	0,8666
MobileNetV1	0,0160	**0,9948**	0,0271	0,9920
MobileNetV2	0,0226	0,9929	0,0767	0,9795

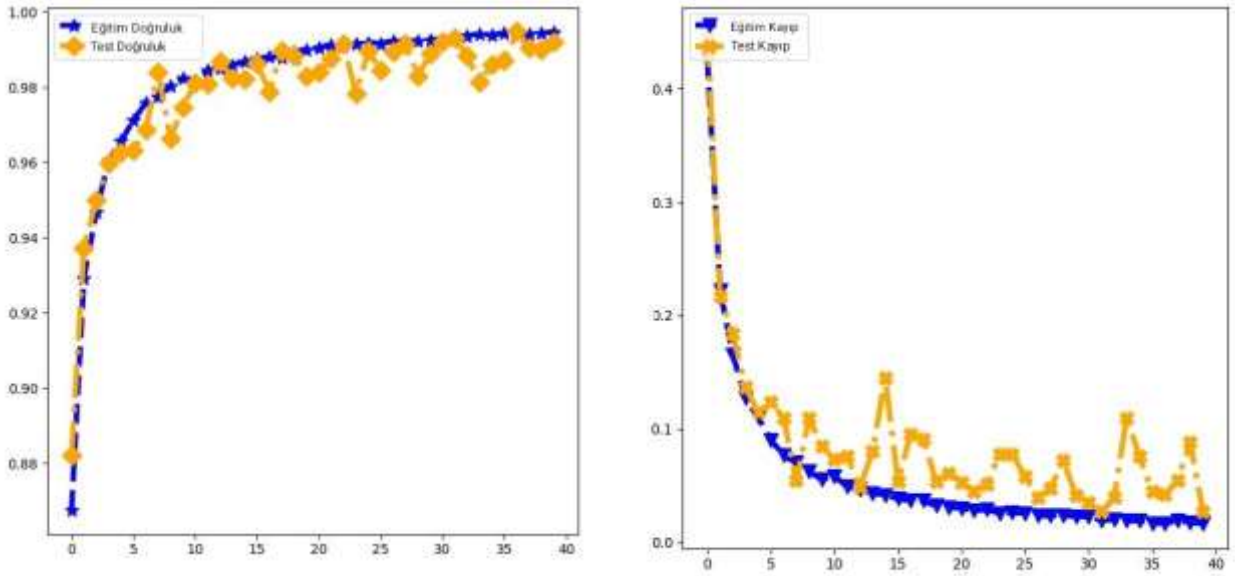
Bitki hastalıklarının tespit edilmesinde en yüksek doğruluğu veren MobileNetV1 M3 modeli olduğunda modelinin performansının detaylı değerlendirilebilmesi için doğruluk ve kayıp grafikleri Şekil 4'de sunulmuştur. Makine öğrenmesinde sıklıkla kullanılan modelin sınıflandırma performansını değerlendirmek için ROC eğrisi analizi de yapılmıştır. ROC eğrisi, doğru pozitif oranını (TPR) yanlış pozitif oranına (FPR) karşı çizer ve modelin farklı eşik değerlerindeki performansını gösterir. Grafikte her bir sınıf için ayrı bir ROC eğrisi çizilmiş ve tüm sınıflar için AUC değerleri hesaplanmıştır. AUC'nin 1.0 olması, modelinin sınıflar için etkin bir performans sergilediğini ve doğru sınıflandırma yaptığını göstermektedir. Elde edilen ROC grafiği Şekil 5'de sunulmuştur. Ayrıca, karmaşıklık matrisi ise Şekil 6'de sunulmuştur.

Şekil 4 incelendiğinde, aşırı öğrenme sorunu bulunmadığı görülmektedir. Şekil 6'da sunulan karmaşıklık matrisi incelendiğinde ise modelin 42 farklı bitki hastalığı ve sağlıklı durum sınıflarını sınıflandırma etkin ve kararlı bir yöntem olduğu görülmektedir.

Makine Öğrenmesi modellerinin değerlendirilmesinde doğruluk haricinde Kesinlik (Precison), Duyarlılık (Sensitivity) ve F1 skoru metrikleri de kullanılmaktadır. Kesinlik metriği pozitif etiketlerine sahip etiketlerinin pozitif olarak bulunabilme oranı olarak tanımlanmaktadır. Duyarlılık ise modelin pozitif örneklerin ne kadarını pozitif olarak tahmin etmen oranına karşılık gelmektedir. F1 skoru ise kesinlik ve duyarlılık oranlarının harmonik ortalaması olarak hesaplanır. Çizelge 7'de MobilNetV1 için Kesinlik, Duyarlılık ve F1 skoru sonuçları her bir sınıf için sunulmuştur. Çizelge 7 incelendiğinde önerilen model Kesinlik için en düşük 0,92 oranına sahipken, Duyarlılık için en düşük 0,94 oranına sahiptir. F1 skoru için ise en düşün oran 0,96'dır. Kesinlik, Duyarlılık ve F1 skor metrikleri için en yüksek oran 1,00 olarak hesaplanmıştır. Çizelge 7 genel olarak değerlendirildiğinde, önerilen yöntem ile her bir sınıfın diğer sınıflara göre oldukça ayrıştırılabilir olduğu görülmektedir.

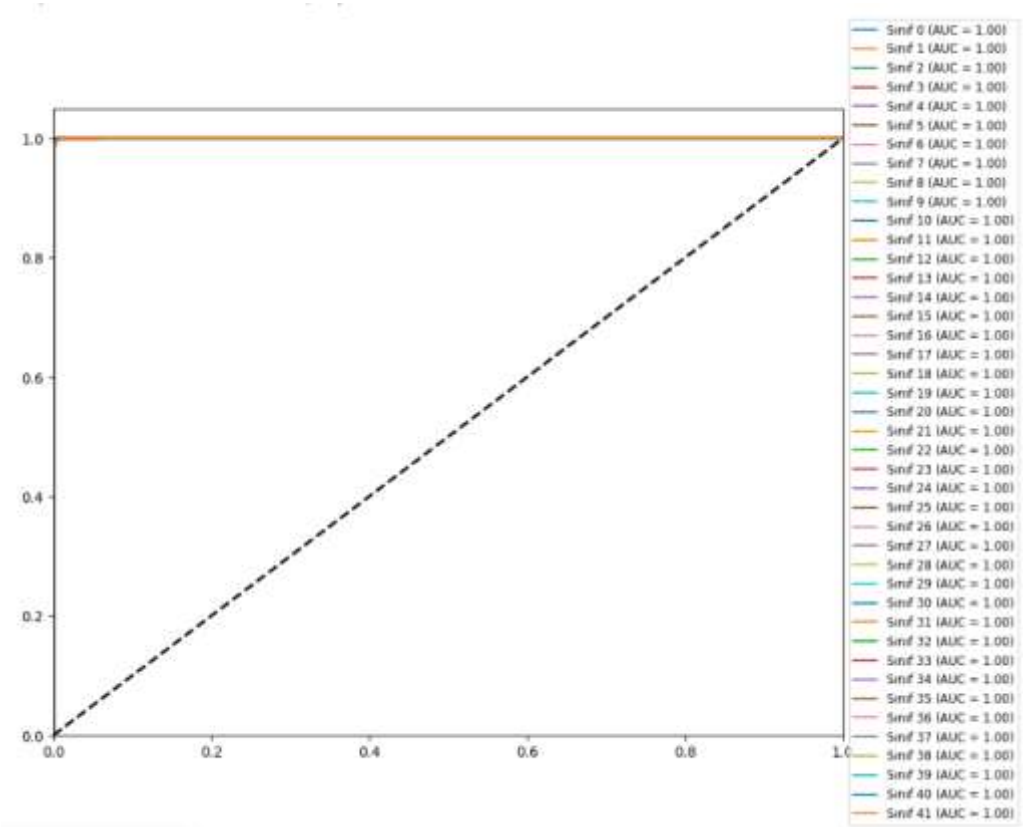
Elde edilen bulgular genel olarak değerlendirildiği de önerilen yöntemin bitki yaprak hastalıklarının sınıflandırılmasında %99,2 ile mükemmele yakın doğruluk gösterdiği görülmektedir. Fakat, bu sonuçların literatürde daha önce yayınlanan diğer çalışmalar ile adil bir karşılaştırma yapılabilmesi adına temel veri setinin kullanıldığı çalışmalar derlenmiş ve Çizelge 8'de karşılaştırmalı olarak rapor edilmiştir. Çizelge 8 incelendiğinde ResNet, DenseNet ve Özel CNN modellerinin kullanıldığı görülmektedir. Önerilen yöntem ile %99,2 doğruluk oranı ile rakip yöntemlerden daha başarılı sonuçlar alındığı görülmektedir. (Chohan ve ark., 2020) tarafından gerçekleştirilen çalışmada CNN modeli %98,3 doğruluk değeri edilmiştir. (Chellapandi ve ark., 2021) tarafından

yürütülen bir diğer çalışmada ise %99 doğruluk oranı elde edilmiştir. Bu iki çalışma önerilen yöntemden nispeten geride kalmıştır. Önerilen yöntem diğer rakiplerden ise görece daha başarılıdır.

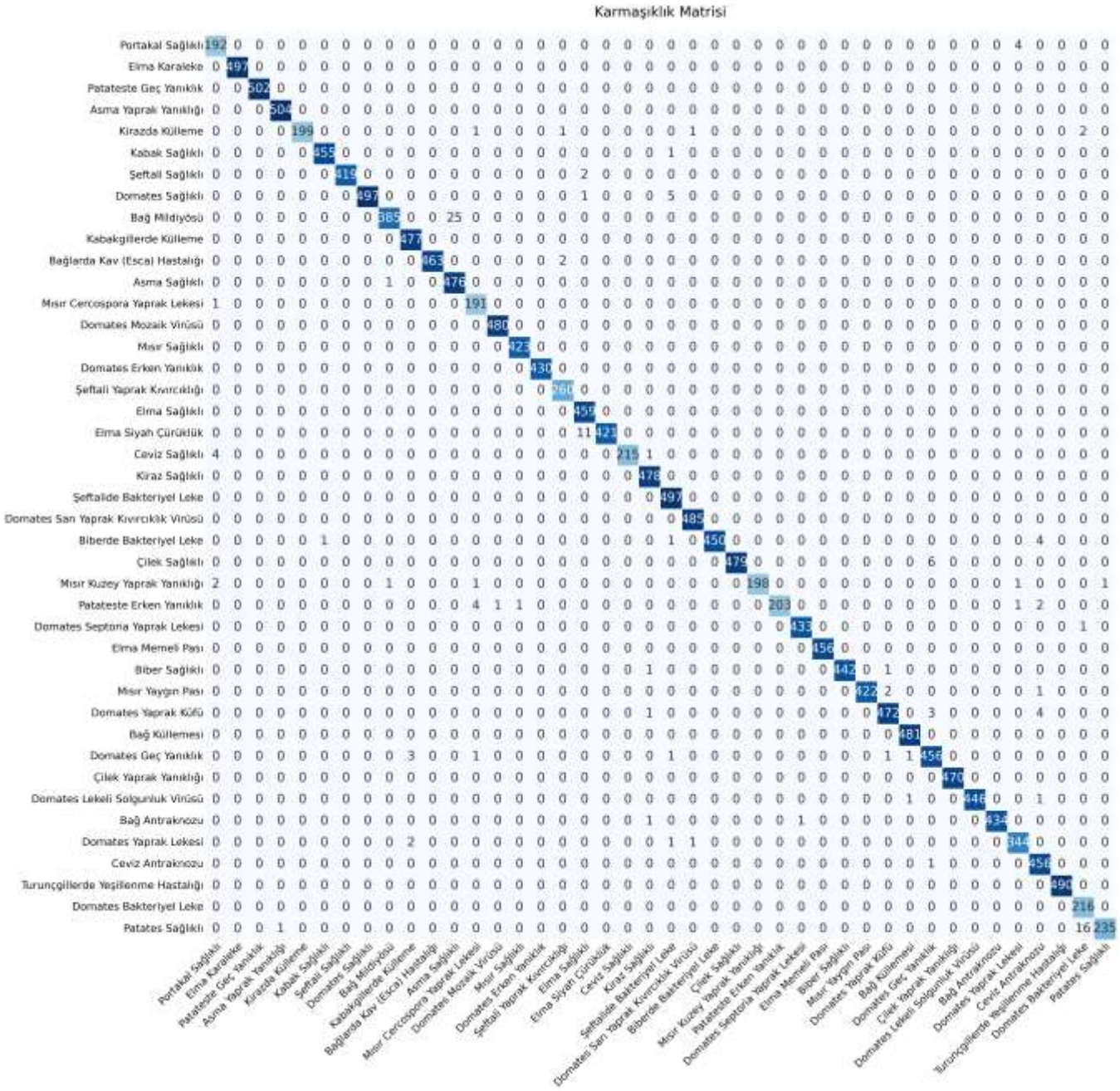


(a) Doğruluk (b) Kayıp

Şekil 4. MobileNetV1 M3 modeli üzerinden elde edilen Doğruluk(a) ve Kayıp Grafiği(b)
Figure 4. MobileNetV1 obtained Accuracy (a) and Loss Graph (b) on the M3 model



Şekil 5. AUC – ROC Eğrisi Sonuçları
Figure 5. AUC – ROC Curve Results



Şekil 6. MobileNetV1 M3 modeli üzerinden Karmaşıklık Matrisi
Figure 6. Confusion Matrix of MobileNetV1 on the M3 Model

Önerilen bitki yaprak hastalıkları tahmin modelinin başarımının görüntüler üzerinden değerlendirilmesi de önemli bir göstergedir. Şekil 7'de veri setinde bulunan örnek görüntülerdeki hastalıkların gerçek ve tahmin sonuçlar görülmektedir.

Test aşamasında, veri setinde bulunmayan ve modelin daha önce karşılaşmadığı bitki türleri de değerlendirilmiştir. Bu, modelin gerçek dünya koşullarında ne kadar genelleme yapabildiğini ve bilinmeyen bitki türlerini doğru bir şekilde sınıflandırma yeteneğini ölçmek için yapılmıştır. Veri setinde bulunmayan gerçek görüntülerin sınıf tahminleri Şekil 8' de sunulmuştur.

Geliştirilen yapay zeka tahmin modelinin son kullanıcı için erişime açılabilmesi adına tasarlanan web sitesi giriş sayfasının ekran görüntüsü Şekil 9'da sunulmuştur. Bu sayfa üzerinden bitki resmi sisteme giriş olarak uygulanmaktadır. Sistem tahmininde bitkinin hastalıkla bulaşık olduğu belirlenmesi durumunda belirlenen hastalık etmeniyle mücadele yöntemlerinin önerildiği Şekil 10'da görülen sonuç sayfasına yönlendirme yapılır. Bitki sağlıklı ise Şekil 11'de görülen bitki sağlıklıdır sonucu verilmektedir. Bu yaklaşım, modelin çıktılarını bir arayüzle birleştirerek, çiftçilere ve bitki uzmanlarına hızlı ve etkili bir şekilde bitki hastalıkları hakkında bilgi

sunmaktadır. Böylece, hastalıkların erken teşhisi ve uygun tedavi yöntemlerinin uygulanması mümkün olur, tarımsal verimlilik artacağı ön görülmektedir.

Çizelge 7. MobileNetV1 için M3 modeli üzerinden Kesinlik, Duyarlılık ve F1-Skor Sonuçları
Table 7. The Results of Precision, Sensitivity, and F1-Score for MobileNetV1 on the M3

	Kesinlik	Duyarlılık	F1- Skoru
Elma Sağlıklı	1,00	1,00	1,00
Elma Karaleke	1,00	1,00	1,00
Elma Siyah Çürüklük	1,00	1,00	1,00
Elma Memeli Pası	1,00	0,98	0,99
Kiraz Sağlıklı	1,00	1,00	1,00
Kirazda Külleme	1,00	1,00	1,00
Mısır Sağlıklı	1,00	1,00	1,00
Mısır Cercospora Yaprak Lekesi	0,99	0,94	0,97
Mısır Yaygın Pası	0,99	1,00	0,99
Mısır Kuzey Yaprak Yanıklığı	0,95	1,00	0,97
Asma Sağlıklı	1,00	1,00	1,00
Bağ Antraknozu	0,96	0,98	0,97
Bağ Mildiyösü	0,96	0,99	0,98
Bağ Küllemesi	1,00	0,99	0,99
Bağlarda Kav (Esca) Hastalığı	1,00	1,00	1,00
Asma Yaprak Yanıklığı	1,00	1,00	1,00
Portakal Sağlıklı	0,99	1,00	0,99
Turunçgillerde Yeşillenme Hastalığı	1,00	0,99	0,99
Şeftali Sağlıklı	1,00	0,97	0,99
Şeftalide Bakteriyel Leke	0,97	1,00	0,98
Şeftali Yaprak Kıvırcıklığı	1,00	0,98	0,99
Biber Sağlıklı	0,98	1,00	0,99
Biberde Bakteriyel Leke	0,99	1,00	1,00
Patates Sağlıklı	1,00	0,99	0,99
Patateste Erken Yanıklık	1,00	1,00	1,00
Patateste Geç Yanıklık	1,00	0,99	0,99
Kabak Sağlıklı	1,00	0,96	0,98
Kabakgillerde Külleme	1,00	1,00	1,00
Çilek Sağlıklı	1,00	1,00	1,00
Çilek Yaprak Yanıklığı	1,00	1,00	1,00
Domates Sağlıklı	1,00	1,00	1,00
Domates Bakteriyel Leke	1,00	0,99	1,00
Domates Erken Yanıklık	0,99	0,98	0,99
Domates Geç Yanıklık	0,98	0,98	0,98
Domates Yaprak Küfü	1,00	1,00	1,00
Domates Mozaik Virüsü	1,00	1,00	1,00
Domates Septoria Yaprak Lekesi	1,00	1,00	1,00
Domates Lekeli Solgunluk Virüsü	0,98	0,99	0,99
Domates Yaprak Lekesi	0,97	1,00	0,99
Domates Sarı Yaprak Kıvırcıklık Virüsü	1,00	1,00	1,00
Ceviz Sağlıklı	1,00	0,98	0,96
Ceviz Antraknozu	0,92	1,00	0,96

Çizelge 8. Önerilen Yöntemin, önceki çalışmalar ile karşılaştırılması
Table 8. Comparison of the Proposed Method with previously proposed methods

Referans	Metod	Doğruluk Skoru
(Rao ve ark., 2022)	Bi-CNN	%94,98
(Geetharamani ve Pandian, 2019)	CNN	% 96,46
(Chohan ve ark., 2020)	CNN	% 98,3
(Sagar ve Jacob, 2021)	ResNet50	% 98,2
(Chellapandi ve ark., 2021)	DenseNet	%99
Önerilen Yöntem	MobileNetV1	**% 99,20**

Şekil 7. Önerilen Yöntemin Tahmin Sonuçları
Figure 7. Prediction Results of Proposed Method.

Şekil 8. Veri Setinde Olmayan Resimlerin Test Sonuçları
Figure 8. The results of testing Images not included in the Data Set

Şekil 9. Bitki Resim Yükleme Formu
Figure 9. Plant Image Upload Form

Şekil 10. Hastalıklı Bitki Sonucu
Figure 10. Diseased Plant Result Screen

Şekil 11. Sağlıklı Bitki Sonuç Ekranı
Figure 11. Healthy Plant Result Screen

SONUÇ ve ÖNERİLER

Bitki hastalıklarının tespiti için geliştirilen yapay zeka modeli, yüksek işlem hızı ve sınıflandırma doğruluğu sağlamaktadır. Bu çözüm, bitki yapraklarının hastalıklı mı yoksa sağlıklı mı olduğunu hızlı ve etkili bir şekilde belirleyebilir, böylece bitki hastalıklarının erken teşhis edilmesine ve tedavi edilmesine olanak tanır. Ayrıca, kullanım kolaylığı ve sonuçlara hızlı erişim imkanı sunarak, çiftçilerin ve bitki uzmanlarının uygulamalarında verimliliği arttırabilir. Bu amaç için literatür de yaygın kullanılma sahip VGG16, VGG19, AlexNet, MobileNetV1 ve MobileNetV2 yöntemleri transfer öğrenme modeli olarak kullanılmıştır. MobileNetV1 en başarılı yöntem olduğu görülmüştür. Modelleme sonucunda etkin olan MobileNetV1 üzerinden son kullanıcı için web uygulaması geliştirilmiştir.

Geliştirilen web uygulaması, bitki hastalıklarının tespitinde çiftçilere ve bitki uzmanlarına büyük kolaylık sağlayacaktır. Uygulamanın sağladığı hızlı ve doğru sonuçlar, tarımsal verimliliği artırarak ekonomik kazançların artmasına katkıda bulunur. Gelecekte, daha yüksek epoch sayıları kullanılarak modelin doğruluğu artırılabilir ve veri seti daha fazla bitki türü ve hastalığını kapsayacak şekilde geliştirilebilir. Ayrıca, bulanık derin sinir ağları ve dikkat tabanlı derin öğrenme modellerinin kullanımıyla daha yenilikçi çalışmalar yapılabilir. Bu tür yaklaşımlar, daha kapsamlı ve etkili uygulamalar sunarak tarım sektöründe önemli ilerlemeler sağlayabilir ve yenilikçi çözümlerin öncüsü olabilir.

Araştırmacıların Katkı Oranı Beyanı

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Seed Beetle (Chrysomelidae: Bruchinae) Species, Occurrence Rate and Damage in Vetch Cultivation Areas of Eleşkirt (Ağrı) District

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ABSTRACT
This study was carried out to determine the seed beetle species in vetch cultivating areas their infestation rates, and weight loss in vetch seeds in the 17 villages of Eleşkirt district of Ağrı province in 2019-2020. Seed beetle adults were collected from common vetch (*Vicia sativa* L.) and Hungarian vetch (*Vicia pannonica* Crantz.) by sweeping net. In addition, they were obtained by culturing the vetch seeds harvested from the fields. As a result of the study, 10 species belonging to the genera *Bruchidius* Schilsky, 1905 (1 species), *Bruchus* Linnaeus, 1767 (6 species), and *Spermophagus* Schoenherr, 1833 (3 species) of subfamily Bruchinae (Coleoptera: Chrysomelidae) were identified. *Bruchus affinis* J. A. Frölich 1799 and *Spermophagus calystegiae* (Lukjanovitch & Ter-Minassian 1957) are first found in the Eleşkirt district. Among the 5.887 adult individuals collected, the most dominant species were *Bruchus brachialis* Fåhraeus 1839 (52.03%) and *Bruchus rufimanus* Boheman 1833 (43.49%). The infestation rate and weight loss in vetch seeds were found as 1.03% and 0.51%, respectively.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Chrysomelidae
Bruchinae
Eleşkirt
Seed beetle
Vetch

Eleşkirt (Ağrı) İlçesinin Fiğ Ekim Alanlarındaki Tohum Böceği (Chrysomelidae: Bruchinae) Türleri, Bulunma Oranı ve Zararları

ÖZET
Bu çalışma fiğ ekim alanlarındaki tohum böceği türleri, bulunuş oranları, bulaşıklık oranları ve ağırlık kayıplarının tespit edilmesi amacıyla Ağrı iline bağlı Eleşkirt ilçesinin merkezi ve ilçeye bağlı 17 köyünde, 2019-2020 yıllarında yürütülmüştür. Tohum böceği erginleri, adi fiğ (*Vicia sativa* L.) ve Macar fiği (*Vicia pannonica* Crantz.) bitkileri üzerinden atrap ile toplanarak, ayrıca tarlalardan hasat edilen fiğ tohumlarının kültüre alınmasıyla elde edilmiştir. Çalışmanın sonucunda, Bruchinae (Coleoptera: Chrysomelidae) altfamilyasından *Bruchidius* (1 tür), *Bruchus* (6 tür) ve *Spermophagus* (3 tür) cinslerine bağlı 10 tür tespit edilmiştir. *Bruchus affinis* J. A. Frölich 1799 and *Spermophagus calystegiae* (Lukjanovitch & Ter-Minassian 1957) türleri Eleşkirt ilçesinden ilk kez tespittir. Toplanan 5.887 ergin birey arasından dominantlığı en yüksek türler, *Bruchus brachialis* (%52.03) ve *Bruchus rufimanus* (%43.49) olmuştur. Eleşkirt ilçesinde tohum böceklerinin fiğ tohumlarında bulaşıklık oranı ve fiğ tohumlarındaki ağırlık kaybı sırasıyla %1.03 ve %0.51 olarak tespit edilmiştir.

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INTRODUCTION
Family Fabaceae (Leguminosae), called legumes, is one of the largest families in the world. Of the 250 thousand flowering plant species considered to exist, 12 thousand are legumes and distributed within approximately 600 genera. Legumes have been one of the important food sources of humankind since ancient times. The presence of

legume seeds in the Egyptian pyramids and in grave excavations in our country is a clear indicator of this (Serin & Tan, 2008). It is stated that vetch, legume forage plant, has approximately 140-150 species worldwide. Vetch is especially known as a native plant of Asian, European, and Mediterranean countries (Avcıoğlu et al., 2009). Important vetch species in Turkey: *Vicia sativa* L., *V. villosa* Roth, *V. pannonica* Crantz., *V. narbonensis* L. and *V. ervilia* (L.) Willd. (Ekiz et al., 2011).

Vetch is a fodder plant that is not selective in terms of soil requirements. It can be grown for grass production, grain production, and grazing or as a green manure plant. Its green and dry grass is high quality and nutritious. Since its grains contain a high amount of crude protein, it can be used extensively in animal nutrition (Elçi, 2005; Ertekin & Çakmakçı, 2020). There is a high crude protein content of 14.0-14.9% in vetch dry grass, depending on harvesting time and vetch type, and 20.2-27.7% in its grains. The remaining vetch straw after grain production also contains digestible crude protein approximately 2.0-3.5% (Çetin, 2016).

Vetch is rich in nutritional value and has a positive effect on the soil in which they are grown. The importance of this plant is increasing today, as the air's free nitrogen bonding properties to the soil, environmentalism and the popularity of sustainable agriculture increase. *Rhizobium* bacteria, which live in common with legumes, enrich the soil layers where their roots spread by binding the nitrogen that is free in the air but cannot be directly used by living things to the environment in which they live (Şehirali, 1988). The vetch is also one of the best rotation plants because it fixes nitrogen into the soil (Kalkan & Avcı, 2020).

According to the data of 2023 in our country, the cultivation area of the plants used in animal nutrition is 19 044 837 decares and their production is 56 764 701 tons. Among the plants used in animal nutrition, vetch has a share of 14.90% with a cultivation area of 2 836 285 decares and 6.55% with its 3 717 866 tons of green grass production. The cultivation area of the fodder plants produced in the Eastern Anatolia Region in 2023 is 5 651 963 da and the production is 9 286 756 tons. Vetch cultivation is carried out in the area of 504 049 da and 438 944 tons of green grass are produced. In Ağrı province, the vetch cultivation area as green grass was 35.708 da and the production amount was 16.310 tons. In Ağrı provinces, the ratio is 8.91% of vetch cultivation area and 7.08% of green grass production in the Eastern Anatolia Region (Anonim, 2023).

Legume plants, which have such an important place in the agricultural structure of the country, are attacked by many pests during the field and storage periods. Among these pests, seed beetles feeding on legume grains have an important place due to the weight and germination losses they cause. Seed beetles, which started to be harmful to the crop during the field period, can continue their damage after harvest and cause a high amount of product losses. Because of these features, seed beetles are described as “Big Protein Consumers” (Yücel, 1985). Many researchers have carried out scientific studies on seed beetles in different habitats, but no study has been found on the species, distribution, and damage of seed beetles in the vetch in Eleşkirt district of Ağrı province. This study was carried out to identify seed beetle species in vetch cultivation areas and to contribute knowledge to the local farmers to be able to get higher yield and quality vetch production opportunities in Eleşkirt district.

MATERIAL and METHOD

Material

The main materials of the research are seeds of *Vicia sativa* L. and *V. pannonica* Crantz. The seed beetles were obtained by culturing vetch seeds and collected by sweeping net from vetch plants. Reared materials are used for the identification of seed beetle species.

Obtaining Species Belonging to the Subfamily Bruchinae

Field observation surveys were carried out to determine the species of seed beetles associated with vetch plants in the center of Eleşkirt district and 17 belonging villages. Three fields in different directions were determined to represent each village and study area. Values were calculated based on 54 fields at least twice during the vegetation season. The samples were taken for two consecutive years, in May, June, and July, during the flower and capsule binding periods of the plants. In the surveys, a sweeping net was used (30 cm diameter, 75 cm deep, conical shape, handle length 55 cm). At different points that can represent each field, the sweeping net circle was kept perpendicular to the ground and swung at an angle of 180°, once in two steps, as far as possible, on the part of the plant close to the soil. Accordingly, 50 sweeping at each point were shaken at four different points of the field, and the seed beetles entering the sweeping net were taken with an aspirator (beetle-sucking tube) and transferred to a killing bottle with potassium cyanide. The beetles taken from the killing bottle were labeled by putting them in beetle storage envelopes. After that, specimens were mounted on triangle cards by glue (Yücel, 1985). Storage surveys were carried out in village producer storages where field surveys were made. Vetch seed samples were collected from three randomly selected storage sites representing each village. Samples of 500 g were taken from the vetch seeds from different locations and depths of the vetchs that were bagged and stored in bulk.

For each sample, a survey record form containing information such as the district, village, storage owner, for what purpose it was stored, and the year of manufacture was filled in. The samples were brought to the laboratory by placing them in plastic bags together with the registration forms. In the laboratory, these samples were transferred into glass jars separately and the mouths of the jars were closed with the help of a thin tulle fabric in order to meet the oxygen need of the insects that will exit the seeds and prevents their escape. A label was affixed to the jar containing information such as the name of the place where the sample was taken, the date of its collection, from which field it was taken, and the product type. The jars were kept under room conditions for 3 months to determine the weight loss (%) and beetle infestation rates (%) in the grains. Adults obtained from the samples, which were checked daily, were collected with the help of an aspirator, and the number of insects, the date of collection, and information of the village and storage where the insects from the jar belonged were recorded (Yücel, 1985; Kaynaş, 2014). Species collected in the field and reared under storage conditions were identified by a third author [Dr. Melek GÜÇLÜ (Atatürk University, Department of Plant Protection)]. For identification of the specimens, the presence of the single or double denticles on the apical side of the middle tibia, the structure of the segments on the antenna, the denticles status on the lateral side of the pronotum, the patterns formed by the feathers on the elytra, the carina, and apical spines status on the hind tibia were taken into consideration.

Determination of Insect Pest Infestation Rate and Weight Loss in Seeds

Vetch seeds (500 g) in the jars were checked daily. The seed beetles that emerged were collected from the jars with the help of an aspirator. After the completion of insect emergence at the end of 3 months, the intact and perforated seeds in the jars were separated individually, counted, and recorded. The number of perforated seeds was used to determine the infestation rate, and their weights were used to determine the weight loss caused by the seed beetles. The infestation density in seeds was calculated using the following formula (in Equality 1) given by Seçkin (Seçkin, 1981).

$$Infestation\ rate = \frac{N1}{N2} \quad (1)$$

Inequality 1, N1 indicates the number of damaged grains, and N2 indicates the number of grains in the sample.

Undamaged and damaged grains collected from the jars were weighed separately with an electronic scale, and their weights were recorded. The obtained data were used to calculate the weight loss (%). The weight loss in seeds was calculated using the following formula (in Equality 2) given by Yücel (Yücel, 1985).

$$Weight\ Loss\ (\%) = \frac{(U\ x\ Nd)-(D\ x\ Nu)}{U(Nd+Nu)}\ x\ 100 \quad (2)$$

Inequality 2, U is the weight of undamaged grains, Nu is the number of undamaged grains, D is the weight of damaged grains, and Nd is the number of damaged grains. The status of seed beetles in the village was determined by taking the average of the infestation rates and weight loss values obtained from samples collected from three storage sites in each village. The status of the district was determined by averaging the values of all villages.

RESULTS

Seed Beetle Species Detected in Vetches in Eleşkirt District

In this study, a total of 10 seed beetle species (Bruchinae) were determined belonging to six species *Bruchus*, one species *Bruchidius* and three species *Spermophagus*. These species are *Bruchus affinis* J. A. Frölich 1799, *Bruchus brachialis* Fåhraeus 1839, *Bruchus ervi* J. A. Frölich 1799, *Bruchus hamatus* Miller 1881, *Bruchus rufimanus* Boheman 1833, *Bruchus viciae* Olivier 1795, *Bruchidius lutescens* (Blanchard 1844), *Spermophagus calystegiae* (Lukjanovitch & Ter-Minassian 1957), *Spermophagus kuesteri* Schilsky 1905 and *Spermophagus sericeus* (Geoffroy 1785). Totally, 3026 female and 2790 male individuals belonging to the genus *Bruchus,* 29 female and 31 male individuals belonging to the genus *Spermophagu*s, and 2 female and 4 male individuals belonging to the genus *Bruchidius* were collected. In this study, seed beetles belonging to the genera *Bruchidius* and *Spermophagus* were not found in the cultivated seeds. These species were only observed in the flowers of vetch plants in the field surveys. However, species belonging to the genus *Bruchus* were found both in the field and under storage conditions. Locality information, collection and emergence dates and male and female numbers of the detected species are given in Table 1.

Occurrence Rate of Seed Beetle Species Detected in Vetches in Eleşkirt District

Among the 5,887 adult individuals obtained, the most dominant species were *Bruchus brachialis* (Vetch seed beetle) with 3.063 individuals and 52.03% occurrence rate, and *B. rufimanus* (Broad bean seed beetle) with 2.560 individuals and 43.49% occurrence rate. The least common species was *B. Viciae,* with one individual and occurrence rate of 0.02%. The occurrence rates and the numbers of species are listed in Table 2.

Table 1. Detected seed beetle species and their locality, collecting and emergence dates and male and female numbers in Eleşkirt district in 2019-2020.

Çizelge 1. 2019-2020 yıllarında Eleşkirt İlçesi'nden tespit edilen tohum böceği türleri ve bunların lokaliteleri, toplanma ve çıkış tarihleri ile erkek ve dişi sayıları

Species	Locality	Collecting and Emergence Dates	Number of Female and Male
Bruchus affinis J. A. Frölich 1799	Alagün	01.VI.2019	5♀, 3♂
	Dolutaş	23.V.2020	4♀, 5♂
	Düzyayla	16.VI.2019	4♀, 7♂
	Esentepe	22.V.2020	10♀, 5♂
	Goncalı	07.VI.2019	4♀, 3♂
	Haydaroğlu	02.VI.2019	2♀, 4♂
	İkizgeçe	16.V.2020	5♀, 6♂
	Kanatgeren	08.VI.2019	7♀, 3♂
	Mollasüleyman	03.VI.2019	11♀, 8♂
	Palakçayırı	21.V.2020	9♀, 6♂
	Pirabat	14.VI.2019	7♀, 7♂
	Toprakkale	24.V.2020	6♀, 10♂
	Yanıkdere	10.VI.2019	4♀, 2♂
	Yayladüzü	04.VI.2019	5♀, 2♂
	Yelkesen	15.0VI.2019	7♀, 5♂,
	Yücekapı	19.V.2020	9♀, 2♂
Bruchus brachialis Fåhraeus 1839	Alagün	01.VI.2019	11♀, 6♂
		03.I.2020	42♀, 50♂
		04.I.2020	37♀, 27♂
		05.I.2020	53♀, 25♂
		06.I.2020	40♀, 51♂
		07.I.2020	64♀, 29♂
	Dolutaş	23.V.2020	6♀, 8♂
		24.IX.2020	14♀, 16♂
		25.IX.2020	16♀, 21♂
		26.IX.2020	12♀, 19♂
	Esentepe	13.VI.2019	19♀, 9♂
		13.XII.2019	51♀, 33♂,
		14.XII.2019	67♀, 41♂,
		15.XII.2019	59♀, 29♂
		16.XII.2019	43♀, 32♂
	Goncalı	07.VI.2019	-, 5♂
	Haydaroğlu	02.VI.2019	1♀, -
		08.VI.2019	5♀, 17♂
		07.XII.2019	36♀, 42♂
		08.XII.2019	31♀, 57♂
	Kanatgeren	09.XII.2019	73♀, 53♂
		10.XII.2019	62♀, 39♂
		11.XII.2019	40♀, 75♂
		30.V.2020	9♀, 10♂
		21.V.2020	10♀, 5♂
	Palakçayırı	28.IX.2020	38♀, 41♂
		29.IX.2020	50♀, 21♂
		14.VI.2019	1♀, 3♂
	Pirabat	27.XII.2019	64♀, 28♂
		28.XII.2019	30♀, 47♂
	Süzgeçli	18.VI.2019	60♀, 32♂
	Yanıkdere	10.VI.2019	2♀, 2♂
		01.XII.2019	47♀, 71♂
	Yayladüzü	02.XII.2019	46♀, 53♂
		03.XII.2019	44♀, 36♂

		04.XII.2019	67♀, 54♂
		05.XII.2019	63♀, 29♂
		06.XII.2019	43♀, 48♂
		18.XII.2019	30♀, 35♂
		19.XII.2019	37♀, 51♂
	Yelkesen	20.XII.2019	42♀, 40♂
		21.XII.2019	36♀, 53♂
		22.XII.2019	27♀, 52♂
		23.XII.2019	9♀, 23♂
	Yücekapı	24.XII.2019	16♀, 31♂
		25.XII.2019	10♀, 17♂
		26.XII.2019	11♀, 23♂
Bruchus ervi J. A. Frölich 1799	Goncalı	07.VI.2019	5♀, 7♂
Bruchus hamatus Miller 1881	Değirmengeçidi	06.VI.2019	-, 1♂
	Dolutaş	23.V.2020	-, 1♂
	Düzyayla	16.VI.2019	-, 1♂
	Alagün	01.VI.2019	27♀, 38♂
		15.V.2020	22♀, 18♂
		06.VI.2019	36♀, 23♂,
	Değirmengeçidi	27.V.2020	20♀, 12♂,
		20.IX.2020	31♀, 20♂
		21.IX.2020	43♀, 28♂
		23.V.2020	13♀, 31♂
	Dolutaş	24.IX.2020	30♀, 17♂
		25.IX.2020	36♀, 23♂
		26.IX.2020	51♀, 24♂,
		16.VI.2019	28♀, 33♂
	Düzyayla	26.V.2020	13♀, 23♂
		22.IX.2020	33♀, 30♂
	Esentepe	13.VI.2019	19♀, 14♂
		22.V.2020	22♀, 14♂
	Goncalı	07.VI.2019	16♀, 22♂
		28.V.2020	33♀, 17♂
		02.VI.2019	27♀, 15♂
	Haydaroğlu	01.I.2020	59♀, 41♂
		31.V.2020	36♀, 21♂
Bruchus rufimanus Boheman 1833		17.VI.2019	9♀, 14♂
	İkizgeçe	29.XII.2019	54♀, 35♂
		30.XII.2019	51♀, 36♂
		16.V.2020	14♀, 22♂
	Kanatgeren	08.VI.2019	15♀, 6♂
		03.VI.2019	16♀, 29♂
	Mollasüleyman	25.V.2020	48♀, 25♂
		27.IX.2020	38♀, 24♂
	Palakçayırı	11.VI.2019	21♀, 33♂
		21.V.2020	30♀, 16♂
	Pirabat	14.VI.2019	26♀, 32♂
		17.V.2020	15♀, 14♂
	Süzgeçli	18.VI.2019	11♀, 13♂
	Toprakkale	09.VI.2019	26♀, 32♂
		24.V.2020	38♀, 18♂
		10.VI.2019	21♀, 41♂
	Yanıkdere	08.I.2020	26♀, 54♂,
		09.I.2020	32♀, 42♂
	Yayladüzü	04.VI.2019	44♀, 20♂
	Yelkesen	15.VI.2019	52♀, 33♂
		20.V.2020	49♀, 50♂

		05.VI.2019	9♀, 25♂
		23.XII.2019	17♀, 24♂
	Yücekapı	24.XII.2019	20♀, 39♂
		25.XII.2019	33♀, 18♂
		26.XII.2019	37♀, 54♂
Bruchus viciae Olivier 1795	Alagün	01.VI.2019	1♀, -
Bruchidius lutescens (Blanchard 1844)	Toprakkale	09.VI.2019	2♀, 4♂
	Esentepe	13.VI.2019	1♀, -
	Goncalı	28.V.2020	-, 1♂
	İkizgeçe	16.V.202	-, 2♂
	Kanatgeren	08.VI.2019	1♀, 1♂
Spermophagus calystegiae (Lukjanovitch & Ter-Minassian 1957)	Mollasüleyman	03.VI.2019	2♀, -
	Palakçayırı	21.V.2020	-, 2♂
	Toprakkale	24.V.2020	1♀, 1♂
	Yayladüzü	04.VI.2019	-, 1♂
	Yelkesen	15.VI.2019	2♀, -
	Yücekapı	05.VI.2019	1♀, 1♂
	Esentepe	13.VI.2019	1♀, -
	Goncalı	28.V.2020	-, 1♂
Spermophagus kuesteri Schilsky 1905	Haydaroğlu	02.VI.2019	-, 1♂
	İkizgeçe	16.V.2020	1♀, -
	Toprakkale	24.V.2020	1♀, -
	Yelkesen	15.VI.2019	-, 1♂
	Değirmengeçidi	27.V.2020	-, 2♂
	Dolutaş	23.V.2020	1♀, 2♂
	Düzyayla	16.VI.2019	-, 2♂
	Esentepe	13.VI.2019	2♀, 2♂
	Goncalı	07.VI.2019	1♀, -
	Haydaroğlu	31.V.2020	-, 1♂
	İkizgeçe	17.VI.2019	-, 2♂
Spermophagus sericeus (Geoffroy 1785)	Kanatgeren	30.V.2020	3♀, -
	Mollasüleyman	03.VI.2019	2♀, -
	Palakçayırı	11.VI.2019	2♀, -
	Pirabat	17.V.2020	1♀, 2♂
	Toprakkale	09.VI.2019	2♀, 1♂
	Yanıkdere	10.VI.2019	-, 1♂
	Yayladüzü	04.VI.2019	-, 2♂
	Yücekapı	05.VI.2019	1♀, -
			3057♀, 2825♂

Table 2. Occurrence rate of seed beetle species detected in vetch cultivation areas of Eleşkirt district in 2019-2020.
Çizelge 2. 2019-2020 yıllarında Eleşkirt İlçesi'nin fiğ ekim alanlarından tespit edilen tohum böceği türlerinin görülme oranı

Species	Number of Seed Beetle	Frequency in Samples (%)
Bruchus affinis J. A. Frölich 1799	187	3.18
Bruchus brachialis Fahraeus 1839	3.063	52.03
Bruchus ervi J. A. Frölich 1799	12	0.20
Bruchus hamatus Miller 1881	3	0.05
Bruchus rufimanus Boheman 1833	2.560	43.49
Bruchus viciae Oliver 1795	1	0.02
Bruchidius lutescens Blanchard 1844	6	0.10
Spermophagus calystegiae Luk. & Ter-Min.1957	17	0.29
Spermophagus kuesteri Schilsky 1905	6	0.10
Spermophagus sericeus Geoffroy 1785	32	0.54
Total	**5.887**	**100.00**

Infestation Rate of Seed Beetles in Vetch Seeds in Eleşkirt District in 2019-2020

The infestation rates of seed beetles in the vetch seeds are shown in Table 3. The highest infestation was detected

in sample number 3 in Yücekapı (2.19%), followed by sample number 2 in Yayladüzü (2.18%), and sample number 3 in Yayladüzü (1.87%). The lowest infestation was detected in sample number 1 in Mollasüleyman village (0.23%), followed by sample number 2 in Düzyayla village (0.24%), and sample number 2 in Mollasüleyman village (0.29%). The highest infestation mean on village/basis were 1.97%, 1.82%, and 1.56% in Yayladüzü, Yücekapı, and Alagün, respectively. The lowest average infestation rates were found in Mollasüleyman and Düzyayla (0.33%), followed by Palakçayırı (0.44%). In the Eleşkirt district, the seed beetle infestation rate of vetch seeds was determined to be 1.03%.

Table 3. Infestation rate (%) of seed beetles in vetch seeds in Eleşkirt district in 2019-2020.
Çizelge 3. 2019-2020 yıllarında Eleşkirt İlçesi'ndeki fiğ tohumlarının tohum böcekleriyle bulaşıklık oranı.

Villages	Storage Number and Infestation Rate (%)			Infestation Mean (%)
	1	2	3	
Alagün	1.79	1.49	1.39	1.56
Değirmengeçidi	0.38	1.10	0.47	0.65
Dolutaş	1.29	1.54	1.39	1.41
Düzyayla	0.33	0.24	0.43	0.33
Center	1.47	1.27	1.09	1.28
Haydaroğlu	0.66	0.44	0.52	0.54
İkizgeçe	1.18	0.76	0.85	0.93
Kanatgeren	1.56	1.50	1.34	1.47
Mollasüleyman	0.23	0.29	0.48	0.33
Palakçayırı	0.45	0.55	0.33	0.44
Pirabat	0.46	0.48	0.40	0.45
Yanıkdere	0.89	0.81	0.76	0.82
Yayladüzü	1.86	2.18	1.87	1.97
Yelkesen	1.26	1.46	1.67	1.46
Yücekapı	1.54	1.73	2.19	1.82
District Mean (%)				**1.03**

Weight Loss Caused by Seed Beetle in Vetch Seeds in Eleşkirt District in 2019-2020

As a result of feeding of seed beetle larvae on vetch seeds, weight losses in vetch seeds are shown in Table 4. The highest weight loss in vetch was detected in sample number 3 in Yücekapı (1.14%), followed by sample number 1 in Alagün (%1.00 and sample 3 (0.99%). The lowest weight loss was detected in sample 2 in Düzyayla (0.12%), followed by sample number 2 in Mollasüleyman 0.13% and sample number 3 in Palakçayırı by 0.14%. The highest weight loss on a village basis was 0.96%, 0.88%, and 0.81% in Yücekapı, Yelkesen and Alagün, respectively. The lowest weight losses were 0.17%, 0.18%, and 0.20% in Düzyayla, Mollasüleyman, and Pirabat, respectively. In Eleşkirt district, the weight loss in vetches was determined to be 0.51%.

Table 4. Weight loss (%) caused by seed beetles in vetch seed samples in 2019-2020 in Eleşkirt district.
Çizelge 4. 2019-2020 yıllarında Eleşkirt ilçesinde fiğ tohumu örneklerinde tohum böceklerinin neden olduğu ağırlık kaybı (%)

Villages	Storage Number and Weight Loss (%)			Weight Loss Mean (%)
	1	2	3	
Alagün	1.00	0.81	0.63	0.81
Değirmengeçidi	0.19	0.52	0.24	0.32
Dolutaş	0.51	0.77	0.73	0.67
Düzyayla	0.17	0.12	0.23	0.17
Center	0.77	0.67	0.52	0.65
Haydaroğlu	0.32	0.24	0.26	0.27
İkizgeçe	0.60	0.40	0.39	0.46
Kanatgeren	0.90	0.83	0.57	0.77
Mollasüleyman	0.13	0.15	0.27	0.18
Palakçayırı	0.22	0.28	0.14	0.21
Pirabat	0.17	0.24	0.19	0.20
Yanıkdere	0.45	0.36	0.40	0.40
Yayladüzü	0.65	0.69	0.73	0.69
Yelkesen	0.74	0.90	0.99	0.88
Yücekapı	0.80	0.93	1.14	0.96
District Mean (%)				**0.51**

Type of Damage Caused by Seed Beetles on Vetch Seeds

Adult seed beetles, which were overwintered in the field or the stored vetch seeds, appeared in the vetch fields in the first week of May, which is the beginning of flowering and capsular attachment of the vetch plant. The adults feed on the flowers, nectar, and pollen of the vetch plant before mating. Adult beetles that feed on the flowers of the vetch plant mate, and females lay their eggs on the vetch capsules. The hatching larva opens a thin gallery in the vetch capsule, enters the vetch seed, feeds in the seed, changes its coat, and completes the pupal stage. It completes its development in the seed and the adult beetles emerge from the seed (Figure 1). After the adults emerged, the damaged seeds perforated as shown in Figure 2.

Figure 1. Vetch seeds infested by seed beetles
Şekil 1. Tohum böcekleriyle bulaşık fiğ tohumları

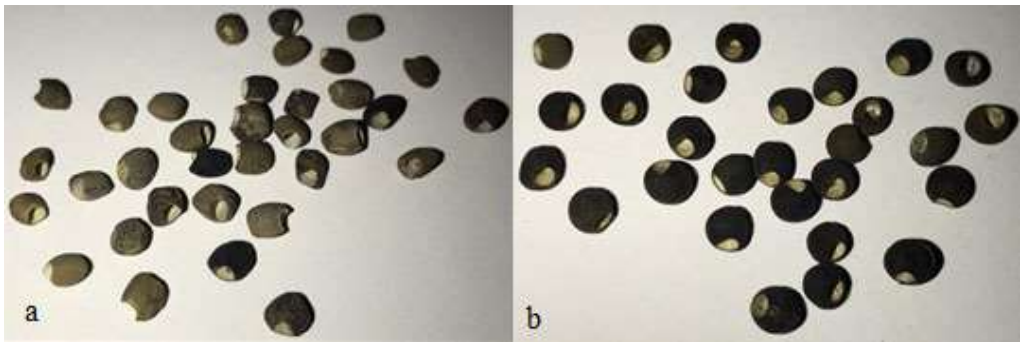

Figure 2. Vetch seeds damaged by seed beetle larvae, *a: Vicia sativa L., b: Vicia pannonica Crantz.*
Şekil 2. Tohum böceği larvaları tarafından zarar gören fiğ tohumları, a: Vicia sativa L., b: Vicia pannonica Crantz.

DISCUSSION

This study was conducted in the center of the Eleşkirt district of Ağrı province and 17 villages in the district. Totally, ten species were determined from the vetch cultivation fields of the Eleşkirt district. All the seed beetle species detected in this study were previously detected in Turkey. Additionally, *Bruchus affinis* and *Spermophagus calystegiae* were detected for the first time in the Eleşkirt district. In addition, the weight losses caused by seed beetles and the infestation rate in the seeds were investigated in vetch cultivation areas.

Bruchus affinis, which was detected in Eleşkirt district of Ağrı province was collected on flowers of *Vicia sativa* and *V. pannonica*. Lodos (1998), stated that *B. affinis* has a rich host list; its main host is *Lathyrus* species, and it also damages plant seeds of genera such as *Lotus*, *Vicia*, *Pisum,* and *Ulex*. Güdek (2020), reported that this species was found in *V. cracca.*

Bruchus rufimanus was collected from the flowers and reared from the seeds of *V. sativa* and *V. pannonica*. It has been found extensively in the seeds of *V. sativa*. Özer (1961), reported that this species is polyphagous and harmful to vetch, lentils, beans, and broad beans. Alkan (1966) stated that it causes damage to chickpeas and vetches. Seçkin (1981), stated that it is only found in bean and black lentil seeds and that this insect causes severe damage to black lentils. Lodos (1998), reported that its main host is a broad bean belonging to the genus *Vicia*, which is found in peas, some *Lathyrus* species, and rarely in beans. György and Merkl (2005) reported that it was found in *V. pannonica*. Güdek (2020) reported that it is found on the flowers of *V. sativa* and *V. pannonica*. In addition to these researchers, this species was detected on *Lathyrus laxiflorus* (Desf.) O. Kuntze, *L. venetus* (Mill.) Woh., *L. cicera* L., *L. vernus* (L.) Bernh., *Pisum sativum* L., *V. bithynica* L., *V. hybrida* L., *V. lutea* L., *V. panonica* Crantz.,

V. peregrina L., *V. faba* L., *V. hirsuta* (L.) Gray., *V. loiseulerii* (M. Bieb.) Litv., *V. narbonensis* L., *Onobrychoides* L. (Delobel and Delobel 2007; Delobel, 2014).

Bruchus ervi and *B. viciae* were collected from flowers of common vetch while, *B. hamatus* was reared from seeds of Hungarian vetch. *B. ervi* was found *Lathyrus* spp., *Lens culinaris* Medik., *Vicia* spp., *V. pannonica* , (Borowiec & Anton, 1993; Decelle & Lodos 1989; Kaynaş, 2014). *Bruchus vicae* was found on *Vicia teneufolia* Roth., *V. sepium* L., *V. angustifolia* L., *Lathyrus sphaericus* Retz., *L. pratensis* L., *L. miniatus* L. *Lens culinaris* (Hoffmann, 1945; Parker, 1957; Decelle & Lodos, 1989). *Bruchus hamatus* was detected on *Vicia variablis* Freyn & Sint., *Lathyrus* spp. (Lukjanovitch & Ter- Minassian 1957). Researchers stated that these seed beetles are harmful to *Vicia* species, so these studies support this study.

Bruchidius lutescens was also collected from the common vetch plant. In the studies, *B. lutescens* was detected on *Onobrychis sativa* L., *O. caput-galli* (L.) Lam. (Abdul-Rassoul et al. 1986; Decelle & Lodos 1989). Güdek (2020) stated that this species was collected from *Onobrychis sativa* (Sainfoin) flowers and obtained from *O. viciifolia* Scop. seed. Sainfoin is one of the forage crops commonly cultivated by producers in Eleşkirt district. It is thought that *B. lutescens* may have flown from sainfon fields to neighboring vetch fields.

Spermophagus calystegiae, *S. kuesteri,* and *S. sericeus* were found on flowers of common vetch and Hungarian vetch plants. These species were found on *Calystegia sepium* L., *C. soldanella* (L.) R.Br., *Convolvulus arvensis* L., *C. althaeoides* L., *C. cantabrica* L., *Medicago sativa* L., *Pimpinella anisum* L., *Carduus* L., *Centaurea* L. and *Vicia sativa* L. (Hoffman 1945; Lukjanovitch & Ter Minassian 1957; Decelle & Lodos 1989; Anton et al. 1997; Güdek, 2020). Studies indicated that the main hosts of these insects is *Convolvulus* and *Calsytegia* species. Weed control is not very common in the agriculture fields of Eleşkirt district, so weeds such as *Convolvulus arvensis* are always seen in the fields. For this reason, it has been observed that *Spermophagus* species, which are found in vetch fields also feed on the flowers of the vetch plant.

The infestation rate and weight loss of seed beetles on vetch seeds were found to be 1.03%, and 0.51%, respectively. Kaynaş (2014) reported that the infestation rate and weight loss were 0.45% and 0.24% for vetch seeds. On the other hand, Yücel (1985), determined that the weight loss in vetch was 2.77%.

Among the 5,887 adult individuals collected, the most dominant species were *B. brachialis* (52.03%) and *B. rufimanus* (43.49%). The least common species were *B. hamatus* (0.05%) and *B. viciae* (0.02%). When the relative occurrence of seed beetles in vetch seeds was examined, the vetch plant which is grown in Eleşkirt district is the main host of *B. brachialis* and *B. rufimanus*.

The highest infestation of seed beetles was detected in Yayladüzü town at 1.97%, followed by Yücekapı town at 1.82% and Alagün village at 1.56%. The lowest infestation was found in Mollasüleyman village and Düzyayla village by 0.33%, followed by Palakçayırı village by 0.44%. The infestation rate of vetch seeds was 1.03% in a total of 45 vetch samples taken from the Eleşkirt district.

The highest weight losses occurred of seed beetle larvae on vetch seeds were determined as 0.96%, 0.88% and 0.81% in Yücekapı town, Yelkesen, and Alagün villages, respectively. The lowest weight losses were found as 0.17%, 0.18%, and 0.20% in Düzyayla, Mollasüleyman, and Pirabat villages, respectively. The weight loss mean was found to be 0.51% for 45 vetch samples taken from Eleşkirt district.

CONCLUSION

According to the obtained results, all vetch cultivation fields of Eleşkirt district are infested by these mentioned seed beetles. Likewise, these pests were found in all storages. Ten species were detected on vetch plants in Eleşkirt district. Among of detected species, *Bruchus* species are found in vetch fields and the seeds in storage. Especially, *Bruchus brachialis* and *B. rufimanus* are common species both on vetch flowers and in vetch seeds in storage. In addition, these species have caused serious damage to vetch seeds in storage. The highest infestation rate was determined as 2.19% in Yücekapı, and the lowest infestation rate was determined as 0.23% in Mollasüleyman village. The most weight loss was found at 1.14% in vetch in Yücekapı, and the lowest weight loss was found at 0.12 in Düzyayla village. It is not considered that these two *Bruchus* species will be significantly harmful to vetch plants grown as green parts for animals in the district. However, it should be taken into account that pest populations can increase based on temperatures and incorrect chemical control applications, and there needs to be monitoring of the populations of these pests. It is estimated that it will cause serious damage if used as a seed in storage. In this context, for the product to be stored against these pests, to prevent increasing the population of the pests and reaching other places must be taken precautions.

As a precaution: the harvest should not be delayed too much, spraying the vetch seeds before they are brought to the storage, cleaning and spraying the empty storage (if the storage is contaminated with the stored product pest) and if pests are detected during the storage period of the vetch seeds, fumigation application can be recommended.

The rate of pest infestation in the field is much lower in late-planted vetches than in early-planted ones. Therefore, late planting is recommended in heavily damaged areas for farmers. It shouldn't be left in heaps in the field for a long time after harvesting. After harvest, the residues left in the field should be buried deeply with a plough and burned. Clean seeds should be used. Infested products, bags or materials shouldn't be placed in the storage. In addition, seeds should be fumigated to prevent contamination of the field from products used as seeds. It is recommended to apply pesticide when the storage is empty, approximately two weeks before placing the product.

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Researchers' Contribution Rate Statement Summary

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

Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

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Soil Nematode Community Analysis of Four Chickpea Cultivated Areas in Aksaray, Türkiye

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ABSTRACT
This study investigates soil nematode communities in four distinct chickpea cultivation areas in Aksaray, Türkiye, to understand their composition, ecological functions, and impact on soil health. Chickpeas, as the nitrogen-fixing crop, play a vital role in sustainable agriculture, supporting soil health and providing economic benefits in rural areas. Soil nematodes, however, including plant-parasitic types like root-knot and lesion nematodes, pose risks to chickpea yield by damaging root systems, thus necessitating effective management strategies. The research took place from May to December 2023 at Düzce University's Nematology Laboratory. Soil samples from four locations (Akgülü, Bağınbaşı, Camili and Göllü) in Aksaray were collected and using the Baermann funnel technique nematodes were exracted. Nematode identification and ecological parameter analyses used for nematode-based biological monitoring were conducted with light microscopy and structured taxonomic keys. The study grouped the nematodes based on feeding behaviors, showing distinct profiles across locations: bacterivores dominated in Akgülü, while Bağınbaşı had a higher prevalence of plant-parasitic nematodes, suggesting soil biodiversity challenges. Analysis of soil food webs indicated a stressed ecosystem in Akgülü and enriched, structured soil in Bağınbaşı, as shown by higher enrichment and structure index values. These patterns highlight the effects of soil management on nematode communities, with biodiversity directly linked to soil health and chickpea productivity. Findings emphasize that integrated pest management, including crop rotation and resistant chickpea varieties, is essential to mitigate the effects of nematodes. The study provides insights into nematode-driven soil dynamics and underscores the need for further research on nematode impacts in various agroecosystems, particularly for sustainable chickpea cultivation.

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Türkiye Aksaray'da Nohut Yetiştirilen Dört Alanda Toprak Nematod Topluluklarının Analizi

ÖZET
Toprak nematod topluluklarının, kompozisyonları, ekolojik işlevleri ve toprak sağlığı üzerindeki etkileri araştırılmıştır. Nohut, azot bağlayıcı bir ürün olarak sürdürülebilir tarımda hayati rol oynamakta, toprak sağlığını desteklemekte ve kırsal alanlarda ekonomik faydalar sağlamaktadır. Ancak kök-ur ve lezyon nematodları gibi bitki paraziti türleri içeren toprak nematodları, kök sistemine zarar vererek nohut verimi için risk oluşturmakta ve bu nedenle etkili yönetim stratejilerini zorunlu kılmaktadır. Bu araştırmanın analizleri Mayıs-Aralık 2023 tarihlerinde Düzce Üniversitesi Nematoloji Laboratuvarında yürütülmüştür. Aksaray iline ait dört lokasyondan (Akgüüu, Bağınbaşı, Camili ve Göllü) toprak örnekleri toplanmış ve nematodları topraktan elde etmek için Baermann huni tekniği kullanılmıştır. Nematodların tanılanması ve ekolojik parametre analizleri, ışık mikroskobu ve yapılandırılmış bir taksonomik teşhis anahtarı kullanılarak gerçekleştirilmiştir. Çalışma, nematodları beslenme davranışlarına göre kategorize etmiş ve lokasyonlar arasında farklı profiller göstermiştir:

Entomoloji

Araştırma Makalesi

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Anahtar Kelimeler
Topluluk analizi
Ekoloji
Nematod çeşitliliği

Akgülü'de bakteriovor nematodlar baskınken, Bağınbaşı'nda bitki-parazit nematodların daha yüksek oranda bulunduğu ve bu durumun toprak biyoçeşitliliği açısından zorluklar oluşturabileceği görülmüştür. Toprak besin ağlarının analizi, Akgülü'de stres altında bir ekosistemi, Bağınbaşı'nda ise daha zengin ve yapısal olarak düzenlenmiş bir toprak yapısını göstermiştir; bu durum, daha yüksek zenginleşme ve yapı indeks değerleri ile ortaya konmuştur. Bu modeller, toprak yönetiminin nematod toplulukları üzerindeki etkilerini vurgulamakta ve biyoçeşitliliğin toprak sağlığı ve nohut verimliliği ile doğrudan bağlantılı olduğunu göstermektedir. Bulgular, nematodların etkilerini azaltmak için ürün rotasyonu ve dayanıklı nohut çeşitleri de dahil olmak üzere entegre zararlı yönetiminin gerekli olduğunu vurgulamaktadır. Çalışma, Aksaray ilindeki bazı nohut ekilmiş alanlardaki nematod kaynaklı toprak dinamikleri hakkında fikir vermekte ve özellikle sürdürülebilir nohut yetiştiriciliği için farklı agroekosistemlerde nematod etkileri üzerine daha fazla araştırma yapılması gerekliliğini vurgulamaktadır.

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INTRODUCTION

Chickpea is a product that plays a crucial role in enhancing the global human food supply. Its nutritional profile has high benefits to for human health, providing a significant amount of protein, fiber, vitamins (such as B vitamins), and minerals (including iron, magnesium, and zinc). Its production can boost local economies, particularly in rural areas where smallholder farmers cultivate them. By serving as both a food source and a cash crop, chickpeas can improve livelihoods, reduce poverty, and foster community development (Merga and Haji, 2019). Moreover, chickpeas play a significant role in sustainable agriculture due to their nitrogen-fixing ability, which improves soil health and reduces the need for chemical fertilizers. This contributes to more sustainable farming practices that can support increased food supply without compromising environmental health. As climate change poses challenges to traditional food production, chickpeas offer a resilient solution due to their hardiness and adaptability to poorer soil conditions and drought. This makes them a strategic crop for ensuring long-term food supplies in changing environmental conditions (Devasirvatham and Tan, 2018).

Türkiye has consistently been one of the leading producers of chickpeas globally over the past decade. According to data from the Food and Agriculture Organization (FAO) and national agricultural statistics, Türkiye's chickpea production has seen fluctuations, with total annual output averaging around 500,000 to 800,000 tons (Muehlbauer and Sarker, 2017). The country benefited from favorable climate conditions and agricultural practices, leading to an increased yield per hectare. Notably, the production peaked in 2021, driven by both domestic demand and export opportunities, particularly to markets in the Middle East and Europe. Over the years, Turkish farmers have also focused on improving crop varieties and cultivation techniques to enhance productivity and sustainability, ensuring that chickpeas remain a crucial component of Türkiye's agricultural sector. However, challenges such as climate change and water scarcity could impact future production levels, necessitating ongoing adaptation and innovation in farming practices (Dellal & Unuvar, 2019).

Plant-parasitic nematodes play a significant role in agriculture and ecosystem dynamics, despite often being viewed as detrimental pests. Plant-parasitic nematodes attack the roots of plants, leading to reduced crop yields and compromised plant health. Their importance lies in their impact on soil microbiomes and nutrient cycling; when they infect plants, they can alter root development and induce stress responses that affect surrounding plant communities (Hugot et al., 2001). Additionally, they serve as indicators of soil health, with their presence reflecting the ecological balance within soil ecosystems. Understanding nematode populations and their interactions can help in developing integrated pest management strategies, promoting sustainable agricultural practices while minimizing crop losses. Consequently, while they are often pests, plant-parasitic nematodes also offer insights into soil ecology and agricultural resilience (Yeates, 2007).

Plant-parasitic nematodes also pose a significant threat to chickpea plants, affecting their growth and yield. The most common nematodes affecting chickpeas include root-knot nematodes and cyst nematodes. In addition to root-knot nematodes and cyst nematodes, chickpea plants can also be affected by other plant-parasitic nematodes such

as lesion nematodes (Pratylenchus spp.) (Behmand et al., 2022; Behmand & Elekcioğlu, 2022). Lesion nematodes feed on plant roots, causing lesions that can lead to reduced water and nutrient uptake, impacting the overall plant health and yield of chickpeas. Dagger nematodes (Xiphinema spp.) are another type of plant-parasitic nematode that can infest chickpeas, transmitting viruses and causing damage to the root system. Infestations can vary in severity depending on nematode species, soil conditions, and plant health, making nematode management crucial for maintaining chickpea crop productivity. Strategies such as crop rotation, resistant varieties, and soil amendments can help mitigate nematode damage in chickpea cultivated fields. These nematodes can weaken the chickpea plants, making them more susceptible to other diseases and environmental stresses. Implementing integrated pest management strategies and maintaining good agricultural practices are essential for minimizing the impact of various plant-parasitic nematodes on chickpea crops (Perry et al., 2024).

A soil food web refers to the complex network of interactions between different organisms in the soil, including plants, fungi, bacteria, and various soil-dwelling animals like nematodes. It represents the flow of energy and nutrients through the soil ecosystem. The enrichment index (EI) is a calculation that provides the location of the food web along the enrichment trajectory (Powell, 2007). It is calculated as $100 \times (e/(e + b))$, where e represents the guilds indicating enrichment (such as Ba1 and Fu2) and b represents the basal conditions guilds. The EI assesses the food web response to available resources and is based on the expected responsiveness of opportunistic non-herbivorous guilds to food resource enrichment. In the context of nematodes, the terms "colonizer" and "persister" are used to describe two different strategies employed by nematodes to survive and reproduce in a given environment: Colonizer nematodes are characterized by their ability to quickly colonize and exploit new environments. They have a high reproductive capacity and can rapidly increase their population size in favorable conditions. These nematodes are often referred to as "r-strategists," emphasizing their focus on rapid reproduction to take advantage of abundant resources. Persister nematodes, on the other hand, exhibit a different strategy where they focus on survival under adverse conditions rather than rapid reproduction (Ferris et al., 2001). These nematodes may have mechanisms that allow them to endure stressful environments, such as drought or extreme temperatures, by entering a dormant or resistant state. They are more resilient to fluctuations in environmental conditions but may have slower reproductive rates compared to colonizer nematodes. Persister nematodes are often associated with a "K-strategist" strategy, emphasizing their ability to persist over time in stable but challenging environments. Understanding these different nematode strategies is important in managing agricultural systems and ecosystems, as it can affect their population dynamics, interactions with other organisms, and responses to environmental changes (Ferris & Bongers, 2009).

This study aims to understand the association between the soil nematode community and chickpea plantations and reveals the comprehensive analysis of soil nematodes from four different chickpea plantations in Aksaray, Türkiye.

MATERIAL and METHOD

This study was conducted at the Nematology Laboratory of the Agricultural Biotechnology Department of Düzce University Faculty of Agriculture between May 2023 and December 2023.

Sampling

Soil samples were collected during a field study in Aksaray province, Türkiye in April 2023. The sampling was conducted in chickpea growing areas of four different points in Aksaray province (Table 1).

Table 1. GPS coordinates of the sampling sites; the location and host plant association.
Çizelge 1. Örnekleme alanlarının GPS koordinatları; konum ve konukçu bitki ilişkisi

Village	Latitude	Longitude	Plant Association	Location
Akgülü	33, 836 55 79	38, 847 31 73	Chickpea	Aksaray
Bağınbaşı	33, 830 712 85	38, 796 159 71	Chickpea	Aksaray
Camili	33,964 640 46	38, 890 602 90	Chickpea	Aksaray
Göllü	33, 826 632 23	38, 847 96 43	Chickpea	Aksaray

A total of 100 samples of the arrangement layout were taken. For each of the four location, 25 soil samples were taken from an area of 15 x 15 cm and from 30 cm of soil depth. All samples were placed in zip-lock bags, stored in portable temperature containers during transportation, and stored in the Düzce University Nematology Laboratory.

Extraction of Nematodes

12 cm diameter petri dishes were prepared using the modified Baermann (1917) funnel technique. After separating the stones, 100 g of fresh soil was analyzed from each sample. Plastic trays were covered with filter paper and incubated for 48 hours to extract nematodes. At the end of 48 hours, alive nematodes were collected. Nematode solutions were heated to 60 °C before expiration and were operated. 4% formalin solution was used for fixation and preservation, and samples were preserved for continuous glass slide preparations. Extractions were labeled with the relevant number, transferred to plastic tubes and stored in the Duzce University Nematology Laboratory. The remaining soil samples are kept in the soil laboratory as a reserve for changes.

Preparation of Nematodes for Light Microscopy

Collected nematodes were rinsed with distilled water to remove any remaining residue. Extracted nematodes were placed in a 1.25 cm deep block containing 96% ethanol in an incubator at 40 °C. A few drops of a glycerol-formalin mixture containing 4% formalin were added and left in the room overnight. The next morning, a few drops of a solution consisting of five parts glycerol and 96% ethanol were added, then the two-thirds of the block were covered with a glass square. At the end of the day, a few drops of a glycerol:ethanol (5:95%) solution were added every other place to ensure the permeation of glycerol. The next day, each nematode was covered with glycerol and continuous glass slide preparations were made (Yoder et al., 2006).

Identification of Nematodes and Analysis of Ecological Parameters

Nematodes were handled manually using an Olympus CH microscope (Olympus Optical, Tokyo, Japan). Classification of nematodes was based on the taxonomic key of De Ley & Blaxter (2004) and additional taxonomic information by Hodda et al. (2006) and Andrássy (2002; 2005). Colonizer-persister soil maturity values were obtained according to the characteristics of nematode life cycle details in accordance with Bongers (1990; 1999). The classification of the reported types of nematodes was determined by Yeates et al. (1993) and Du Preez et al. (2022). To obtain the maturity degree of nematodes in the ecosystem, the structure index and richness index were calculated by Ferris et al. (2001) and Ferris & Bongers (2009). The Nematode Indicator Joint Analysis (Sieriebriennikov et al., 2014) programming system was used to analyze the strength of the food web, feeding type recognition and MI family indices.

RESULTS and DISCUSSION

The results based on the feeding types of nematode assemblage suggested that in chickpea cultivation region in Akgülü, bacterivores were found to be 66.6 % followed by plant parasitic nematodes (14.1%), fungivores (8.9%), omnivores (8.3%), predators (2.2%) and unicellular eucaryote feeders (0.0%); Bağınbaşı region: plant parasitic nematodes (58.2%), fungivores (19.4%), predators (9.2%), bacterivores (9.0%), omnivores (4.1%) and unicellular eucaryote feeders (0.0%); Camili region: plant parasitic nematodes (49.6%), bacterivores (23.7%), omnivores (20.4%), fungivores (6.3%), predators (0.0%) and unicellular eucaryote feeders (0.0%); Göllü region: bacterivores (38.0%), plant parasitic nematodes (37.4%), omnivores (20.4%), predators (2.2%), fungivores (2.0%) and unicellular eucaryote feeders (0.0%) (Fig. 1).

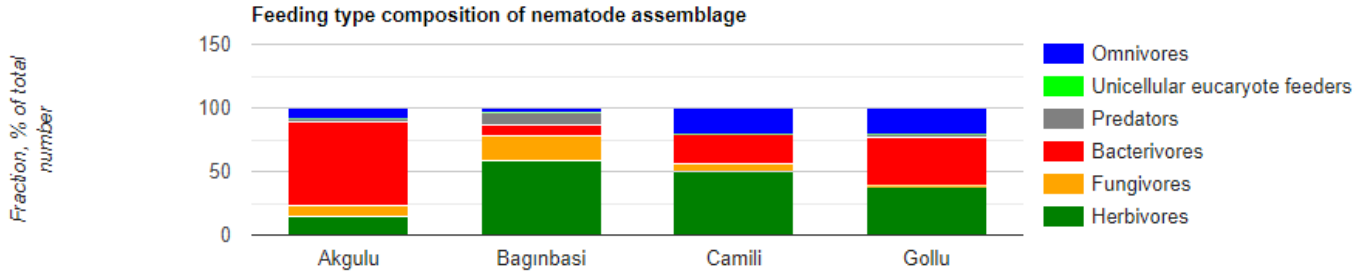

Figure 1. Feeding types of nematodes at different four region in Aksaray, Türkiye.
Şekil 1. Türkiye, Aksaray'daki farklı dört bölgede nematodların beslenme tipleri.

The results based on the classification of plant parasitic nematode feeding type differences, nematode assemblage in Akgülü region were found to be migratory endoparasites 43.9 % followed by epidermal/root hair feeders (33.7%), ectoparasites (22.5%) and sedentary parasites, semi-endoparasites and algal/lichen/moss feeders (0.0%); Bağınbaşı region: migratory endoparasites (29.7%), semi-endoparasites (28.9%), sedentary parasites (23.7%), ectoparasites (15.2%), epidermal/root hair feeders (2.5%) and algal/lichen/moss feeders (0.0%); Camili region: ectoparasites

(46.7%), epidermal/root hair feeders (28.9%), migratory endoparasites (24.4%) and sedentary parasites, semi-endoparasites and algal/lichen/moss feeders (0.0%); Göllü region: semi-endoparasites (35.8%), ectoparasites (29.6%), sedentary parasites (24.4%), migratory endoparasites (10.2%) and epidermal/root hair feeders and algal/lichen/moss feeders (0.0%) (Fig. 2).

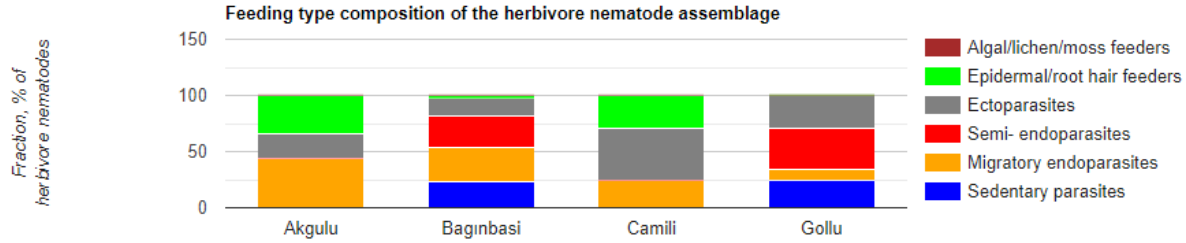

Figure 2. Distribution (%) of feeding types within the plant-parasitic nematodes from Aksaray, Türkiye.
Şekil 2. Türkiye, Aksaray'daki bitki paraziti nematodlar içinde beslenme tiplerinin dağılımı (%).

The results based on the classification of free-living nematode feeding type differences, nematode assemblages in Akgülü region were found to be bacterivores 77.5 % followed by fungivores (10.3%), omnivores (9.6%), predators (2.6%) and unicellular eucaryote feeders (0.0%); Bağınbaşı region: fungivores (46.6%), predators (22.0%), bacterivores (21.7%), omnivores (9.7%) and unicellular eucaryote feeders (0.0%); Camili region: bacterivores (47.1%), omnivores (40.4%), fungivores (12.5%) and predators and unicellular eucaryote feeders (0.0%); Göllü region: bacterivores (60.7%), omnivores (32.5%), predators (3.6%), fungivores (3.2%) and unicellular eucaryote feeders (0.0%) (Fig. 3).

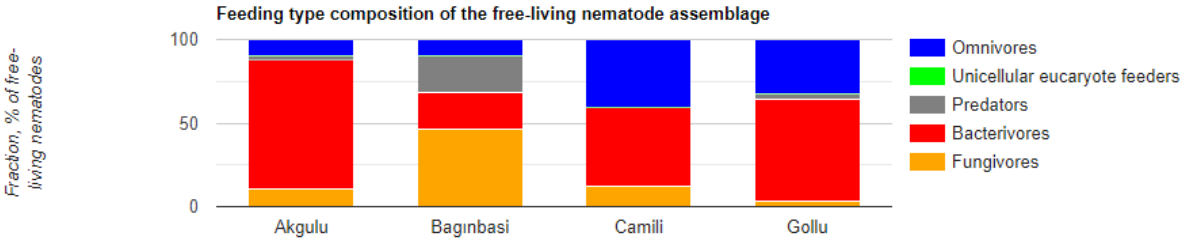

Figure 3. Distribution (%) of feeding types within the free-living nematodes from Aksaray, Türkiye.
Şekil 3. Türkiye, Aksaray'daki serbest yaşayan nematodlar içinde beslenme tiplerinin dağılımı (%).

The results based on the classification of free-living nematode c-p classification differences in Akgülü region were CP2 (87.7%), CP5 (8.8%), CP4 (3.4%), CP1 (0.1%), and CP3 (0.0%); Bağınbaşı region: CP2 (53.6%), CP5 (31.8%), CP4 (14.6%) and CP1 and CP3 (0.0%); Camili region: CP2 (47.1%), CP5 (29.3%), CP4 (23.6%), CP1 and CP3 (0.0%); Göllü region: CP2 (56.1%), CP5 (30.8%), CP4 (13.1%), CP1 and CP3 (0.0%) (Fig. 4).

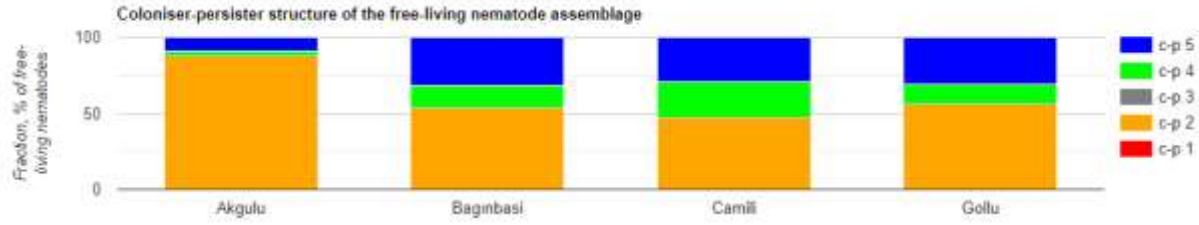

Figure 4. Free-living nematode c-p classification from four region in Aksaray, Türkiye.
Şekil 4. Türkiye, Aksaray'daki dört bölgeden serbest yaşayan nematode c-p sınıflandırması.

The results based on the classification of plant parasitic nematode cp class differences, nematode assemblages in Akgülü region were PP3 (66.3%), PP2 (33.7%) and PP4, PP5 (0.0%); Bağınbaşı region: PP3 (95.5%), PP2 (2.5%),

PP5 (2.0%) and PP4 (0.0%); Camili region: PP3 (52.3%), PP2 (46.5%), PP5 (1.3%) and PP4 (0.0%); Göllü region: PP3 (86.6%), PP2 (13.4%) and PP4, PP5 (0.0%) (Fig. 5).

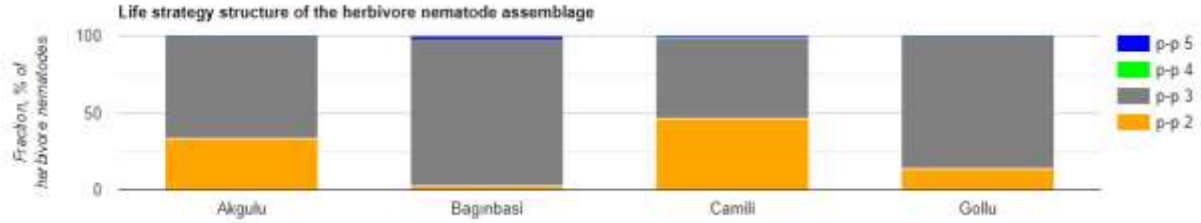

Figure 5. Plant parasitic nematode c-p classification from four region in Aksaray, Türkiye.
Şekil 5. Türkiye, Aksaray'daki dört bölgeden bitki paraziti nematod c-p sınıflandırması.

The enrichment index analysis (EI), results showed the highest enrichment level in the Bağınbaşı region (EI value: 28.15) followed by; the Akgülü region (EI value: 10.25), Göllü region (EI value: 5.13) and Camili region (EI value: 0.0). According to the structure index analysis (SI), results showed the highest structure level in Camili region (SI value: 80.65) followed by; Bağınbaşı region (SI value: 80.30), Göllü region (SI value: 62.71) and Akgülü region (SI value: 39.84).

Food web analysis is constructed to indicate whether the soil community is basal (and inferred stressed), enriched, or structured and stable. As a dynamic and evolving framework, we assess soil food web conditions by analyzing the position of nematode faunal composition within the faunal profile. Results showed that the Bağınbaşı region's nematode assemblage was mature, fertile with a modarete C:N value, high bacterial and fungal activity and suppressive soil. Nematode assemblage of the Akgülü region occurred at degraded, depleted, with high C:N value, more fungal and bacterial activity and some conductive, some suppressive soil types.

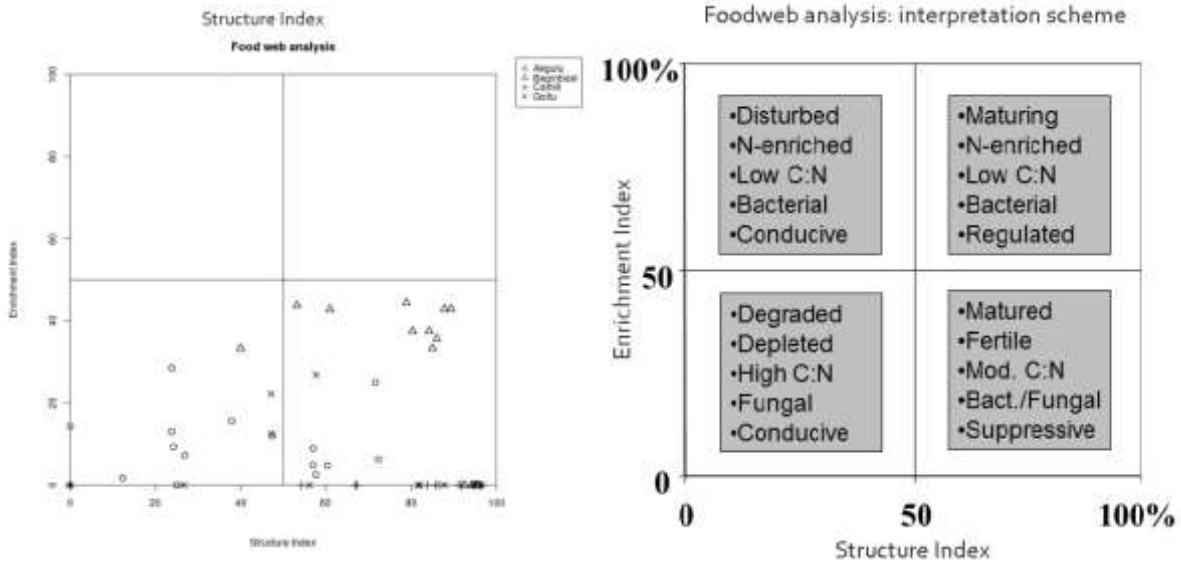

Figure 6. Food web analysis (Enrichment/Structure indices) from four region in Aksaray, Türkiye.
Şekil 6. Türkiye, Aksaray'daki dört bölgeden besin ağı analizi (Zenginleşme/Yapı indeksleri).

The findings of this study provided valuable data to understand the structure and diversity of soil nematode communities in four different chickpea growing areas in Aksaray. The distribution of soil nematodes is shaped by the effects of agricultural practices, environmental conditions and soil properties in these regions. The distribution of nematodes according to different feeding types can be interpreted as an indicator of ecosystem health and can help determine strategies to increase sustainability in agricultural production.

Bacterivorous and Fungivorous Nematodes

The dominance of bacterivorous nematodes (66.6%) in the Akgülü region indicates that the microbial activity of this area is high and the organic matter decomposition process is accelerated. Bacterivorous nematodes play important roles in the soil ecosystem, decompose organic matter through microorganisms and contribute to the

availability of nutrients to plants. This can increase soil fertility and facilitate plant nutrient uptake. At the same time, the low rates of fungivorous nematodes (8.9%) indicate an ecosystem where the fungal population is not dominant. Fungivores can contribute to the control of pathogenic fungi by consuming fungi, so the lower rates of these nematodes may indicate that the risk of pathogenic fungi in the ecosystem is low.

In the Bağınbaşı region, the high rate of plant parasitic nematodes (58.2%) indicates that this area is heavily infested with harmful species. The low levels of bacterivores and omnivores (9.0% and 4.1%) may suggest that the ecosystem is under pressure and biodiversity is low. This situation points to the need to increase biodiversity for sustainable agriculture. Biodiversity naturally keeps harmful nematodes under control, so low rates of bacterivores and omnivores may indicate the lack of such balancing mechanisms.

Omnivorous and Predatory Nematodes

The high rate of omnivorous nematodes (20.4%) in the Camili region suggests that this ecosystem has a more balanced structure. Omnivorous nematodes are generally found in more mature and stable soil ecosystems and are therefore considered an indicator of soil health. The complete absence of predatory nematodes (0.0%) may indicate that another important element of biodiversity is missing in this ecosystem. Predatory nematodes are natural predators that control populations of harmful nematodes, and the absence of these species may lead to uncontrolled proliferation of plant parasitic nematodes.

The balanced distribution of bacterivorous and omnivorous nematodes in the Göllü region (38.0% and 20.4%) indicates that this region is more ecologically stable and biodiversity is better preserved. However, the high rate of plant parasitic nematodes (37.4%) reveals that there is a significant risk threatening plant health in this area. This situation emphasizes the need to develop pest management strategies.

Plant Parasitic Nematodes and Integrated Pest Management

One of the most important findings of the study is that plant parasitic nematodes are dominant in varying proportions among regions. Plant parasitic nematodes can cause significant yield losses, especially in plants that attack the root system, such as chickpeas. It was determined that migratory endoparasites (43.9%) were dominant in the Akgülü region, indicating that the root system was seriously damaged. Similarly, the co-existence of migratory endoparasites and sedentary parasites in the Bağınbaşı region indicates that harmful nematode management should be more comprehensive. These situations necessitate more effective implementation of integrated pest management strategies in chickpea fields; crop rotation, the use of resistant varieties and the application of biological control methods can reduce the effects of these pests.

Soil Food Web Analysis and Soil Health

The fact that the nematode communities in the Akgülü region are largely composed of bacterivorous nematodes (66.6%) indicates that this area has high organic matter transformation and microorganism activity. This finding is consistent with studies indicating that bacterivorous nematodes are dominant in areas with high organic matter content (Ferris et al., 2001). However, the high C: N values in Akgülü indicate that the soil is stressed with some high fungal activities.

In the Bağınbaşı region, it was determined that plant parasitic nematodes were dominant at a rate of 58.2%. This supports studies such as Perry et al. (2024) and Bongers (1990), which emphasize plant health risks in areas where damaging nematode populations are dominant and biodiversity is low. The relatively high rate of omnivorous nematodes (20.4%) in the Camili region indicates that the ecosystem in this region has a more mature and balanced structure.

Ecological and Agricultural Implications of the Results

The high nematode activities determined in the Akgülü and Bağınbaşı regions indicate that integrated pest management strategies should be implemented. Nematode biological indicator models developed by Ferris and Bongers (2009) suggest solutions such as crop rotation and the use of resistant varieties to improve such stressed and unenriched ecosystems.

The 37.4% plant parasitic nematode population detected in the Göllü region represents an important threat that can damage the chickpea root system and lead to yield losses. This finding supports the studies by Perry et al. (2024) that draw attention to the economic effects of plant-parasitic nematodes.

Previous studies showed that nematode research in the Aksaray region of Türkiye focuses on root-knot nematodes that threaten potato and wheat production, particularly the distribution and effects of quarantine species such as Meloidogyne chitwoodi throughout the country (Evlice & Bayram, 2016). Similarly, the wheat gall nematode

Anguina tritici is observed with high infection rates in some provinces of Central Anatolia, especially in provinces such as Aksaray (Elmalı, 2002). Ditylenchus dipsaci was observed in garlic production in Aksaray province (Ateş Sönmezoglu et al., 2019). Unfortunately, there are not many studies on the nematode fauna of the region other than these 3 plant parasitic nematode species.

Protection of Biodiversity and Soil Health

The high enrichment index value (EI: 28.15) in the Bağınbaşı region showed that this area is biologically richer and microorganism activities are more intense. This situation is closely related to diversity indices and soil structure and represents a balanced ecosystem as defined by Ferris et al. (2001).

The low structure index value in the Akgülü region (SI: 39.84) indicates the insufficient biodiversity and low soil health of this area. This finding is consistent with the studies on weak soil systems defined by Yeates (2007). In such areas, it is recommended to implement solutions such as organic matter supplementation, farm gûre use, and biological control measures.

In this study, soil food web analyses were also conducted and these analyses revealed the effects of nematode communities in the field on soil health. The highest enrichment index value (EI: 28.15) was found in the Bağınbaşı region and it was determined that this region has a richer soil food web. Rich soil food webs indicate healthy soils with high biodiversity and microorganism activities. In contrast, lower enrichment and structure index values in the Akgülü and Göllü regions suggest that these regions have more stressed and degraded ecosystems. Especially the low structure index in the Akgulu region (SI: 39.84) indicates that soil health in this area is poor and that remedial agricultural practices are needed.

In conclusion, the data obtained in the study highlight the diversity of nematode communities in different agricultural areas and the effects of these communities on soil health. These findings may guide the development of pest management strategies in chickpea farming. In particular, the use of nonhost crop rotations, resistant varieties against plant parasitic nematodes, and the application of biological control methods may increase sustainability in agricultural production. In addition, this study reveals that further research is needed to better understand the role of soil nematodes in agroecosystems.

Researchers' Contribution Rate Declaration Summary

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

Statement of Conflict of Interest

The authors declare no conflict of interest.

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Analysis of Flavonoids Structural Genes in Between Chalaza and Microphyll a Mutant Natural Green Cotton Fiber

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ABSTRACT
Undesirable coloring pigments in naturally colored fibers can affect their aesthetic and commercial value in the textile industry. In our green cotton breeding program, we identified a gene mutation that causes different colored fibers to form on a single seed. We investigated the expression patterns of flavonoid biosynthesis structural genes to understand the formation of these different colors between the chalazal and microphyll parts of the seed. Significant variations in gene expression levels were observed among the examined flavonoid genes, highlighting the complexity of flavonoid biosynthesis pathways in cotton seeds. In the green fibers on the microphyll part, lower expression levels of enzymes such as *4Cl* (4-coumarate: CoA ligase), *C4h* (cinnamate 4-hydroxylase), *F3h* (flavone 3-hydroxylase), *F3′5′h* (flavonoid 3′5′-hydroxylase), *Ans* (anthocyanidin synthase), Anr (anthocyanidin reductase), and *Ufgt* (UDP-glucose: flavonoid 3-O-glucosyltransferase) were identified as potential factors influencing fiber coloration. Conversely, in the white fibers on the chalazal part, the expression levels of *Chs* (chalcone synthase) and *Chı* (chalcone isomerase) genes were lower than those in the microphyll part. This low expression is thought to be due to a mutation at the beginning of the phenylalanine pathway, preventing the formation of a green color in the white fibers on the chalazal part together with the low synthesis of the *Chı* gene. Understanding the molecular mechanisms behind these mutations is crucial for developing strategies to mitigate their effects and sustain the textile industry. The findings can inform cotton breeding programs to address unwanted coloration issues through genetic interventions, potentially enhancing the aesthetic and commercial value of naturally colored cotton fibers.

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Mutant Doğal Yeşil Pamuk Lifinde Chalaza ve Microphyll Arasındaki Flavonoid Yapısal Genlerin Analizi

ÖZET
Doğal renkli liflerdeki istenmeyen farklı renk pigmentleri, tekstil endüstrisindeki estetik ve ticari değerlerini etkileyebilir. Yeşil pamuk ıslah programımızda, tek bir tohumunda farklı renkli liflerin oluşmasına neden olan bir gen mutasyonu tespit ettik. Tohumun şalazal ve mikrofil kısımları arasındaki bu farklı renklerin oluşumunu anlamak için flavonoid biyosentez yapısal genlerinin ifade modellerini araştırdık. İncelenen flavonoid genleri arasında gen ekspresyon seviyelerinde önemli farklılıklar gözlenmiş, bu da pamuk tohumlarındaki flavonoid biyosentez yollarının karmaşıklığını vurgulamıştır. Mikrofil kısmındaki yeşil liflerde, *4Cl* (4-coumarate: CoA ligaz), *C4h* (sinamat 4-hidroksilaz), *F3h* (flavon 3-hidroksilaz), *F3′5′h* (flavonoid 3′5′-hidroksilaz), *Ans* (antosiyanidin sentaz), *Anr* (antosiyanidin redüktaz) ve *Ufgt* (UDP-glukoz: flavonoid 3-O-glukosiltransferaz) lif renklenmesini etkileyen potansiyel faktörler olarak tanımlanmıştır. Buna karşılık, şalazal kısımdaki beyaz liflerde, *Chs* (chalcone synthase) ve *Chı* (chalcone isomerase) genlerinin ifade seviyeleri mikrofil kısımdakilerden daha

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Pigmentasyon

düşüktü. Bu düşük ifadenin, şalazal kısımdaki beyaz liflerde yeşil renk oluşumunu engelleyen fenilalanin yolunun başlangıcındaki bir mutasyondan kaynaklandığı düşünülmektedir. Bu mutasyonların arkasındaki moleküler mekanizmaları anlamak, etkilerini azaltmak ve tekstil endüstrisini sürdürmek için stratejiler geliştirmek açısından çok önemlidir. Bulgular, genetik müdahaleler yoluyla istenmeyen renklenme sorunlarını ele almak için pamuk ıslah programlarını bilgilendirebilir ve potansiyel olarak doğal renkli pamuk liflerinin estetik ve ticari değerini artırabilir.

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INTRODUCTION

Naturally colored cotton has inherent color in the fiber. With growing consumer demand for environmentally friendly products, naturally colored cotton, which can be used with little or no processing and dyeing steps, is becoming increasingly attractive to the textile industry (Zhao and Wang 2015; Vreeland 1999). Unfortunately, only brown and green naturally colored varieties are currently available, limiting the development of the naturally colored cotton textile market. It may be possible to increase the diversity of available naturally colored fibers through conventional breeding, but this approach is limited by a lack of understanding of the process of cotton fiber color formation. In order to advance the integration of new natural colors into cotton, it is imperative to provide guidance for molecular breeding programs. The fundamental requirement for this is to explore and understand the molecular basis of pigment synthesis and deposition in cotton fibers. Additionally, the biosynthesis of flavonoids has been identified as a critical factor influencing fiber color in naturally colored cotton, with limitations in fiber quality observed in brown and green cotton fibers (Liu et al., 2018). Flavonoids and structural genes are key players in determining cotton fiber color inheritance (Gong et al., 2014). The expression profiles of key genes involved in the flavonoid biosynthetic pathway have been meticulously examined to unravel the intricate processes contributing to the formation of colored cotton fibers (Feng et al., 2013). Notably, investigations into the pigmentation of green-colored cotton fibers have provided novel insights into the underlying mechanisms, presenting potential avenues for the development of cotton varieties with consistently stable green-colored fibers (Sun et al., 2019).

Colored cotton fiber quality is a subject of significant interest due to its distinct characteristics and potential applications in the textile industry. Several studies have investigated the relationship between fiber color and quality traits in colored cotton (Xiao et al., 2014; Zhang et al., 2017). Feng et al. (2015) highlighted a negative correlation between fiber color and quality traits, underscoring the importance of quantitative trait loci (QTL) analysis in colored cotton. Breeders have attempted to cross white cotton with colored cotton to improve the fiber quality of colored cotton, but the results have been unsatisfactory (Yuan et al., 2012) due to mainly a negative correlation between fiber color and fiber quality traits, presumably because of pleiotropic effects of fiber color genes (Wang et al., 2014). Additionally, distant hybridization-sterility between brown cotton cultivars and Sea-island cotton cultivars (white cotton) is an impediment not only for the improvement of fiber quality but also for map-based cloning of fiber color genes in colored cotton (Zhang et al., 1994).

The coloration and quality of cotton fibers are influenced not only by genetic factors but also by environmental and developmental factors. Environmental factors include elements such as light, temperature, water availability, and nutrients. For instance, light intensity and spectrum can affect flavonoid biosynthesis, leading to changes in fiber color. High light intensity may increase flavonoid production, contributing to the formation of darker-colored fibers. Temperature is another crucial factor; lower temperatures can suppress flavonoid synthesis, resulting in lighter-colored fibers. Water stress can alter metabolic processes in the plant, changing gene expression profiles and affecting fiber quality and color. The deficiency or excess of nutrients, particularly elements like nitrogen and phosphorus, can influence flavonoid biosynthetic pathways, thereby altering fiber pigmentation.

Developmental factors encompass the intrinsic processes that affect gene expression throughout the plant's life cycle. The developmental stages of cotton fibers can cause significant changes in gene expression profiles. For example, the high expression of certain flavonoid genes during the fiber elongation stage can directly impact fiber quality and color. Additionally, changes in gene expression associated with plant aging can lead to variations in fiber pigmentation. Considering these factors together allows for a better understanding of the color and quality

characteristics of cotton fibers and enables the optimization of these traits through breeding programs.

An undesirable mutant line arose during plant breeding efforts due to the presence of mutations that some common types of undesirable mutant cotton lines encountered in our cotton breeding, which was aimed to improve the fiber quality of green cotton. Variability in fiber color distribution combined with color variations in the cotton lint can result in yarn irregularities, including thick and thin spots, slubs, and color streaks. These inconsistencies compromise yarn quality and aesthetics in the textile industry. Undesirable gene mutations in F lines through generations in plant breeding can spontaneously occur and/or inadvertently persist propagated due to several factors. During the formation of the F_2 and subsequent generations, the segregation of alleles according to Mendelian genetics can result in the reappearance or increased frequency of undesirable traits carried in the parent lines (Mackay et al., 2020; Khatodia et al., 2016; Ookawa et al., 2010; Xu et al., 2015; Li et al., 2015). When desirable and undesirable genes are located close to each other on the same chromosome, it can be difficult to separate them through recombinant. This linkage can cause undesirable traits to persist in breeding lines (Didelot et al., 2010). Genetic recombination during meiosis can create new allele combinations, potentially leading to the expression of undesirable traits (Mercier et al., 2015). Of course, desirable mutations can be introduced into elite lines through backcrossing. This involves crossing a hybrid with one of its parents or an individual genetically similar to its parent to maintain the majority of the elite line's genome while incorporating the new trait. Generally, crossing plants produce F1 hybrids that exhibit heterosis, where undesirable mutations are masked by the presence of dominant desirable alleles from both parents (Boerma and Walker, 2005). In our study, green cotton with low fiber quality was crossed with white cotton belonging to high fiber quality to increase its fiber quality a little more. Then, green lines in each F generation were backcrossed with white fiber (male, recurrent) cotton (4 generations, after obtaining F_1). After the 5th year, the backcrossed lines selected were left to self-fertilize until the F_{10} generation. After selections up to the F_{10} generation, it was determined that one of the 4 green lines did not resemble the green cotton that was introduced into the hybrid as the parent and that different colored fibers were produced in the seeds of that line (Figure 1). Of course, we did not examine the genes that caused the color difference in the seeds of this line throughout its generations. The reason why this mutant green cotton line was chosen, was most likely because it given a greenish color and had higher fiber quality than other lines throughout generations up to the F_{10} generation.

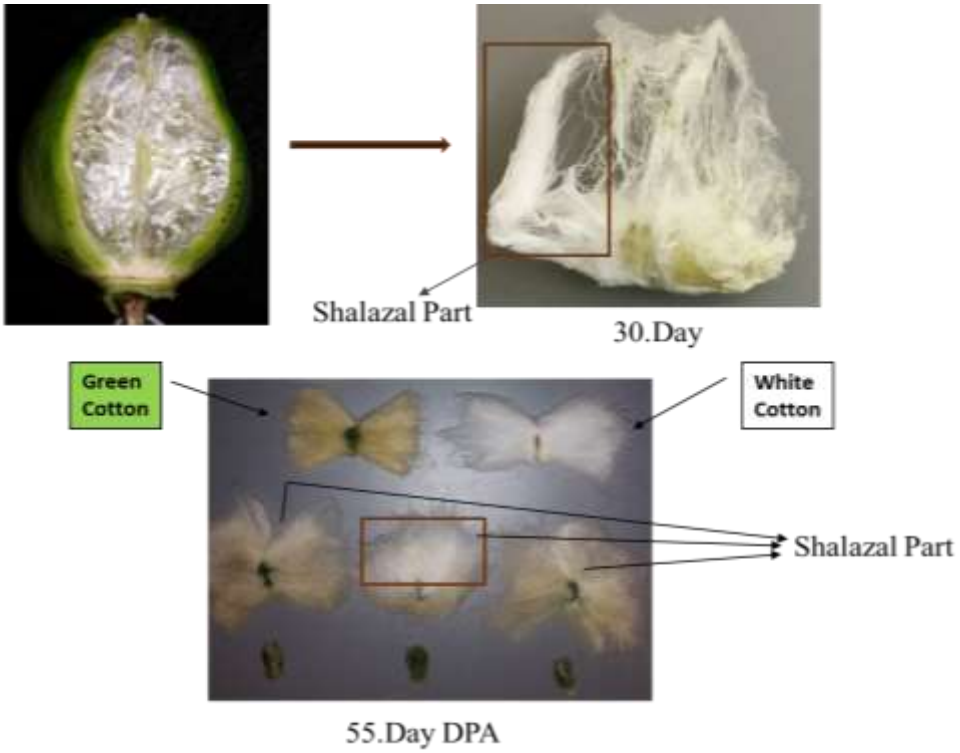

Figure 1. The mutation that occurred in fibers on a seed in the green cotton variety before/after the opening of the boll.

Şekil 1. Yeşil lif rengine sahip pamuk genotipinde koza açılmadan önce/açıldıktan sonra tohum üzerindeki liflerde meydana gelen mutasyon.

The occurrence of gene mutations in green cotton has been a subject of interest in recent research. Studies have demonstrated the feasibility of gene mutation in cotton using the CRISPR/Cas9 system, with a specific focus on genes involved in chloroplast development (Gao et al., 2017), furthermore, genome sequencing has provided novel insights into the molecular mechanisms underlying virescent mutation in cotton (Gao et al., 2021). Additionally, gene expression analyses have revealed divergent patterns between brown and green color cotton, particularly in the activation of genes encoding enzymes for the synthesis of caffeic acid derivatives, lignin, and lignan in the developing fibers of green cotton (Li et al., 2020). Moreover, the expression levels of certain structural genes related to flavonoid metabolism were found to be substantially higher in brown cotton lines compared to green cotton lines (Canavar & Rausher, 2021). These findings collectively indicate the presence of distinct genetic mechanisms underlying the pigmentation and metabolic processes in green cotton.

The aim of this research focuses on a mutation in the breeding green cotton line that was noticed during our previous study (Figure 1). In this mutation, it was observed that different color pigments were detected in the fibers of the same seed. In this context, the focus of the research is to determine the flavonoid gene expression in the fibers in the chalaza and micropyle parts of the mutation and to identify the point where the mutation starts. This scientific study is being conducted to understand the effects of this mutation at the molecular level and to develop strategies to mitigate these effects. Furthermore, identifying the specific point at which the mutation starts to develop is vital to maintain the quality of cotton fibers and ensure the sustainability of the textile industry.

MATERIALS and METHODS

Plant materials and sample collection

Advanced green fiber F_{10} generation breeding line (Line number is 12) was produced by crossing white cotton (female; N84 cultivar) with green-colored cotton (male; green cultivar). An unregistered cultivar was used in this study: a green fiber cotton cultivar (Green) from Azerbaijan (Gürel, Akdemir, & Karadayı, 2001). After the F_1 generation, the Green line (female) was backcrossed by crossing with white cotton (male, recurrent) for 4 generations in an introgression program. After 4 years of backcrossing, green breeding lines were produced by selfing pollination up to F_{10} generations. Seeds of the green cotton line were planted in three replicate pots (51-L volume, biological replicates), three seeds per pot, in the greenhouse of Duke University, Durham, NC, at temperatures of 30–32 °C during the day and 20–22 °C at night, and at 16 h of daily light.

Plants were thinned to one seedling per pot after germination. We ended up sampling three biological replicates (i.e., one plant from each of the three blocks) for gene expression analysis. Successive temporal samples for a biological replicate were taken from the same individual. According to Kim & Triplett (2001) and Zhang, Li, Wang, & Chee (2008), cotton fiber goes through four main developmental stages: fiber start (−3 to 5 d post-anthesis [DPA]), elongation (5–25 DPA), secondary cell wall production (15–45 DPA), and maturity or dehydration (45–70 DPA). On the day of anthesis (0 DPA), flowers and developing cotton bolls were tagged and labeled. In this study, cotton bolls and ovaries were harvested on the 30th day, because the beginning of light coloration can be seen at this stage after the day post-anthesis. Ovules were quickly dissected from the ovaries on ice. White fibers on chalaza and light green fibers on microphyll on a seed at the 30th DPA were quickly separated using forceps and also all parts of the seed such as cotyledon, epicotyl, hypocotyl, embryo, and radicle were removed to get only the seed coat, where the fiber is formed, was taken. Then immediately stored at −80 °C until RNA extraction.

RNA extraction, cDNA synthesis, and semi-quantitative PCR

Total RNA was extracted using the Sigma Aldrich Spectrum plant total RNA kit from a part of the seed (white fiber, green fiber, and seed coat) for 30. DPA treatment. RNA was quantified spectrophotometrically using Nano Drop-1000 (Thermo Scientific). Because extraction of different tissues yielded different RNA concentrations, we diluted all extracts to the same concentration for subsequent analyses.

Double-strand complementary DNA (cDNA) was synthesized from total RNA using MultiScribe reverse transcriptase kits of Applied Biosystems using random primers according to the manufacturer's instructions. To synthesize double-strand cDNA from total RNA, the 2× Reverse Transcription Master Mix (RT) was prepared as follows: 10× RT buffer (2 µl), 10× RT random primers (2 µl), 25× dNTP mix (100 mM, 0.8 µl), MultiScribe reverse transcriptase 50 U μl^{-1} (1 µl), and nuclease-free H_2O (4.2 µl) were combined for a total reaction of 10 µl. Ten microliters of 2× RT master mix and 10 µl of the RNA sample were pipetted into each tube, pipetting up and down two times to mix on the ice box. The thermal cycler conditions (Bio-Rad) were 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, and 4 °C for ∞.

4Cl, 4-coumarate:CoA ligase; *Anr*, anthocyanidin reductase; *Ans*, anthocyanidin synthase; *C4h*, cinnamate 4-hydroxylase; *Chı*, chalcone isomerase; *Chs*, chalcone synthase; *Dfr*, dihydroflavonol 4-reductase; *F3h*, flavone 3-hydroxylase; *F3'h*, flavonoid 3'-hydroxylase; *F3'5'h*, flavonoid 3'5'-hydroxylase; *Lar*, leucoanthocyanidin

reductase; *Pal*, phenylalanine ammonia-lyase; *Ubq7*, ubiquitin gene; *Ufgt*, UDP-glucose: flavonoid 3-O-glucosyltransferase;

Table 1. Sequences of the primers used for real-time PCR analysis.
Çizelge 1. Real-time PCR analizi için kullanılan primerlerin dizileri.

Genes	Primers	Sequences (5' to 3')	Accession number (Sequence ID)	Amplicon length (bp)
GhPal	PAL-F	AGCTTGGAACTGGGTTGTTG	XM_016878448	134
	PAL-R	AGCACCATTCCAACCCTTTA		
GhC4h	C4H-F	TTTGGGTCGTTTGGTACAGA	XM_016868687	137
	C4H-R	AAAATTGCCTTGGCTTAGCA		
Gh4cl	4CL-F	AAGGTGCACTTTGTTCATGC	NM_001327242	148
	4CL-R	CGTTGCAATTTAAAAGCCAAAT		
GhChs	CHS-F	CAGAGGAAGGACTGGAGTGG	XM_016823419	86
	CHS-R	AGCAGCAACACTATGGAGCA		
GhChi	CHI-F	ATGGAGTTTCTCCTCCAGCA	XM_016810061	85
	CHI-R	GGTTTTTCACTGTCGACTCCA		
GhF3h	F3H-F	CTGAAGAAGCTGGCCAAAGA	NM_001327494	99
	F3H-R	TGCAAGGATTTCCTCCAATG		
GhF3'h	F3'H-F	AGTGGGAGTTGGCTGATGGATT	NM_0013227514	155
	F3'H-R	CTCCTCACCCTGAAACGACAAC		
GhF3'5'h	F3'5'H-F	AAACATGGATGAGGCCTTTG	NM_001327621	111
	F3'5'H-R	GCAAGGGATGTGCTTAGGAA		
GhDfr	DFR-F	CATGTTCGTAGGAGCTGTCG	NM_001327665	118
	DFR-R	GGTAGGCACTCAATTGTTGAAA		
GhLar	LAR-F	GAATGAGCCATTCCGAACAT	XM_016880783	135
	LAR-R	GCTTCGACTACTGGCTTTGG		
GhAns	ANS-F	ACAATGCTAGTGGGCAGCTT	EF187442	139
	ANS-R	GCAGTTGCCTTGCATACTCA		
GhAnr	ANR-F	TGGGATCGAGGAAATCTACG	NM_001327416	95
	ANR-R	ACCATAATCATTGGGGAAGC		
GhUfgt	UFGT-F	AAGCAGATAGCGGTGGAGAA	XM_016885447	118
	UFGT-R	GCCTCCAACACCAAATTTTTC		
GhHiston3	His3-F	CAGGAAATTGCCTTTCCAGA	XM_016885274	113
	His3-R	GTATGCCTCTGCAGCTTCCT		
GhUBQ7	UBQ7	AAGCCCAAGAAGATCAAGCA	DQ116441	115
	UBQ7	CGCATTAGGGCACTCTTTTC		

The 13 flavonoid enzyme coding genes and two control genes examined in this study, along with their abbreviations used in this publication, are shown in Table 1. The polymerase chain reaction (PCR) primers used to amplify 85-to-154-bp fragments of the genes *Pal*, *C4h*, *4cl*, *Chs*, *Ch1* (XM_016810061), *F3h*, *F3'5'h*, *Dfr*, *Lar* and *Anr* were obtained from Xiao et al. (2014). The primers of F3'h (NM_0013227514), Ans (EF187442), His3 (XM_016885274), and UBQ7 (DQ116441) were designed using gene sequences obtained from the cotton genome by BLAST (Basic Local Alignment Search Tool, National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Canavar and Rausher 2021). Primers (forward and reverse) were synthesized by Integrated DNA Technologies. The PCR was performed using Phusion DNA polymerase (New England Biolabs) according to the manufacturer's instructions. The amplification protocol consisted of 40 cycles of

initial denaturation at 94 °C for 3.5 min followed by 63 °C for 30 s and 72 °C for 2 min (Bio-Rad MyCycler thermal cycler PCR). The PCR products were identified electrophoretically by running on a 1% agarose gel containing SybrRsafe (8.0 µl, Invitrogen). Gels were viewed with a ChemiDoc MP imaging system 170-8280 (Bio-Rad).

Quantitative RT-PCR analysis of gene expression levels

Complementary DNA was diluted to 2.0 ng µl for use in quantitative PCR (qPCR) reactions. Quantitative reverse-transcription PCR was performed in a total volume of 20 µl with 10 µl DyNAmo HS SYBR Green (Thermo Scientific), 1 µl forward primer (0.5 µM), 1 µl reverse primer (0.5 µM), 2.0 µl cDNA template, and 6.0 µl double-distilled H_2O (total volume of 20 µl). Reactions were run on a Roche LightCycler thermocycler according to the manufacturer's instructions. The PCR amplification used a 15-minute initial denaturation step at 95 °C, followed by 45 cycles with a 10-s denaturing step at 94 °C and an annealing step for 30 s at 58 °C. All experiments involved three biological replicates for each cotton genotype and stage, and each biological replicate was performed in duplicate (technical replicate).

The threshold cycle (Ct) values were reported as a mean for each replicate and the fold changes of transcription levels of target genes relative to the reference genes (UBQ7 [ubiquitin gene] and Histon 3) were analyzed by the comparative Ct ($2^{-\Delta\Delta Ct}$) method. The Ct values were used to calculate ΔCt and ΔΔCt values (Balasubramanian et al., 2016). The ΔCt was calculated by subtracting the Ct values of UBQ7 and Histone 3 (two control genes) from the Ct values of the flavonoid synthesis gene within the same stage, whereas ΔΔCt was calculated by subtracting the ΔCt values of fiber stages at 30 DPA. Hence, the results were presented as relative expression of flavonoid genes in different stages normalized to that for the seed coat. Normalized fold expression among the stage of 0 DPA of the samples was calculated with seed coat as a standard. The possibility of contamination by genomic DNA was ruled out by running the PCR reactions on isolated RNA.

Statistical Analysis

To determine whether gene expression levels were different in 2 different fiber samples taken from the chalazal and microphyll of seed, expression levels, and analysis of variance (ANOVA) were performed in the "JMP®, Version <16.0>. SAS Institute Inc., Cary, NC, 1989–2023" statistical program. The difference between the means was determined by LSD Student-t (0.05).

RESULTS and DISCUSSION

When the results of the quantitative RT-PCR analysis of gene expression levels were examined, it was also observed that the genes were present in all three seed parts (White fiber on chalaza and green fiber on microphyll and seed coat) (Figure 2). The expression levels of 13 flavonoid genes (*Pal, C4h, 4Cl, Chs, F3'5'h, F3'h, F3h, Lar, Dfr, Ans, Anr,* and *Ufgt*) were statistically different in parts of the seeds (green part, white part and seed coat) (Table2).

When the averages were compared according to the regions where the samples were taken, it was determined that the highest expression level of the *Pal* gene was observed in green fiber, even though it was in the same statistical group as white fiber. The lowest expression of *Pal* was found in the seed coat. The *C4h* gene expression was statistically highest in white fiber and lowest in the seed coat. When *4Cl* gene expression was evaluated statistically, no difference was found between green fiber and seed coat, but white fiber had a higher expression level. It was observed that the gene was synthesized mostly in the seed coat and no difference was found in the samples taken according to fiber color. *F3'5'h* flavonoid gene expression was found to be significantly higher in white fiber samples and 46.5% more than in green fiber samples. No difference was found in *F3'h* flavonoid synthesis according to the sampled seed regions. *F3h* gene synthesis was highest in the white fiber section, while green fiber and seed coat were statistically in the same group. However, it was expressed 48.1% more in the white fiber section. When *Lar* flavonoid gene expression averages were examined, it was seen that the green fiber section came to the fore. It was 59.67% more in the green fiber section than in the white fiber section. While *Dfr* expression was found in both colored fiber regions, it was the least in the seed coat (Figure 2).

The results of our expression analyses revealed several major patterns. First, genes of the anthocyanin pathway, are expressed at detectable levels in fibers on the seed of green cotton line with green and white. All parts of the seed and color types thus should be capable of producing anthocyanins if anthocyanidins are produced. Several studies have underscored the pivotal role of flavonoid genes in cotton fiber development (Peng et al., 2020). Tan et al. (2013) revealed that the dominance of flavonoid gene expression during fiber elongation stages significantly impacts fiber development, particularly through the flavonoid naringenin's retardation effect. Canavar & Rausher (2021), further elucidated the importance of these genes by demonstrating their high expression levels during fiber elongation, suggesting their substantial involvement in this developmental phase. Gong et al. (2014) expanded, proposing a potential link between highly expressed flavonoid genes during fiber elongation and fiber quality,

highlighting their significance in cotton fiber development and quality regulation. Collectively, these findings underscore the multifaceted roles of flavonoid genes, not only in pigment formation but also in various aspects of fiber development and elongation, emphasizing their importance in regulating fiber quality and coloration across different developmental stages.

Table 2. Variance analysis of Relative expression levels taken from white part, green part, and seed coat flavonoid genes at stages of 30 DPA fiber development. *4Cl*, 4-coumarate: CoA ligase; *Anr*, anthocyanidin reductase; *Ans*, anthocyanidin synthase; *C4h*, cinnamate 4-hydroxylase; *Chı*, chalcone isomerase; *Chs*, chalcone synthase; *Dfr*, dihydroflavonol 4-reductase; *F3h*, flavone 3-hydroxylase; *F3'h*, flavonoid 3'-hydroxylase; *F3'5'h*, flavonoid 3'5'-hydroxylase; *Lar*, leucoanthocyanidin reductase; *Pal*, phenylalanine ammonia-lyase; *Ubq7*, ubiquitin gene; *Ufgt*, UDP-glucose: flavonoid 3-O-glucosyltransferase; ** P-value is less than 0.01 ($p < 0.01$).
* P-value is generally less than 0.05 ($p < 0.05$).

Çizelge 2. Beyaz lif, yeşil lif ve tohum kabuğunda, 30 DPA lif gelişim aşamasında elde edilen flavonoid genlerinin ifade seviyelerinin varyans analizi. . *4Cl*, 4-coumarate: CoA ligase; *Anr*, anthocyanidin reductase; *Ans*, anthocyanidin synthase; *C4h*, cinnamate 4-hydroxylase; *Chı*, chalcone isomerase; *Chs*, chalcone synthase; *Dfr*, dihydroflavonol 4-reductase; *F3h*, flavone 3-hydroxylase; *F3'h*, flavonoid 3'-hydroxylase; *F3'5'h*, flavonoid 3'5'-hydroxylase; *Lar*, leucoanthocyanidin reductase; *Pal*, phenylalanine ammonia-lyase; *Ubq7*, ubiquitin gene; *Ufgt*, UDP-glucose: flavonoid 3-O-glucosyltransferase; ** P-value 0.01 ($p < 0.01$).* P-value 0.05 ($p < 0.05$).

Source	Df	Mean Square											
		PAL	C4H	4CL	CHS	CHI	F3'5'H	F3'H	F3H	LAR	DFR	ANS	ANR	UFGT
Part of Seed	2	0.677***	3.08***	8.23***	0.168***	0.03**	0.363***	0.0066	0.563**	31.18***	64.77***	0.078**	1.01***	0.18*
Replication	2	0.0008	0.01	0.009	0.0008	0.002	0.016	0.0057	0.0096	0.0006	0.367	0.01	0.0135	0.588
Error	4	0.008	0.005	0.021	0.0009	0.003	0.004	0.0015	0.012	0.196	0.244	0.0058	0.0067	0.018

Table 3. qPCR results of expression levels of 13 genes related to flavonoid genes in three different regions of cotton seeds harvested on the 30th day, Lsd student-t test (0.05).

Çizelge 3. 30. günde hasat edilen pamuk tohumlarının üç farklı bölgesinde flavonoid genlerle ilgili 13 genin ifade düzeylerinin qPCR sonuçları, Lsd student-t testi (0.05).

Genes	White fibre on chazal	Green fibre on microphyll	Seed Coat
PAL	1.68 A	1.91 A	1.00 B
C4H	2.96 A	1.52 B	1.00 C
4CL	4.02 A	1.32 B	1.00 B
CHS	0.53 C	0.78 B	1.00 A
CHI	0.80 B	0.89 AB	1.00 A
F3'5'H	1.29 A	0.60 C	1.00 B
F3'H	0.94 ns	1.06 ns	1.00 ns
F3H	1.58 A	0.76 B	1.00 B
LAR	4.44 B	7.44 A	1.00 C
DFR	8.58 A	9.45 A	1.00 B
ANS	1.20 A	0.88 B	1.00 C
ANR	2.16 A	1.53 B	1.00 C
UFGT	1.49 A	1.30 AB	1.00 B

In addition, to investigate the relationship between flavonoid biosynthesis levels and cotton fiber properties, Jia et al. (2022) conducted a study that identified significant correlations between differentially expressed genes related to flavonoid biosynthesis, such as *Pal, 4Cl, C4h, F3h, F3'h*, and F3'5'h, and total flavonoid content. This highlights the regulatory importance of these genes in flavonoid production, which can impact the properties of cotton fibers. Additionally, Wang et al. (2021) explored the phenylpropanoid biosynthesis pathway in bamboo, emphasizing the roles of *Pal, 4Cl*, and *C4h* genes in directing phenylpropanoid intermediates towards lignin or flavonoid biosynthesis. Understanding these mechanisms is crucial for comprehending how different genes influence the synthesis of compounds that contribute to fiber properties in cotton. By synthesizing insights from studies on flavonoid biosynthesis pathways and gene expression related to cotton fiber properties, a comprehensive understanding of how genes like *C4h* and *4Cl* influence flavonoid levels and, consequently, fiber characteristics can be achieved. These findings are essential for unraveling the intricate relationship between gene expression, flavonoid biosynthesis, and the quality of cotton fibers.

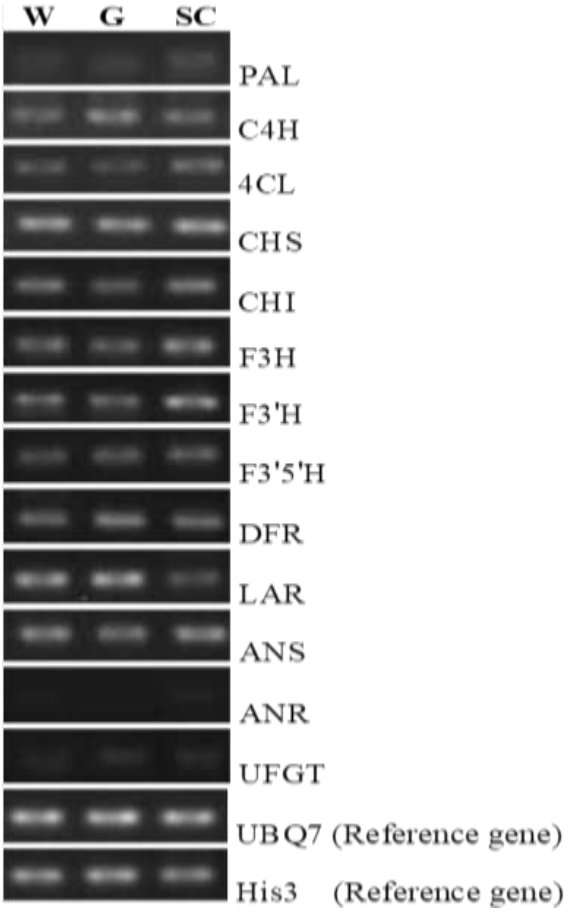

Figure 2. The results of the quantitative RT-PCR analysis of gene expression (the gel image of one replication). W: White cotton, G: Green cotton, SC: Seed coat.

Şekil 2. Gen ifadesinin kantitatif RT-PCR analizinin sonuçları (bir replikasyonun jel görüntüsü). W: Beyaz pamuk, G: Yeşil pamuk, SC: Tohum kabuğu.

The relationship between suberin and naringenin in green cotton fibers can be elucidated through a combination of studies. Suberin, a key component of the cell wall, has been identified as a monomer of glycerol (Moire et al., 1999). It is insoluble and lipophilic, contributing to the structural integrity of the cell wall (Moire et al., 1999). Studies have shown that suberin deposition can be altered by specific inhibitors, affecting the dimensions of suberin lamellae in green cotton fibers (Schmutz et al., 1996). On the other hand, naringenin, a flavonoid, has been found to impact fiber development in cotton. It can significantly retard fiber development, indicating its regulatory role in the growth processes of cotton fibers (Tan et al., 2013). Additionally, naringenin has been associated with pigment formation variations in cotton fibers, with its upregulation observed in certain cotton lines (Lv et al., 2023). The presence of suberin in green cotton fibers has been confirmed through ultrastructural and chemical analyses, highlighting its suberized nature (Yatsu et al., 1983).

The reduced expression of the *Chs* and *Chı* genes in white fibers, leading to decreased pigment formation, suggests a potential lack of suberin formation due to mutations affecting these genes. Notably, one of the *Chı* genes directly synthesizes *F3'5'H,* which is associated with suberin formation (Graça, 2015). Suberin is a lipophilic macromolecule found in specialized plant cell walls, providing insulation and protection where needed (Graça, 2015). Mutations affecting the expression of the *Chı* gene associated with *F3'5'h* synthesis may disrupt suberin formation, potentially influencing the color and quality of cotton fibers. Because Also, Few reports on the formation of the suberin layer of green cotton fiber suggest that the color of the fiber cannot be expressed when the synthesis of the suberin substances is inhibited during the fiber development (Schmutz et al 1993). The observation of lower expression levels of these genes in white fibers at 30 DPA highlights the importance of gene regulation in fiber development and coloration. The study's focus on a later developmental stage provides insights into the potential impact of mutations occurring during the peak synthesis period of these genes on their later expression levels (Fernandes et al., 2017). Understanding the molecular mechanisms underlying gene expression dynamics in colored cotton fibers is crucial for unraveling the genetic basis of fiber traits and color inheritance.

Furthermore, the link between gene expression, suberin formation, and pigment synthesis underscores the intricate regulatory networks governing fiber development. The study's findings contribute to the broader understanding of gene regulation in cotton fibers and shed light on the potential role of mutations in modulating gene expression and fiber characteristics.

Chemical analysis has further revealed the composition of suberin layers in green fibers, with a significant proportion of monomers being 22-hydroxydocosanoic acid (Sun et al., 2019). This underscores the importance of suberin in the structural composition of green cotton fibers. In the study conducted by Canavar and Rausher (2021), it was stated that *C4h* and *4Cl* flavonoid biosynthesis were at the highest, medium, and lowest levels, respectively, in cotton with brown, green, and white fiber colors. It was also observed that the synthesis of these two flavonoids was a part of the subuerin mechanism. In the same study, it was revealed that the synthesis of F3h, F3'h, and F3'5' was highest in colored cotton (due to the naringenin mechanism). However, in our study, unlike expected, an increase in the synthesis of these flavonoids in the white fiber region was observed. In Figure 3, this study, the pathway formed as a result and the synthesis rates of flavonoids in white fiber, green fiber, and seed coat are given. These results suggest that the mutation may have occurred at one or both of the two points where these genes are synthesized.

The potential effects of gene expression differences on fiber elongation in cotton, particularly concerning genes such as *4CL* (4-coumarate-CoA ligase), *C4H* (cinnamate-4-hydroxylase), *F3H* (flavanone-3-hydroxylase), *F3'H* (flavonoid 3'-hydroxylase), and *F3'5'H* (flavonoid 3',5'-hydroxylase), are significant in understanding the molecular mechanisms underlying fiber development. These genes are crucial in the flavonoid biosynthesis pathway, which has been linked to various aspects of plant growth, including fiber elongation. Research indicates that the expression of genes involved in flavonoid biosynthesis, such as C4H and F3H, is associated with the regulation of fiber elongation in cotton. For instance, (Yoo & Wendel, 2014) highlighted that several genes related to fiber elongation, including those in the flavonoid biosynthesis pathway, were over-expressed in domesticated cotton varieties compared to their wild counterparts. This suggests that enhanced expression of these genes may contribute to improved fiber characteristics, including elongation and quality (Yoo & Wendel, 2014).

Moreover, the enzymes encoded by F3H, F3'H, and F3'5'H are integral to the accumulation of flavonoids, which can influence cell wall properties and, consequently, fiber elongation. The synergistic action of these enzymes in the biosynthesis of anthocyanins and other flavonoids has been shown to affect the structural integrity of the cell wall, thereby impacting fiber growth. However, the reference provided Wang et al. (2021) does not directly support the claim regarding fiber elongation in cotton, as it focuses on anthocyanin regulatory networks in walnuts (Wang et al., 2021). Therefore, this citation has been removed. Additionally, the role of ethylene in promoting fiber elongation has been documented, with genes such as GhXB38D being implicated in this process. Ethylene signaling enhances the expression of genes involved in cytoskeleton construction and cell wall synthesis, which are essential for fiber elongation. Song (2023) discusses how ethylene treatment promotes fiber cell elongation in cotton by increasing the expression of relevant genes (Song, 2023). Furthermore, the expression of expansin genes, which are known to facilitate cell wall loosening, is also influenced by the flavonoid biosynthesis pathway. Expansins work in concert with the structural components of the cell wall to promote elongation. The identification of expansin genes that are co-expressed with flavonoid biosynthesis genes underscores the interconnectedness of these pathways in regulating fiber elongation. Lv et al. (2020) provide insights into the role of expansin genes in fiber cell growth, indicating their importance during the elongation stages of cotton fiber development (Lv et al., 2020).

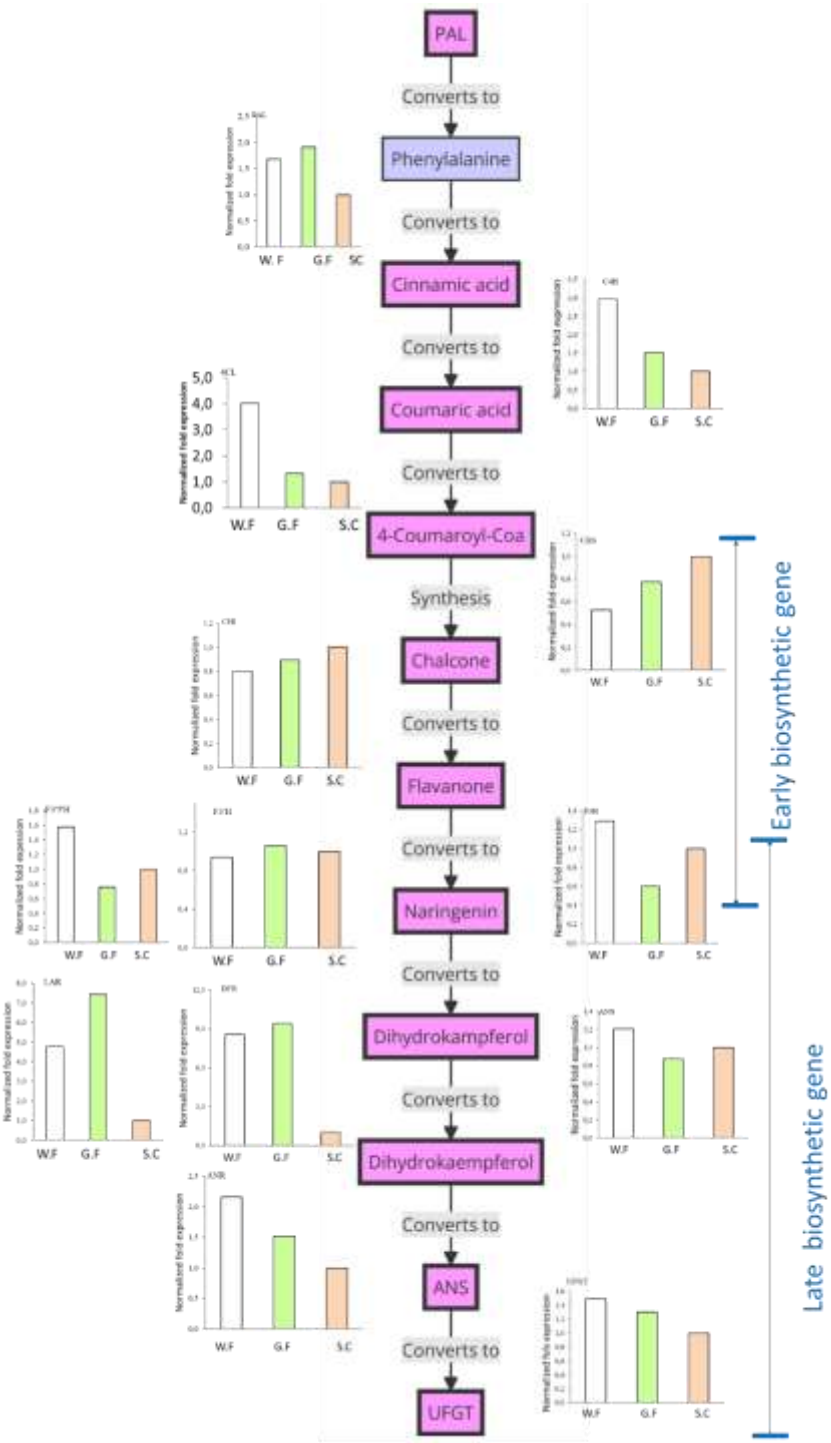

Figure 3: Diagram of the flavonoid pathway in green cotton fibers and expression patterns of flavonoid-related genes.Relative expression of flavonoid genes during fiber development in green cultivar. UBQ7 and His3 were used as internal controls to normalize the expression data. The fold expression of each gene was normalized to the expression of that gene in green cotton at the stage of 55 DPA (days post-anthesis). The significance of differences at a given stage of flavonoid genes is seen. Enzyme abbreviations: 4Cl, 4-coumarate:CoA ligase; Anr, anthocyanidin reductase; C4h, cinnamate 4-hydroxylase; Chi, chalcone isomerase; Chs, chalcone synthase; Dfr, dihydroflavonol 4-reductase; F3h, flavone 3-hydroxylase; F3'h, flavonoid 3'-hydroxylase; F3'5'h, flavonoid 3'5'-hydroxylase; Lar, leucoanthocyanidin reductase; Pal, phenylalanine ammonia-lyase; UBQ7, ubiquitin gene; Ufgt, UDP-glucose:flavonoid 3-glucosyltransferase.

Figure 3: Yeşil pamuk liflerinde flavonoid yolunun diyagramı ve flavonoid ile ilişkili genlerin ifade desenleri. Yeşil çeşitte lif gelişimi sırasında flavonoid genlerinin göreceli ifadesi. İfade verilerini normalize etmek için UBQ7 ve His3 iç kontrol genleri olarak kullanıldı. Her genin katlanma ifadesi, 55 DPA (çiçeklenme sonrası gün) aşamasındaki yeşil pamuktaki ifadesine göre normalize edildi. Enzim kısaltmaları: 4Cl, 4-coumarate:CoA ligase; Anr, anthocyanidin reductase; C4h, cinnamate 4-hydroxylase; Chi, chalcone isomerase; Chs, chalcone synthase; Dfr, dihydroflavonol 4-reductase; F3h, flavone 3-hydroxylase; F3'h, flavonoid 3'-hydroxylase; F3'5'h, flavonoid 3'5'-hydroxylase; Lar, leucoanthocyanidin reductase; Pal, phenylalanine ammonia-lyase; UBQ7, ubiquitin gene; Ufgt, UDP-glucose:flavonoid 3-glucosyltransferase.

The findings of the study regarding gene expression differences in flavonoid biosynthesis pathways in cotton have substantial implications for cotton breeding programs. Understanding the genetic mechanisms that influence fiber color and quality can facilitate targeted genetic modifications, ultimately leading to the development of cotton varieties with desirable traits. Specifically, the manipulation of flavonoid gene expression can be harnessed to enhance fiber color and quality, which are critical attributes in the textile industry (Xhing et al., 2022).

Recent research has demonstrated that specific genes involved in flavonoid biosynthesis play a pivotal role in determining fiber characteristics. For instance, the identification of key regulatory genes can inform breeding strategies aimed at improving fiber length and strength, which are essential for high-quality cotton production (Lv et al., 2023) By leveraging genetic engineering techniques such as CRISPR/Cas9, breeders can precisely edit these genes to enhance fiber properties, thereby accelerating the breeding process and increasing the efficiency of developing superior cotton varieties (Ullah et al., 2012).

Moreover, the integration of findings related to gene expression into existing breeding programs can enhance genetic diversity within cotton populations. This is particularly important given the current trend of genetic uniformity resulting from the monoculture of a few successful cultivars (Ullah et al., 2012). By introducing genetic variability through the targeted manipulation of flavonoid biosynthesis genes, breeders can create cotton varieties that not only exhibit improved fiber quality but also demonstrate greater resilience to environmental stressors such as drought and salinity (Zhu et al., 2013). This is crucial in the context of climate change, where cotton crops are increasingly exposed to abiotic stresses that can adversely affect yield and quality (Hassan et al., 2020). In conclusion, the insights gained from this study provide a robust foundation for advancing cotton breeding programs. By focusing on the genetic underpinnings of fiber color and quality through the modulation of flavonoid biosynthesis pathways, breeders can develop high-quality, diverse-colored cotton varieties that meet the evolving demands of the textile industry. The application of modern genetic tools will further enhance the ability to produce cotton that is not only aesthetically appealing but also resilient to the challenges posed by environmental stressors.

In our study, we examined gene expression levels in fibers on the 30th day post-anthesis (DPA). Literature typically indicates that these genes peak in synthesis between DPA 5 and 14, shortly after flowering. This is supported by numerous studies, including those by Lacape et al. (2012), Gilbert et al. (2013), and Canavar and Rausher (2021). However, we conducted our analysis at 30 DPA due to the visibility of colored bolls at this stage. It is possible that mutations occur much earlier, during the peak synthesis period of these genes, affecting their later expression.

CONCLUSION

The results of this study prove that a mutation in the flavonoid biosynthesis pathway has impacted the production of key enzymes such as *4Cl/C4h* and *F3h, F3'h, and F3'5'h*. This mutation will undoubtedly lead to the formation of differently colored fibers on a single seed, presenting significant challenges for breeding programs in terms of fiber consistency and readability. The low expression of the *CHS* and *CHI* genes, which is attributed to a mutation at the beginning of the phenylalanine pathway, is a crucial finding. This mutation prevents the formation of green color in the white fibers on the chalazal part, due to the reduction in synthesis of the *CHI* gene. These insights are invaluable for breeding programs, as they reveal the genetic mutations affecting fiber color.The study emphasizes the necessity for further research to investigate the practical applications of these findings in cotton breeding. Notably, certain genes demonstrated preferential expression in specific seed regions, indicating differential regulation of flavonoid synthesis. These findings indicate that mutations in flavonoid biosynthesis pathways may contribute to variations in fiber color and quality in cotton, offering new avenues for enhancing cotton fiber diversity and quality through targeted breeding strategies.

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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Women Entrepreneurship in Organic Agriculture: The Case of Türkiye

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ABSTRACT

Organic agriculture, whose importance is increasing day by day, is a form of production that prohibits the use of harmful substances in agricultural production and prioritizes soil health in order to protect human health and the ecological system. As in every subject, entrepreneurs are needed for the development of the sector in organic agriculture. In this context, while women entrepreneurs contribute to the production process on the one hand, on the other hand, they gain social and economic self-sufficiency. The aim of this research is to determine the entrepreneurial levels of women engaged in entrepreneurial activities in organic agriculture. In the research, the survey technique was used within the scope of the quantitative research method. The population of the research consists of women entrepreneurs engaged in organic agriculture activities in different regions and provinces of Türkiye. 532 women entrepreneurs selected by judgmental sampling method were determined as the sample of the research. According to the results of the research, it was determined that there was a significant difference in general agricultural entrepreneurship levels in terms of education level and marital status, but not in terms of age, geographical region, and industry experience, while there was a significant difference in organic agriculture entrepreneurship levels s in terms of age and education level, but not in terms of marital status, geographical region, and industry experience. In conclusion, this research is valuable in terms of revealing the current situation of women entrepreneurship in organic agriculture, which is an extremely important sector.

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Organik Tarımda Kadın Girişimciliği: Türkiye Örneği

ÖZET

Günümüzde önemi her geçen gün artan organik tarım, insan sağlığını ve ekolojik sistemi korumak için tarımsal üretimde zararlı maddelerin kullanımını yasaklayan ve toprak sağlığını ön planda tutan bir üretim biçimidir. Her konuda olduğu gibi organik tarımda da sektörün gelişimi için girişimcilere ihtiyaç bulunmaktadır. Bu bağlamda kadın girişimciler bir yandan üretim sürecine katkı sağlarken diğer yandan da kendilerine sosyal ve ekonomik açıdan öz yeterlilik kazandırmaktadır. Bu araştırmanın amacı, organik tarımda girişimcilik faaliyetinde bulunan kadınların girişimcilik eğilimlerini belirlemektir. Araştırmada nicel araştırma yöntemi kapsamında anket tekniği kullanılmıştır. Araştırmanın evrenini Türkiye`nin farklı bölge ve illerinde organik tarım faaliyetinde bulunan kadın girişimciler oluşturmaktadır. Yargısal örnekleme yöntemi ile seçilen 532 kadın girişimci araştırmanın örneklemi olarak belirlenmiştir. Araştırmanın sonuçlarına göre genel tarımsal girişimcilik düzeyinde eğitim düzeyi ve medeni durum açısından anlamlı bir farklılığın olduğu, yaş, coğrafi bölge ve sektör deneyimi açısından ise anlamlı bir farklılığın olmadığı, organik tarım girişimcilik düzeyinde ise yaş ve eğitim düzeyi açısından anlamlı bir farklılığın olduğu, medeni durum, coğrafi bölge ve sektör deneyimi açısından ise anlamlı bir farklığın olmadığı belirlenmiştir.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 02.08.2024
Kabul Tarihi : 18.12.2024

Anahtar Kelimeler
Organik tarım
Tarımsal girişimcilik
Kadın girişimciliği
Girişimcilik düzeyi

Sonuç olarak, bu araştırma son derece önemli bir sektör olan organik tarımda kadın girişimciliğinin mevcut durumunu ortaya koyması açısından değerlidir.

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INTRODUCTION

Today, the rapid population growth in the world is constantly increasing the need for food. The difficulty of the food industry in meeting this demand brings along the search for new production techniques. Since the early 20th century, emerging and rapidly developing technology also affected the agricultural sector and led to the transformation of this sector. Innovations in the agricultural sector also affect the production process. In terms of the agricultural sector, production refers to the yield obtained from the soil. In accordance with the new world order and human needs, producers strive to obtain the maximum level of product in a short time, and at this point, they benefit from technological opportunities. This leads to an increase in the use of chemicals in the food industry. These chemicals, fertilizers, hormones, and inorganic substances that increase production and thus profit, disrupt the ecological balance and threaten the environment and human health. In order to eliminate or minimize the effects of this negative situation, humanity is turning to organic agriculture as a form of production that does not harm human health, protects the ecosystem, and does not destroy natural resources. Organic agriculture is a form of production that essentially prohibits the use of chemical pesticides and fertilizers, but adopts the principle of organic and green fertilization, alternation, soil conservation, increasing plant resistance, and using biological control, and prioritizes product quality rather than production increase (DOKAP, 2018). Saving on chemical input costs, contractual purchase guarantee, creation of a new employment area, increasing demand for quality/healthy products and high prices are among the important advantages of organic agriculture. The disadvantages of this production method include the lack of specialized personnel equipped in organic agriculture, the negative impact of modern production in nearby regions, and fluctuations in supply (Hatunoğlu Durmaz, 2010). Therefore, as in every field, entrepreneurship is undoubtedly needed for the development of the sector in organic agriculture.

Entrepreneurship is one of the key concepts of the economy. Before the concept of entrepreneurship, it is necessary to talk about the entrepreneur who performs this activity. An entrepreneur is a person who transforms existing capital into investment by taking various risks and producing goods and services to meet human needs. According to Zimmerer and Scarborough (1998), an entrepreneur is a person who evaluates opportunities by calculating the risk and creates a new business or enterprise by collecting the necessary resources to create capital in order to reach profit under uncertainty. Entrepreneurs are characterized by risk-taking, seeing opportunities and needs, pooling resources, being open to innovation and evaluation, starting new businesses, and producing new products (Çetinkaya Bozkurt & Alparslan, 2013; Demiryürek & Demir, 2018). Entrepreneurship is an organized form of entrepreneurial activity. In other words, the establishment and execution of an enterprise by taking into account the possibility of profit/loss and the sum of the activities in this process are considered entrepreneurship. While entrepreneurship was initially accepted as a simple commercial activity, today, in parallel with technological developments, it is expanding into a field where elements such as brands, patents, and knowledge are used as capital to create added value. On the other hand, it is possible to come across examples of entrepreneurship in the local context that aim to develop in rural areas within the context of regional development. One of these examples is the initiatives carried out by women, who are disadvantaged in many aspects of society, in the field of organic agriculture. It can be said that the activities carried out by women entrepreneurs in this field make significant contributions to them. On the other hand, women are an important part of society and have an important role in the fulfillment of many services in society. The socio-economic process has undergone change and transformation with the entry of women into working life. While the presence and status of women in business life continue to increase day by day, the phenomenon of entrepreneurship has also been affected by this situation and women entrepreneurs have emerged. More than one concept is used to define women entrepreneurs. In general, the terms "businesswoman" and "woman entrepreneur" are used synonymously. However, a woman entrepreneur means "someone who does business on her own behalf". A businesswoman is understood as a woman who works in a workplace. Women entrepreneurs are creative, courageous, visionary, self-confident, open to innovation, highly skilled in achieving economic independence, and able to create employment for others by establishing and operating a business by adapting to family and social life. Since women entrepreneurs have a greater tendency to take risks, it can be said that, unlike other women, they are more determined and more willing to plan their future (Keskin, 2014). Entrepreneurial women are not only those who establish and run a business but also those who manage their own careers. They are intelligent, independent, and assertive individuals who can fight against

difficulties to achieve their goals, constantly review their expectations and business endeavors, and have a significantly high social awareness (Moore, 2000).

There are many studies on women's entrepreneurship in the national and international literature, and in the literature review, women's entrepreneurship has been examined within the scope of rural development, agriculture, and organic agriculture. Alston (2003) tried to reveal the contribution of women to the agricultural sector, tried to explain the dimensions of this contribution, and focused on the process of women entrepreneurs receiving the return for their labor. She emphasized that agriculture in Australia is at a critical point in terms of globalization and taking part in the world market and therefore women's entrepreneurship in agriculture is very important. Şahin (2006) examined the profile of women entrepreneurship in Konya province and concluded that women entrepreneurs engage in entrepreneurial activities as a necessity to meet family needs. Marchesoni and De Ros (2009) concluded that although the number of women entrepreneurs in the province of Trento in Italy is increasing, it is not yet at the desired level and that women are making good use of the opportunities offered by new fields of activity in marketing despite their disadvantaged position in the agricultural sector. Davaslıgil (2011) examined the role of women's entrepreneurship in rural development and concluded that social, cultural, and economic factors such as education level, number of children, migration status, marital status, and wage level are determinants in women's entrepreneurship. Erem Kaya and Atsan (2013) found that rural women who are young and have high incomes have higher levels of adoption of organic agriculture. In addition, educational status, land ownership, frequency of watching developments related to agriculture on television programs, and participation in training were determined as other factors affecting rural women's adoption of organic agriculture. Zaridis et al. (2015) stated that with the development of the agricultural sector, the role of rural women in agricultural production and organization has developed and this encourages women's entrepreneurship. They concluded that the level of vocational education of rural women is effective on their entrepreneurial tendencies and that women entrepreneurs face some economic and managerial problems. Marangoz et al. (2016) stated that there are many factors that lead women to entrepreneurship in rural areas such as the lack of other options for work, the desire to be their own boss, the inheritance of the family business, taking on the responsibility of economic livelihood due to the death of the spouse and success motive. Karaturhan et al. (2018) found that factors such as being raised in a family engaged in farming, education level, number of children, working in agricultural work, receiving vocational training, being open to innovations, taking part in projects related to women, being aware of organic agriculture, and having personal income affect the likelihood of rural women to adopt organic agriculture. Bakay et al. (2020), based on the example of the Demirci district of Manisa province, concluded that women's entrepreneurship provides many advantages such as self-confidence, economic freedom, strong communication, social environment, power, activism, dignity, and a sense of success. Aggarwal and Johal (2021) stated that rural women entrepreneurship is recognized as an important value in academic research and government policies. They also found that women's entrepreneurship has become popular in recent years, the most articles in this field have been published in India, the most cited studies are from the United Kingdom, and the existing studies focus on the factors affecting women's entrepreneurship.

When the statistics of the Ministry of Agriculture and Forestry of the Republic of Türkiye are analyzed, it is understood that Türkiye is making progress in organic agriculture every year and the export of organic products has increased compared to the previous year. For example, it is seen that the amount of organic product exports increased from 51,320,336 kg in 2022 to 59,185,999 kg in 2023 (T.R. Ministry of Agriculture and Forestry, 2024). However, it can be said that women have an important role in organic agriculture in Türkiye as in the world, and in this context, the number of women entrepreneurs is increasing day by day. While the rate of women entrepreneurs was 13.1% in 2002, it increased to 17.4% in 2023 (With Female Entrepreneurs, 2024). The main objective of this research is to determine the entrepreneurial levels of women engaged in entrepreneurial activities in the organic agriculture sector in Türkiye. In this context, the research has 3 sub-objectives: (1) To examine women's general agricultural entrepreneurship levels in terms of demographic variables (earning income, personal satisfaction, gaining social status, providing employment opportunities, contributing to the national economy, providing social benefits and desire to work independently, etc.), (2) To examine women's entrepreneurial levels towards organic agriculture in terms of demographic variables (transforming organic agriculture knowledge into practice, implementing innovations in the field of organic agriculture and finding solutions to problems, etc.), (3) To determine women's thoughts about the consequences of being an entrepreneur (reflections of entrepreneurship in women's life). This research is considered to be important in terms of revealing the profile of women entrepreneurship in organic agriculture, which is an extremely important sector in terms of both human and environmental health. It is extremely important for women who are disadvantaged in Türkiye to gain economic competence and social status by taking part in the organic agriculture sector as entrepreneurs.

MATERIAL and METHOD

In the research, the general agricultural entrepreneurship levels, organic agriculture entrepreneurship levels, and thoughts on the results of being an entrepreneur of women who are engaged in entrepreneurship activities in the organic agriculture sector in Türkiye were investigated using the survey technique. In this context, it was examined whether the general agricultural and organic agriculture entrepreneurship levels of women differ according to the demographic variables of age, education level, marital status, geographical region, and industry experience. In line with the relevant literature and the objectives of the study, 2 main hypotheses and 10 sub-hypotheses were developed:

H_1: The general agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of demographic variables.

H_{1a}: The general agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of age.

H_{1b}: General agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of education level.

H_{1c}: The general agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of marital status.

H_{1d}: The general agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of geographical region.

H_{1e}: General agricultural entrepreneurship levels of women entrepreneurs show a significant difference in terms of industry experience.

H_2: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of demographic variables.

H_{2a}: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of age variable.

H_{2b}: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of education level.

H_{2c}: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of marital status.

H_{2d}: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of geographical region.

H_{2e}: Organic agriculture entrepreneurship levels of women entrepreneurs show a significant difference in terms of industry experience variable.

A quantitative research method was used in the study. In this context, the questionnaire technique was preferred in the data collection phase. The questionnaire form consists of four sections. In the first part; there is a "Demographic Information Form (DIF)" consisting of 12 questions aiming to determine the demographic characteristics of the participants. In the second part, there is the "General Agricultural Entrepreneurship Scale (GAES)" which aims to measure the general agricultural entrepreneurship levels of the participants and consists of 14 items. The third section includes the "Organic Agricultural Entrepreneurship Scale (OAES)", which aims to measure the participants' organic agricultural entrepreneurship levels and consists of 10 items. In the fourth section, there is the "Consequences of Being an Entrepreneur Scale (CBES)" consists of 10 items, that aim to determine the participants' thoughts on the consequences of being an entrepreneur. In the development of GAES and OAES, Can and Engindeniz (2017) *"Young people's agricultural entrepreneurship tendencies: A sample research in Türkiye"* and Esmer and Gıdık (2020) *"A Research on the determination of agricultural entrepreneurship tendencies of organic agriculture students*" were used. A 5-point Likert-type scale 1: Not important, 2: Less important, 3: Undecided, 4: Important, 5: Very important) was used to rate these scales. In the development of CBES, Şahin (2006) research titled *"The woman entrepreneurs and an application about the profile of woman entrepreneurs in Konya"* was utilized. This scale is graded on a 5-point Likert scale (1: Strongly disagree, 2: Disagree, 3: Undecided, 4: Agree, 5: Strongly agree) scale was used. In the analysis process of the study, an average score level was obtained for these scales and these scores were used in all analyses.

The population of the research consists of women entrepreneurs engaged in organic agriculture activities in Türkiye. In this context, 532 women entrepreneurs selected by convenience sampling method were determined as the sample of the research. In the literature, with a confidence level of 95% and a margin of error of 5%, the minimum sample size over the maximum population is 384, and accordingly, it can be said that the sample size is sufficient (Yazıcıoğlu & Erdoğan, 2004). Ethics committee approval was obtained from the Bayburt University

Ethics Committee in order to conduct research on the determined sample (Date: 31/05/2021 and Decision Number: 2021/118). The survey form was delivered to the participants electronically between November 7-23, 2021 and feedback was provided.

Statistical analysis of the data was performed with the SPSS 22.0 package program. Cronbach's Alpha (α) values were examined to test the reliability of the scales, and as a result of the reliability analysis, it was seen that GAES (0.914), OAES (0.960) and CBES (0.966) were highly reliable according to Cronbach's Alpha (α) values (Kayış, 2009).

RESULTS and DISCUSSION

Since the scales used in the study were developed by utilizing other scales in the literature, Exploratory Factor Analysis (EFA) was used to test the construct validity of the scales. EFA is an analysis technique that aims to explain the factor structure of a newly developed or translated scale (Yaşlıoğlu, 2017). EFA results are given in Table 1.

Table 1. EFA results
Çizelge 1. Açıklayıcı Faktör Analizi (AFA) bulguları

Scales	Factor means	Explained cumulative variance (%)	KMO	Sig.
GAES	0.684	48.91	0.833	0.000
OAES	0.744	74.45	0.887	0.000
CBES	0.776	77.60	0.871	0.000

When the EFA results in Table 1 are examined, it is seen that the KMO values of the scales are well above the level of 0.5, indicating that the scales are in good condition in terms of sampling adequacy (Field, 2009). In addition, the factor means of the scales are above 0.40 (Hair Jr et al, 2014). These results prove that all scales have construct validity.

The frequency (f) and percentage (%) results regarding the demographic variables of age, marital status, nature of the place where the participants grew up, educational level, geographical region of residence, entrepreneurship training status, and industry experience are given in Table 2.

Table 2 shows that 69.7% of the participants were married and 30.3% were single. 36.2% of the participants were between the ages of 18-30, 38% between 31-45, 13.2% between 46-60, and 12.6% between 61 and above, indicating that more than half of the participants belonged to the young and middle age group. It was determined that 35.3% of the participants live in rural areas and 64.7% in urban areas, and it is noteworthy that there is a high rate of women entrepreneurs engaged in organic agriculture in cities. It is noteworthy that 44% of the participants are primary school graduates, 9.9% are secondary school graduates, 26.3% are high school graduates, 17.7% are undergraduate graduates, 2.1% are postgraduate graduates, and 19.8% of women entrepreneurs have undergraduate and postgraduate education. This situation shows that organic agriculture is a field of endeavor and earning for people with high levels of education as well as people with low levels of education living in rural areas. Of the participants, 30.4% live in Marmara, 15.8% in Central Anatolia, 13.2% in Mediterranean, 12.8% in Aegean, 11.1% in Southeastern Anatolia, 9.4% in Black Sea and 7.3% in Eastern Anatolia. In general, it can be said that the number of women entrepreneurs engaged in organic agriculture decreases as we move from west to east. The proportional distribution of the participants according to the regions is very different, and it can be said that this is due to the fact that women in the western regions have a higher awareness of organic agriculture. It is also possible to say that this is a result of the sample. It is seen that 16% of the participants have received entrepreneurship training and 84% have not received entrepreneurship training, and it is thought that very few of the women entrepreneurs have received training on entrepreneurship and this is due to the prevalence of entrepreneurship training and women's awareness on this issue. When the sector experience results are examined, it is understood that 35.3% of the participants have less than 1 year, 19% of the participants have 1-5 years of experience, 13.2% of the participants have 6-10 years of experience, 32.5% of the participants have 11 years or more of organic agriculture sector experience, approximately 1/3 of the participants are new to organic agriculture and approximately 1/3 of the participants are very experienced in this field.

Table 3 presents the frequency (f) and percentage (%) results of the sources of venture capital, size, types, fields of activity, and employee qualifications/number of enterprises established by women entrepreneurs in the organic agriculture sector.

Table 2. Demographic results
Çizelge 2. Demografik bulgular

Demographic variables		f	%
Marital status	Married	371	69.7
	Single	161	30.3
	Total	532	100
Age	18-30	193	36.2
	31-45	202	38.0
	46-60	70	13.2
	61 and above	67	12.6
	Total	532	100
Nature of place of growth	Rural area	188	35.3
	Urban region	344	64.7
	Total	532	100
Education level	Primary school	234	44.0
	Middle school	53	9.9
	High school	140	26.3
	License	94	17.7
	Postgraduate	11	2.1
	Total	532	100
Geographical region	Marmara	162	30.4
	Central Anatolia	84	15.8
	Mediterranean	70	13.2
	Aegean	68	12.8
	Southeast Anatolia	59	11.1
	Black Sea	50	9.4
	Eastern Anatolia	39	7.3
	Total	532	100
Entrepreneurship training status	Yes	85	16.0
	No.	447	84.0
	Total	532	100
Industry experience	Less than 1 year	188	35.3
	1-5 years	101	19.0
	6-10 years	70	13.2
	11 years and above	173	32.5
	Total	532	100

Table 4. Results regarding enterprises
Çizelge 3. İşletmelere ilişkin bulgular

Variables		f	%
Source of venture capital	Equity	340	63.9
	Debt/credit	145	30.3
	Grant	47	8.8
	Total	532	100
Enterprise size	Small scale	457	85.9
	Medium scale	62	11.7
	Large scale	13	2.4
	Total	532	100
Enterprise type	Agricultural production	455	85.5
	Agricultural product processing	60	11.3
	Agricultural marketing	17	3.2
	Total	532	100
Field of activity	Crop production	395	74.3
	Crop and animal production	77	14.4
	Animal production	48	9.0
	Beekeeping	12	2.3
	Total	532	100
Employee qualification/number	Herself/himself	126	23.7
	Family	13	2.4
	1-5	279	52.4
	5 and above	59	11.1
	Changing	56	10.5
	Total	532	100

When Table 3 is analyzed, it is seen that 63.9% of women entrepreneurs used equity as venture capital, 30.3% borrowed/loaned, and 8.8% applied for grants, and it is understood that a significant portion of women entrepreneurs established their enterprises with equity. It is seen that 85.9% of the enterprises are small-scale, 11.7% are medium-scale, 2.4% are large-scale, and most of the enterprises are small-scale. It can be said that 85.5% of the enterprises are agricultural production enterprises, 11% are agricultural product processing enterprises and 3.2% are agricultural marketing enterprises, and these ratios are in parallel with the results of enterprise size. 74.3% of the enterprises are engaged in crop production (olive, vegetable, and fruit), 14.4% in both crop production and animal production (two fields), 9% in animal production, and 2.3% in beekeeping. It is seen that in 23.7% of the enterprises, women entrepreneurs work only by themselves, while in 2.4% of the enterprises they work together with their families. In addition, 52.4% of the enterprises have 1-5 employees, 11.1% have 5 or more employees, and this situation varies in 10.5% of the enterprises. It is possible to say that this situation is due to the fact that most of the enterprises are small-scale enterprises and seasonal changes in labor demand.

Descriptive statistical results including frequency (f), mean, standard deviation (SD), skewness, and kurtosis values for the general agricultural and organic agriculture entrepreneurship levels of the participants are given in Table 4.

Table 4. Descriptive statistical results
Çizelge 4. Tanımlayıcı istatistiki bulgular

Scales	f	Mean	SD	Skewness	Kurtosis
GAES	532	3.65	1.315	-0.301	-0.683
OAES	532	3.82	1.285	-0.660	-0.263
CBES	532	3.44	1.469	-0.229	-1.193

When Table 4 is examined, it is seen that the participants' general agricultural entrepreneurship levels (X=3.65±1.315) and organic agriculture entrepreneurship levels (X=3.82±1.285) are close to the level of "4: Important". It is understood that women's thoughts on the results of being an entrepreneur (X=3.44±1.469) are similarly close to the level of "4: Agree". Accordingly, it can be said that the participants' entrepreneurial levels and their thoughts on the consequences of being an entrepreneur are above the middle level. In addition, whether the data are normally distributed was evaluated by looking at the skewness and kurtosis values. Whether the data are normally distributed was evaluated by looking at the skewness and kurtosis values. According to Tabachnick and Fidell (2013), skewness and kurtosis values should be between -1.5 and +1.5 for the data to show normal distribution. When the values in Table 4 are examined, it is understood that all three-scale data are normally distributed. Therefore, it was deemed appropriate to use parametric tests (Independent Sample T-test and ANOVA Analysis) to test the hypotheses. The frequency (f) and percentage (%) results of the general agricultural entrepreneurship levels of the participants on the basis of scale items are given in Table 5 (Scale Question: Please rate your general objectives for being an entrepreneur in the agricultural sector according to their importance).

Table 5. General agricultural entrepreneurship level
Çizelge 5. Genel tarımsal girişimcilik düzeyi

Items/frequency and percentage	Not important		Less important		Undecided		Important		Very important	
	f	%	f	%	f	%	f	%	f	%
Ensure high profits	81	15.2	25	4.7	179	33.6	78	14.7	169	31.8
	91	17.1	21	3.9	161	30.3	93	17.5	166	31.2
Providing personal fulfillment	91	17.1	49	9.2	157	29.5	33	6.2	202	38.0
	96	18.0	44	8.3	231	43.4	55	10.3	106	20.0
Contributing to the national economy	35	6.6	22	4.1	116	21.8	83	15.6	276	51.9
Gaining social status	95	17.9	18	3.4	170	31.9	61	11.5	188	35.3
	74	13.9	23	4.3	122	22.9	67	12.6	246	46.3
Working independently	33	6.2	44	8.3	115	21.6	46	8.6	294	55.3
	33	6.2	5	0.9	179	33.6	92	17.3	223	42.0
Providing employment opportunities	147	27.6	47	8.8	151	28.4	108	20.3	79	14.9
	44	8.3	11	2.1	121	22.7	51	9.6	305	57.3
Staying motivated and working	64	12.0	10	1.9	122	23.0	64	12.0	272	51.1
	12	2.3	44	8.3	139	26.1	82	15.4	255	47.9
Personal tastes and preferences	14	2.6	15	2.8	172	32.3	54	10.2	277	52.1

According to Table 5, the items with the highest rate of "very important" are "meeting the needs of the family (57.3%)", "personal tastes and preferences (55.3%)", "meeting individual needs (52.1%)", "working independently (51.9%)" and "making good use of time (51.1%)". According to these findings, although women mostly engage in entrepreneurial activities to provide for the family, it is understood that they also engage in entrepreneurial activities for other purposes such as personal tastes and preferences such as hobbies, individual needs, the desire to work independently and to make good use of time. In this context, Şahin (2006) stated that women entrepreneurs mostly engage in entrepreneurial activities as a necessity and that factors such as personal satisfaction and self-realization remain weak in entrepreneurship preference.

The frequency (f) and percentage (%) results of the participants' organic agriculture entrepreneurship levels on the basis of scale items are given in Table 6 (Scale Question: Please rate your objectives for becoming an entrepreneur in the organic agriculture sector according to their importance).

Table 6. Organic agriculture entrepreneurship level
Çizelge 6. Organik tarım girişimcilik düzeyi

Items/frequency and percentage	Not important		Less important		Undecided		Important		Very important	
	f	%	f	%	f	%	f	%	f	%
To transform organic agriculture knowledge into practice	93	17.5	2	0.4	136	25.6	80	15.0	221	41.5
Contributing to the organic agriculture sector	36	6.7	53	10.0	149	28.0	67	12.6	227	42.7
Offering organic agricultural products to consumers	57	10.7	7	1.3	192	36.1	44	8.3	232	43.6
To utilize the organic agricultural resources in the country	69	13.0	14	2.6	25	4.7	220	41.4	204	38.3
Applying innovations in the field of organic agriculture	44	8.3	5	0.9	145	27.3	81	15.2	257	48.3
Agricultural production according to organic conditions	88	16.5	4	0.8	147	27.6	68	12.8	225	42.3
Contributing to the spread of organic agriculture	46	8.6	49	9.2	127	23.9	91	17.1	219	41.2
Promoting organic agriculture	34	6.4	5	0.9	172	32.3	70	13.2	251	47.2
To produce solutions to problems in the field of organic agriculture	46	8.6	12	2.2	153	28.8	94	17.7	227	42.7
Increasing awareness and awareness of organic agriculture	15	2.8	1	0.2	155	29.1	83	15.6	278	52.3

According to Table 6, it is seen that the issues that the participants see at the "very important" level are "to increase the awareness and consciousness of organic agriculture (52.3%)", "to implement innovations in the field of organic agriculture (48.3%)" and "to popularize organic agriculture (47.2%)" respectively. Accordingly, it can be said that women engage in entrepreneurship activities in organic agriculture mostly to promote organic agriculture, to increase awareness of organic agriculture, to implement innovations in the field of organic agriculture, and to popularize organic agriculture. In addition, it is seen that the option of "utilizing the organic agriculture resources in the country" is at the level of "important (41.4%)". Karaturhan et al. (2018) found that factors such as being open to innovations and being conscious about organic agriculture affect the likelihood of women adopting organic agriculture.

The frequency (f) and percentage (%) results of the participants' thoughts on the consequences of being an entrepreneur on the basis of scale items are given in Table 7 (Scale Question: Please mark the extent to which you agree with the following statements about the consequences of being an entrepreneur in the organic agriculture sector).

Table 7. Consequences of being an entrepreneur
Çizelge 7. Girişimci olmanın sonuçları

Items/frequency and percentage	Strongly disagree		Disagree		Undecided		Agree		Strongly agree	
	f	%	f	%	f	%	f	%	f	%
Being an entrepreneur increased my self-confidence.	111	20.9	14	2.6	8	1.5	213	40.0	186	35.0
Being an entrepreneur helped me understand the world better.	75	14.1	181	34.0	29	5.5	66	12.4	181	34.0
Being an entrepreneur has improved my human relations.	39	7.3	135	25.4	87	16.4	68	12.8	203	38.1
Being an entrepreneur helped me learn the realities of life.	48	9.0	120	22.6	19	3.6	132	24.8	213	40.0
Being an entrepreneur provided me with economic independence.	51	9.6	123	23.1	54	10.2	123	23.1	181	34.0
Being an entrepreneur enabled me to express myself better.	88	16.5	122	22.9	45	8.5	93	17.5	184	34.6
Being an entrepreneur gave me a social identity.	55	10.3	170	32.0	39	7.3	98	18.4	170	32.0
Being an entrepreneur allowed me to dream.	49	9.2	123	23.1	60	11.3	95	17.9	205	38.5
Being an entrepreneur made me a role model.	53	9.9	136	25.6	39	7.3	103	19.4	201	37.8
Being an entrepreneur increased my reputation within the family.	65	12.2	142	26.7	64	12.0	50	9.4	211	39.7

When Table 7 is examined, it is seen that the options with the highest thoughts of the participants regarding the results of being an entrepreneur are "becoming an entrepreneur enabled me to learn the facts of life (40%)", "becoming an entrepreneur increased my reputation in the family (39.7%)", "becoming an entrepreneur enabled me to dream (38.5%)" and "becoming an entrepreneur improved my human relations (38.1%)", respectively. Accordingly, it can be said that as a result of becoming an entrepreneur, women have learned the facts of life, their reputation in the family has increased, they have started to dream and their relationships with people have improved. In addition, it can be understood from the related findings that being an entrepreneur increases women's self-confidence. Similarly, Bakay et al. (2020) concluded that being an entrepreneur provides advantages to women in many areas such as self-confidence, economic independence, communication skills, and social prestige.

Whether the general agricultural and organic agriculture entrepreneurship levels of the participants differ in terms of age variable was evaluated by ANOVA analysis and the results of the analysis are given in Table 8 (H_{1a} and H_{2a}).

Table 8. ANOVA analysis results according to age variable
Çizelge 8. Yaş değişkenine göre ANOVA analizi bulguları

Scales	Age	N	Mean	F	Sig.
GAES	18-30	193	-	0.37	0.657
	31-45	202	-		
	46-60	70	-		
	61 and above	67	-		
OAES	18-30	193	4.01	0.20	0.037
	31-45	202	3.95		
	46-60	70	3.67		
	61 and above	67	3.65		

When Table 8 is examined, it is determined that there is no significant difference in general agricultural entrepreneurship levels in terms of age variable (Sig.>0.05), while there is a significant difference in organic agriculture entrepreneurship levels (Sig.<0.05). Therefore, H_{1a} is rejected and H_{2a} is accepted. In this case, looking at the averages, it can be said that women's organic agriculture entrepreneurship levels decrease with increasing age. Erem Kaya and Atsan (2013) concluded that young women have higher levels of adoption of organic agriculture.

Whether the general agricultural and organic agriculture entrepreneurship levels of the participants differ in terms of education level was evaluated by ANOVA analysis and the results of the analysis are given in Table 9 (H_{1b} and H_{2b}).

Table 9. ANOVA analysis results according to education level variable
Çizelge 9. Eğitim düzeyi değişkenine göre ANOVA analizi bulguları

Scales	Education level	N	Mean	F	Sig.
GAES	Primary School	234	4.09	8.19	0.046
	Middle School	53	3.91		
	High School	140	3.67		
	License	94	3.36		
	Postgraduate	11	3.22		
OAES	Primary School	234	4.23	8.01	0.021
	Middle School	53	4.04		
	High School	140	3.95		
	License	94	3.37		
	Postgraduate	11	3.51		

When Table 9 is examined, it is determined that there is a significant difference (Sig.<0.05) in both general agricultural and organic entrepreneurship levels in terms of education level, and H_{1b} and H_{2b} are accepted. In this case, when the averages are considered, it can be said that women's general agricultural and organic agriculture entrepreneurship levels differ slightly in terms of education level. Zaridis et al. (2015) concluded that the level of vocational education of rural women is effective on their entrepreneurial levels.

Whether the general agricultural and organic agriculture entrepreneurship levels of the participants differ in terms of marital status was evaluated by Independent Sample T-Test and the test results are given in Table 10 (H_{1c} and H_{2c}).

Table 10. T-test results according to marital status variable
Çizelge 10. Medeni durum değişkenine göre t-testi bulguları

Scales	Marital status	N	Mean	Homogeneity of variances	F	Sig.	t	Sig. (2-tailed)
GAES	Married	371	3.91	Homogeneous	0.753	0.387	-2.498	0.014
	Single	161	3.39	Not homogeneous			-2.509	0.013
OAES	Married	371	-	Homogeneous	0.109	0.742	-1.517	0.132
	Single	161	-	Not homogeneous			-1.498	0.137

When Table 10 is examined, the fact that Sig. values of general agricultural and organic agriculture entrepreneurship levels are greater than 0.05 showing that the variances are homogeneous. In this case, when Sig. (2 tailed) values are examined, it is understood that there is a significant difference in general agricultural entrepreneurship levels in terms of marital status variable (Sig2.<0.05), while there is no significant difference in organic agriculture entrepreneurship levels (Sig2.>0.05). Therefore, H_{1c} is accepted and H_{2c} is rejected. Therefore, when the averages are examined, it is seen that the general agricultural entrepreneurship levels of married women are at a lower level, but it can be said that this situation is due to the social and economic concerns of married women. Davaslıgil (2011) stated that marital status affects women's entrepreneurial levels.

Whether the general agricultural and organic agriculture entrepreneurship levels of the participants differ in terms of geographical region variable was evaluated by ANOVA analysis and the results of the analysis are given in Table 11 (H_{1d} and H_{2d}).

When Table 11 is examined, it is determined that there is no significant difference (Sig.>0.05) in both general agricultural and organic entrepreneurship levels in terms of geographical region, and H_{1d} and H_{2d} are rejected. Accordingly, it is possible to say that women's general agricultural and organic agriculture entrepreneurship levels are similar in terms of geographical region.

Whether the general agricultural and organic agriculture entrepreneurship levels of the participants differ in terms of industry experience variable was evaluated by ANOVA analysis and the findings of the analysis are given in Table 12 (H_{1e} and H_{2e}).

Table 11. ANOVA analysis results by geographical region variable
Çizelge 11. Coğrafi bölge değişkenine göre ANOVA analizi bulguları

Scales	Geographical region	N	F	Sig.
GAES	Mediterranean	162	7.41	0.103
	Eastern Anatolia	84		
	Aegean	70		
	Southeast Anatolia	68		
	Central Anatolia	59		
	Black Sea	50		
	Marmara	39		
OAES	Mediterranean	162	6.83	0.241
	Eastern Anatolia	84		
	Aegean	70		
	Southeast Anatolia	68		
	Central Anatolia	59		
	Black Sea	50		
	Marmara	39		

Table 12. ANOVA analysis results according to industry experience variable
Çizelge 12. Sektör deneyimi değişkenine göre ANOVA analizi bulguları

Scales	Industry Experience	N	F	Sig.
GAES	Less than 1 year	188	4.41	0.351
	1-5 years	101		
	6-10 years	70		
	11 years and above	173		
OAES	Less than 1 year	188	7.05	0.673
	1-5 years	101		
	6-10 years	70		
	11 years and above	173		

When Table 12 is examined, it is determined that there is no significant difference in both general agricultural and organic entrepreneurship levels in terms of industry experience (Sig.>0.05) and H_{1e} and H_{2e} are rejected. Accordingly, it can be said that women's entrepreneurial levels are similar in terms of organic agriculture experience.

The acceptance/rejection status of all hypotheses developed within the scope of the research on whether women's general agricultural and organic agriculture entrepreneurship levels differ according to the demographic variables of age, education level, marital status, geographical region, and industry experience are shown in Table 13.

Table 13. Acceptance/rejection of hypotheses
Çizelge 13. Hipotezlerin kabul/ret durumları

Hypothesis	Accept/Reject
H1	Partially acceptance
H_{1a}	Rejection
H_{1b}	Acceptance
H_{1c}	Acceptance
H_{1d}	Rejection
H_{1e}	Rejection
H2	Partially acceptance
H_{1e}	Rejection
H_{2a}	Acceptance
H_{2b}	Acceptance
H_{2c}	Rejection
H_{2d}	Rejection
H_{2e}	Rejection

When Table 13 is analyzed, some of the sub-hypotheses (H_{1b}, H_{1c}, H_{2a}, H_{2b}) are accepted and some of them (H_{1a}, H_{1d}, H_{1e}, H_{2c}, H_{2d}, H_{2e}) are rejected. Accordingly, it can be said that the entrepreneurial levels of women in organic agriculture partially differ in terms of demographic variables. Therefore, H_1 and H_2 are partially accepted. In support of this finding, Davaslıgil (2011) concluded that social and economic factors such as education level, marital status, number of children, and wage amount are effective on women's entrepreneurial activities.

CONCLUSION

The endangerment of human health, the deterioration of ecological balance, and the remembrance of the importance of the relations between humans, nature, and other living things have led people to healthy production and consumption. Organic agriculture, which is defined as a way of obtaining high nutritional value and healthy products by not using harmful substances in the production process and processing the soil correctly, has become an important sector in Türkiye as well as in the world, showing development day by day. Entrepreneurship, which promises innovation and progress in terms of its nature, is an important phenomenon for the organic agriculture sector as in every sector. An entrepreneur is defined as a person who transforms his/her capital into investment in order to gain profit or benefit by producing goods and/or services by taking some risks, and entrepreneurship is defined as this activity itself. In order to be a successful entrepreneur, it is necessary to have some characteristics. Rational behavior, foresight, seizing opportunities, communication skills, and crisis management are some of these characteristics. In recent years, it has been observed that women have become entrepreneurs in many fields and have gained social/economic gain in this way. One of these areas is the agricultural sector. It can be seen that especially women living in rural areas and somehow involved in agricultural production do not get a return for their labor. It is highly likely that rural women will prevent labor exploitation through entrepreneurship. In addition, women living in these regions are in a disadvantaged position due to various reasons such as education, living conditions, and gender roles. For this reason, entrepreneurship for rural women is not only a source of income but also a gateway to life. For rural women, entrepreneurship can be said to be the beginning of a new story whose protagonists are themselves.

The main objective of this research conducted in Türkiye is to determine the entrepreneurial levels of women in organic agriculture. In this context, the sub-objectives of the research are to determine the general agricultural entrepreneurship levels of women, to reveal their entrepreneurship levels in organic agriculture, and to determine their thoughts about the results of being an entrepreneur. According to the results of the hypotheses developed in line with these objectives, it was determined that there was a significant difference in terms of education level and marital status in general agricultural entrepreneurship levels, while there was no significant difference in terms of age, geographical region, and industry experience. In organic agriculture entrepreneurship levels, it was determined that there was a significant difference in terms of age and education level, while there was no significant difference in terms of marital status, geographical region, and sector experience. On the other hand, it was determined that women generally engage in entrepreneurship activities to provide for the family, personal tastes and preferences such as hobbies, individual needs, desire to work independently and to make good use of time. In terms of organic agriculture, it has been determined that they engage in entrepreneurship activities in order to promote organic agriculture, increase awareness of organic agriculture, implement innovations in the field of organic agriculture, and popularize organic agriculture. Within the scope of the results of being an entrepreneur, it was concluded that as a result of becoming an entrepreneur, women learned the realities of life, their reputation increased within the family, they started to dream, their relationships with people improved and their self-confidence increased. These results reveal that entrepreneurship provides significant benefits for women not only in terms of economic but also social and personal development.

Although many factors have an impact on the transition to organic agriculture, it can be said that the most important factor is “health”. The fact that many diseases, especially cancer, are increasing day by day and that these diseases rank high in the causes of death requires questioning the quality of the food consumed and consumption behaviors. In this regard, experts state that the prerequisite for a healthy diet is natural nutrition. This situation reveals the importance of organic agriculture once again. In another respect, organic agriculture has opened an economic door for women living in disadvantaged positions in rural areas. As in many examples, women entrepreneurs earn economic gains by marketing the products they produce in the enterprises they have established with their initiatives. These entrepreneurial women are able to become economically independent and socialize together. In this respect, they can be role models for many similar women in Türkiye. Therefore, this research, it was tried to raise awareness by revealing the current situation of women's entrepreneurship in organic agriculture and to contribute to the development of both fields by linking the organic agriculture sector and women's entrepreneurship. As a result of the research, it has been observed that women entrepreneurs have achieved significant gains in many aspects of their activities in the field of organic agriculture and offer significant outputs to society. For this reason, it is recommended to organize training on organic agriculture for women,

support projects on this subject, carry out activities to raise awareness on the subject, support women entrepreneurs, for the state to provide resources to those concerned, make tax regulations on organic products and to ensure media visibility. On the other hand, it can be said that it would be very useful to conduct qualitative research using face-to-face interview techniques in order to examine the entrepreneurial levels of women in organic agriculture in depth in future studies.

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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 declare that there is no conflict of interest between them.

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Analysis of Trend, Growth, and Instability Index in Output, Harvested Area, and Yield of Oil Palm Fruit in Nigeria

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ABSTRACT
The research examined the trend, growth rate, and instability index related to the output, harvested area, and yield of oil palm fruit in Nigeria. Utilizing secondary data sourced from the Food and Agricultural Organization (FAO) covering the period from 1961 to 2022, the study revealed inconsistent patterns in the trend of output, harvested area, and yield of oil palm fruit across the specified timeframes. The compound growth rates identified were 1.11% for output, 1.03% for harvested area, and 0.08% for yield. The Cuddy-Della Valle instability index (CDI) and the Coppock Instability Index (COI) were calculated at 13.87 and 46.57 for output, 13.87 and 46.02 for harvested area, and 2.52 and 37.88 for yield, respectively. These instability indices were relatively low, indicating limited activity within the oil palm fruit sub-sector in Nigeria. A decomposition analysis of the total effect of the output showed that the area effect accounted for 94.20%, the yield effect for 3.19%, and the interactive effect for 2.61%. The findings indicate that the area effect is the predominant factor driving the growth of oil palm fruit production in Nigeria. Consequently, it is recommended that various programs such as; small grower schemes, oil palm farmers' cooperatives, and off-takers scheme be implemented within the sub-sector to stimulate increased activities, production, and yield. Also, the oil palm fruit farmers should be provided with quality seeds and financial resources in addition to broadening research efforts aimed at developing high-yield varieties.

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Nijerya'da Yağ Palmiyesi Meyvesinin Üretim, Hasat Alanı ve Verimindeki Eğilim, Büyüme ve Istikrarsızlık Indeksinin Analizi.

ÖZET
Araştırmada, Nijerya'daki yağ palmiyesi meyvesinin çıktısı, hasat alanı ve verimi ile ilgili eğilim, büyüme oranı ve istikrarsızlık endeksi incelenmiştir. Gıda ve Tarım Örgütü'nden (FAO) alınan ve 1961-2022 dönemini kapsayan ikincil veriler kullanılarak yapılan çalışma, belirtilen zaman dilimleri boyunca yağ palmiyesi meyvesinin çıktısı, hasat alanı ve verimi eğiliminde tutarsız kalıplar ortaya koymuştur. Belirlenen bileşik büyüme oranları çıktı için %1.11, hasat alanı için %1.03 ve verim için %0.08'dir. Cuddy-Della Valle istikrarsızlık endeksi (CDI) ve Coppock İstikrarsızlık Endeksi (COI) sırasıyla çıktı için 13.87 ve 46.57, hasat alanı için 13.87 ve 46.02 ve verim için 2.52 ve 37.88 olarak hesaplanmıştır. Bu istikrarsızlık endeksleri nispeten düşük olup, Nijerya'daki yağ palmiyesi meyvesi alt sektöründe sınırlı faaliyet olduğunu göstermektedir. Çıktının toplam etkisinin ayrıştırma analizi, alan etkisinin %94.20, verim etkisinin %3.19 ve etkileşimli etkinin %2.61 olduğunu gösterdi. Bulgular, alan etkisinin Nijerya'da yağ palmiyesi meyvesi üretiminin büyümesini yönlendiren baskın faktör olduğunu göstermektedir. Sonuç olarak, artan faaliyetleri, üretimi ve verimi teşvik etmek için alt sektörde küçük yetiştirici planı, yağ palmiyesi çiftçileri kooperatifi, alıcı planı gibi çeşitli programların uygulanması önerilmektedir. Ayrıca, yağ palmiyesi meyvesi üreticilerine, yüksek verimli çeşitlerin geliştirilmesine yönelik

Tarım Ekonomisi

Araştırma Makalesi

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Anahtar Kelimeler
Büyüme
İstikrarsızlık endeksi
Yağ palmiyesi meyvesi
Eğilim
Nijerya

araştırma çalışmalarının artırılmasının yanı sıra, kaliteli tohum temini ve finansal kaynakların sağlanması da önemlidir.

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INTRODUCTION

The oil palm tree *(Elaeis guineensis)* is one of the major cash crops in southern Nigeria (PIND, 2011; Patrick et al., 2013; Ojo et al., 2017; Akpan et al., 2020). During the pre-independent era, the country contributed nearly 50% of the global palm oil exports. By the mid-1960s, Nigeria commanded approximately 43% of the global market share of palm oil (PIND, 2011). However, the prominence of Nigeria in palm oil production diminished as Indonesia, Malaysia and Thailand emerged as leaders, largely due to Nigeria's increased emphasis on crude oil extraction (Olufemi, 2015; Busari et al., 2022). Currently, Nigeria ranks fifth in global palm oil production, generating about 1.50 million metric tons annually, which constituted about 2% of the total global output in 2023 (FAO, 2024). Despite Nigeria's fluctuating oil palm fruit production, the sub-sector's importance is growing due to its role in job creation, industrialization, and rural income generation (Akpan et al., 2019; Udoka et al., 2019). The oil palm crop produces various derivatives, with palm oil being the most widely used and a staple in many Nigerian diets. The palm oil is rich in carotenoids, vitamins, tocopherols, fatty acids, vitamin E, and emulsifiers among others (Gonzalez-Diaz and García-Núñez, 2021).

The demand for oil palm fruit and its derivatives in Nigeria has shown a consistent rise over the years. For instance, the domestic consumption of palm oil, primarily for food purposes, was recorded at 1.65 million metric tons in 2020, increasing to 1.71 million metric tons in 2021 (USDA, 2022). In contrast, domestic production has been inconsistent and fallen short of demand with about 1.275 million metric tons in 2020 and 1.400 million metric tons in 2021, resulting in supply deficits of 0.375 million metric tons and 0.310 million metric tons for the respective years (USDA, 2022). These supply shortfalls have led to an increased demand for imports, which poses significant financial challenges for the nation's economy. Should this trend persist without suitable interventions, it could lead to the neglect of other sectors, exacerbating more, the poverty crisis in Nigeria (Abbas et al., 2018, Ephraim et al., 2022). Presently, with a population exceeding 200 million, the demand is anticipated to grow alongside the ongoing supply deficiencies. The USDA (2022) reports that Nigeria is the largest consumer of palm oil in Africa, with an annual consumption of 1.79 million metric tons, followed by Egypt at 1.225 million metric tons in 2022. In 2020, the production of oils and fats in Sub-Saharan Africa reached 6 million metric tons, while domestic consumption was 11.2 million metric tons, resulting in an import requirement of 7.4 million metric tons (USDA, 2022). Furthermore, a report from 2019 indicated that Nigeria's total consumption of fats and oils had risen to approximately 3 million metric tons, with palm oil accounting for 44.7% of this figure (PWC, 2019).

The Nigerian government has initiated various strategies aimed at revitalizing the declining fortunes of oil palm fruit production, which is primarily dominated by smallholder farmers (Patrick et al., 2013, Udoka et al., 2019; Akpan et al., 2019). These interventions have included import restrictions, financial support for stakeholders, and infrastructural enhancements, among other measures. Notably, in 2015, the Federal Government (FG) prohibited the importation of palm kernel and palm oil products to boost domestic production (CBN, 2015). In 2019, the FG implemented a closure of its land borders to enforce the ban on imported palm oil derivatives. Furthermore, approximately ₦30 billion was allocated as loans to oil palm farmers to improve their productivity. The CBN also introduced the Anchored Borrower Program (ABP) in 2015, aimed at providing indirect financial assistance to small-scale oil palm farmers to enhance overall production. In 2020, the CBN allocated ₦34.3 billion to significant palm oil enterprises with the goal of expanding cultivated land from 20,000 hectares in 2020 to 100,000 hectares by 2025, thereby increasing production and creating employment opportunities for the youth. Despite these efforts, the anticipated results have not materialized, as Nigeria's prominent position in the global oil palm fruit and derivative market remains elusive. The annual growth rate of output continues to be marginal (FAO, 2024). In 2019, major palm oil companies listed on the Nigerian Stock Exchange (NSE) reported a decline in revenue. Additionally, global market dynamics have hindered the FG's objectives to enhance oil palm fruit and derivative production. For instance, the average price of crude palm oil fell from US$751 per metric ton in 2017 to US$601 per metric ton in 2019, representing a decline of 19.97% (Vanguard, 2020).

The implementation of various intervention programs within the sub-sector, coupled with the influence of external factors, has created a pressing need to examine the trends and characteristics of instability in the production, yields, and harvested areas of oil palm fruit in the country. While the imperative to enhance oil palm fruit

production, yields, and harvested areas is clear due to their significant role in the nation's economic development, the associated instability in these variables may lead to several detrimental consequences. For example, such instability can discourage investment in oil palm fruit production due to the perceived risks and increasing uncertainties. Furthermore, the income of farmers and their ability to make optimal farming decisions are adversely affected by rising instability in output, yield, and cultivated land. Additionally, fluctuations in farm output and yield can disrupt price stability, influence consumer preferences, and impact the purchasing power of low-income households (Akpan, 2012, Akpan et al., 2012a; Akpan et al 2012b, Akpan et al 2012c).

As noted by Abu and Adakole (2017), Ikuemonisan et al. (2023), and Antia-Obong et al. (2024), various arable crops in Nigeria have showed differing levels of compound growth rates and instability in output, harvested area, and yields across the different time frames. This underscores the significance of the time component in crop production and yields, alongside the inherent instability within the agricultural sector. The production decomposition analyses conducted by Abu and Adakole (2017) and Ikuemonisan et al. (2020) for some arable crops in Nigeria yielded mixed results, highlighting the importance of area, yields, and their interaction effects on the variability of crop output. A comprehensive examination of the data concerning oil palm fruit is particularly timely, given the critical importance of food security for the Nigerian population. While some researchers (Akpan, 2019; Akpan et al., 2024) have reported a positive compound growth rate in oil palm fruit and its derivatives production in Nigeria, such findings are insufficient and necessitate updates to accurately reflect the current circumstances.

As asserted by Akpan et al 2012b, Akpan et al 2012c and Rani et al., (2021), the fluctuations in agricultural production, arable land, and crop yields are increasingly influenced by a multitude of factors, including rising farm insecurity, banditry, terrorism, conflicts between herdsmen and farmers, erratic rainfall patterns, inadequate irrigation, a surge in natural disasters, and corrupt agricultural policies, among others. It is evident that instability is a prevalent issue within the agricultural systems of numerous developing nations, including Nigeria. Given that agricultural practices remain largely reliant on natural conditions, such as weather, it is reasonable to conclude that farm outputs, the extent of harvested land, and crop yields will continue to experience significant variability over time. Understanding the true nature of growth rates and the levels of instability in the production, harvested area, and yields of oil palm fruit is essential for informing policymakers about effective strategies for policy development. To fulfill this primary aim, the study specifically examines the trends, growth rates, and instability indices of oil palm fruit production, harvested area, and yield in Nigeria. The study also identifies the contributions of yield, area, and interaction effects on the output of oil palm fruit in the country.

MATERIALS AND METHOD

Study Area

The research was carried out in Nigeria, a country rich in agricultural, marine, and forest resources. The extensive availability of both human and natural resources facilitates the cultivation of a diverse array of agricultural products. Over sixty percent of the population is engaged in agricultural activities, which include the production of cassava, groundnuts, oil palm, cotton, rubber, cocoa, rice, maize, aquaculture and artisanal fishing, coconut, livestock, yams, various beans and legumes, sorghum, carrots, and a variety of vegetables, among others.

Data source

The research utilized secondary data obtained from Food and Agricultural Organization (FAO), extended from 1961 to 2022.

Model Specification

Analytical Techniques

The study utilized a compound growth rate to analyze the growth rate in oil palm fruit production, harvested land area, and yield. This approach was selected based on the expectation that the subsector comprising the oil palm fruit output production, harvested land area development, and yield would display exponential growth trends over the years, influenced by diverse intervention policies and programs implemented by the federal and other tiers of governments to enhance the productivity of the subsector.

(a) Measuring a compound growth rate of variables

To estimate the exponential growth rate in oil palm fruit output, harvested area, and yield, equation 1 was specified according to (Udoh and Akpan 2019; Akpan et al., 2024) as thus:

$$log_e\left(Y_o, Y_a, Y_y\right) = \delta_0 + \delta_1 t + U_t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, Y_o, Y_a, Y_y are the output in tons, harvested area in hectares, and yields in tons/ha of oil palm fruit across the years under review. Variable "t" represents the time variable measured in years. An Ordinary Least Squares (OLS) estimation method was employed to generate the parameter δ_1 required for estimating the compound growth rate of each dependent variable. The use of the OLS technique was deemed necessary due to its simplicity and suitability as the estimation method for the specified growth rate model. Given a simple compound growth model as in equation 2, and comparing it with the exponential growth model in equation 1; a compound growth rate is derived as shown in equation 3,

$Y_t = Y_0(1+r)^t$ ……(2)

Comparing with an exponential equation

$r = \left(e^{\delta_1} - 1\right) x\ 100$ Or (antilogb_1 – 1) x 100……(3)

Where r is the measure of a compound growth rate or exponential growth rate for a specified variable expressed in a percentage.

Measuring series instability index

The study used the coefficient of variation (COV), Cuddy-Della Valle index (CDI), and Coppock Instability Index (COI) to assess the instability in output, yield, and harvested land area of oil palm fruit in Nigeria. The estimation details of each of the estimates are provided in the subsequent sections.

Coefficient of Variation (COV)

The Coefficient of Variation (COV) is the most common index used to measure variability/instability in series. It assesses the relative dispersion of data around the mean value. The index is known to overestimate the level of instability in time series which is characterized by long-run trend. It does not explain properly the trend component inherent in a time series. A higher COV indicates higher variability and vice visa.

$$COV = \frac{standard\ deviation}{mean} \quad \ldots\ldots(4)$$

Cuddy-Della Valle index (CDI)

The Cuddy-Della Valle index de-trends the annual series and shows the exact direction of the instability (Cuddy and Valle, 1978). It eliminates the influence of trends in the coefficient of variation (CV) by utilizing the coefficient of determination. Hence, it is a better measure of instability in agricultural production, harvested area, and yields compared to the coefficient of determination (Wasem, 2001). A low value of this index indicates low instability in series and vice-versa. The CDI expression is presented as;

$$CDI = CV\sqrt{1 - R^2} \quad \ldots\ldots(5)$$

Where CV is the coefficient of variation in percent, and R^2 denotes the coefficient of determination obtained from time trend regression on output, harvested area, and yield of oil palm fruit in the country. The levels of instability are categorized within the following ranges: Low instability = (from 0 – 15); Medium instability = (greater than 15, but less than 30) and High instability = (>30). Note that, an adjusted coefficient of determination can also be used.

Coppock Instability Index (COI)

The Coppock (1962) instability index measures instability through log variance method. The higher the Coppock instability index represents a higher instability and vice versa.

$$Coppock\ Instability\ Index\ (COI) = Antilog\left(\sqrt{logV} - 1\right)x\ 100 \quad \ldots\ldots(6)$$

Where,

$$logV = \frac{1}{N-1}\sum(logX_{t+1} - logX_t - M)^2 \quad \ldots\ldots(7)$$

$$M = \frac{1}{N-1}\sum(logX_{t+1} - logX_t) \quad \ldots\ldots(8)$$

Where,
X_t = Time series variable under consideration (log of output/area/yield) in period t.
M = Mean value of the first differences of logarithm
N = Total number of observations
V = Value of Variance log obtained by substituting the values of first differences and M in equation 7.

Oil palm Output Decomposition

The analysis of the growth rate and instability indices do not account for the relative contribution of the harvested area and yields as well as the interaction component to the total production of oil palm fruit. The need for decomposition of the oil palm fruit output is necessary to isolate the relative contributions of the yield and area effects as the interactive effect on the oil palm fruit production. Therefore, the decomposition analysis was carried out to achieve this objective. The initial assumption is as follows:

Production (total effect) = Yield effect + Area effect + Interaction effect

$$P = \frac{A_0 \Delta Y * 100}{\Delta P} + \frac{Y_0 \Delta A * 100}{\Delta P} + \frac{\Delta Y \Delta A * 100}{\Delta P} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Where,
A_0 = Harvested area in the base year
ΔA = Current harvested area minus the base area
Y_0 = Yield in the base year
ΔY= Current yield minus the base yield
ΔP = Current production minus base production
All analyses specified in the study are done for seven (7) periods i.e. 1961–1970, 1971–1980, 1981–1990, 1991–2000, 2001–2010, 2011–2022, and 1961 – 2022.

RESULTS and DISCUSSION

Trend Analyses of output, harvested area, and yield of oil palm fruit in Nigeria

The trend diagrams representing the production, yield, and harvested area of oil palm fruit in Nigeria from 1961 to 2022 are illustrated in Figures 1, 2, and 3, respectively. The production of oil palm fruit displayed a fluctuating pattern, characterized by significant peaks and troughs throughout the examined timeframe. Starting at 6.75 million tons in 1961, the oil palm production saw a decline until 1964, when it reached 6.5 million tons. From 1965 onwards, the trend continued to decline on average, eventually peaking at 6.8 million tons in 1997. After this peak, a gradual increase was noted until 2004, culminating in approximately 8.7 million tons. However, between 2005 and 2015, the country experienced stagnation in oil palm fruit production. In contrast, from 2016 to 2022, the sector experienced a resurgence, characterized by a steady increase in output, which reached a peak of 12.68 million tons in 2022.

The harvested area (ha) for oil palm fruit production exhibited a pattern that closely aligns with the annual production figure This relationship is a result of policies implemented to boost oil palm fruit output, which concurrently affected the area of land harvested. Likewise, the yield trend of oil palm fruit has reacted to various policies aimed at significantly altering the output of this agricultural sub-sector within the country. For example, between 1961 and 1984, the yield stagnated due to multiple factors, including the sector's overall neglect by different government tiers, largely driven by the lucrative returns from crude oil extraction (Eme & Fakayode, 2013). This timeframe coincided with the pre-structural adjustment program (pre-SAP) period, during which agricultural production was not given priority. The import substitution policies of this era were plagued by corruption, lack of sincerity, and substantial instability in the country's macroeconomic environment. Additionally, the palm oil fruit industry faced a shortage of skilled labor, as many young individuals migrated to urban centers or oil-rich regions in pursuit of better prospects (Yakub, 2008; Aloko, 2023). The government also encountered obstacles related to land acquisition, environmental repercussions, and community opposition in executing its plantation initiatives (Ekenta and Ajala, 2017). Moreover, environmental issues, such as the destruction of groves due to development projects, intensified the challenges faced by the industry, leading to decreased palm oil production and adversely affecting local farmers (Okolo et al., 2019). Furthermore, oil palm fruit production faced significant hurdles during this period due to the civil war in the late 1960s and early 1970s, which primarily impacted the regions producing oil palm fruit.

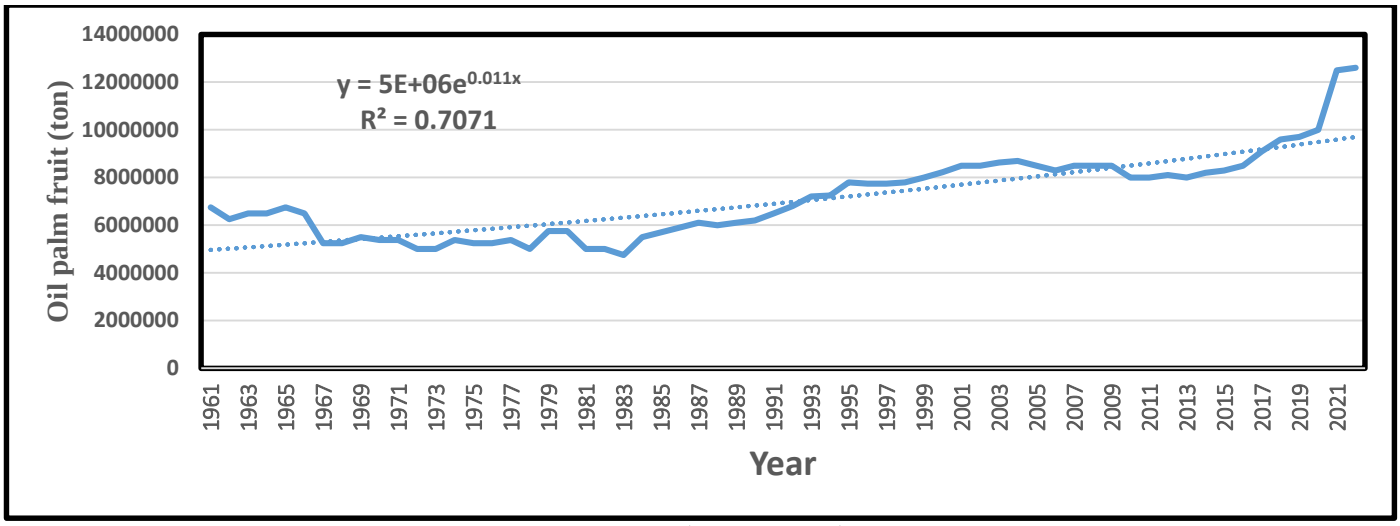

Figure 1: Trend in oil palm fruit production in Nigeria (1961 – 2022)
Şekil 1. Nijerya'da palmiye yağı meyvesi üretimindeki eğilim (1961 – 2022)

As a result, the country lost its significant position in global oil palm fruit production, leading to a scenario where domestic demand exceeded local supply. From 1985 to 2022, the yield of oil palm fruit consistently exceeded 2.5 tons per hectare, although it was characterized by significant fluctuations. This phenomenon can be partially explained by the structural adjustment program policies implemented from 1986 to 1993, which fostered private investment in the sub-sector through the privatization and commercialization of agricultural production and processing. Throughout this period, substantial investments were made in the sector, bolstered by government incentives and the introduction of programs designed to improve financing for small and medium-sized oil palm farmers in the country.

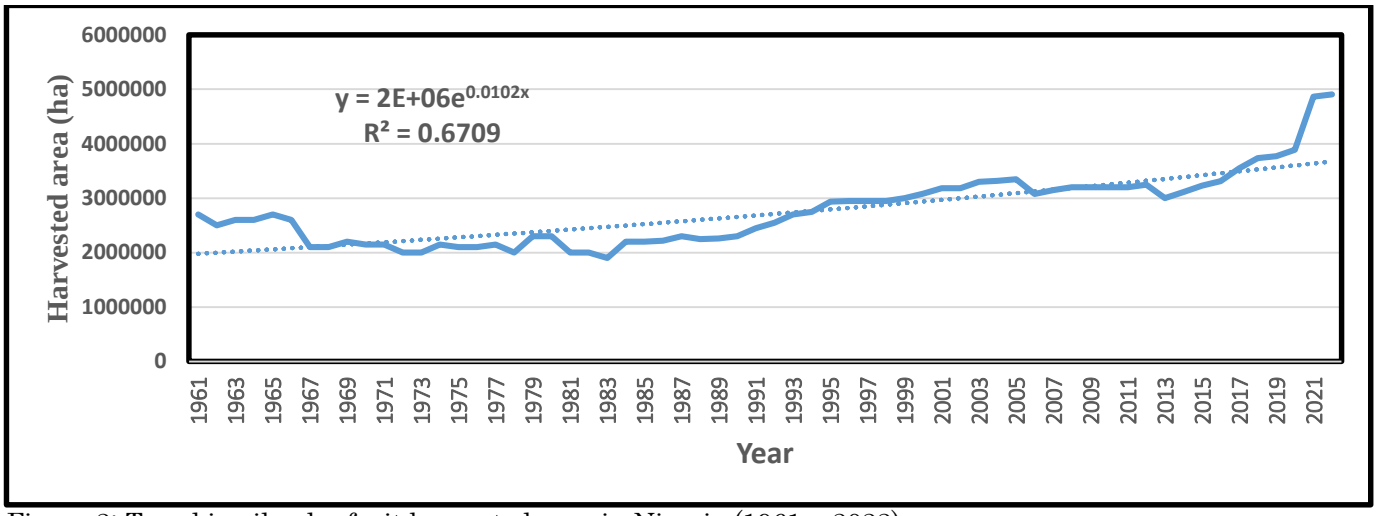

Figure 2: Trend in oil palm fruit harvested area in Nigeria (1961 – 2022)
Şekil 2. Nijerya'da palmiye yağı meyvesi hasat alanındaki eğilim (1961 – 2022)

The Structural Adjustment Programme was implemented alongside various policies that diminished governmental involvement in agricultural production while encouraging privatization (Ojo 1989, Nwosu 1992, Michael and Murat 2019, Shehu et al., 2021). Despite a slight and unsustainable increase in oil palm fruit yields from 1985 to 2022, these yields remained lower than the average outputs recorded in Malaysia and Indonesia.

The Compound growth rate an Instability Index in output, area, and yield of oil palm fruit in Nigeria

The coefficients of variability, compound growth rate (CGR), Cuddy-Della Valle instability index (CDI), and Coppock Instability Index (COI) pertaining to the output, harvested area, and yield of oil palm fruit in Nigeria for the periods 1961–1970, 1971–1980, 1981–1990, 1991–2000, 2001–2010, 2011–2022, and the overall span from 1961 to 2022 are detailed in Tables 1, 2, and 3, respectively.

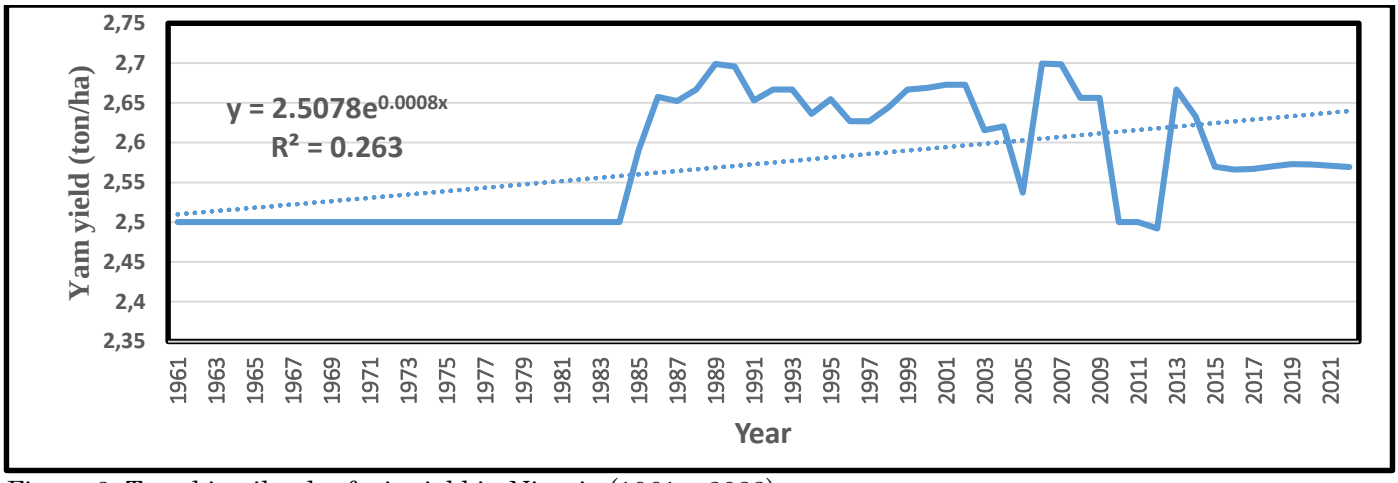

Figure 3: Trend in oil palm fruit yield in Nigeria (1961 – 2022)
Şekil 3. Nijerya'da palmiye yağı meyve verimindeki değişim (1961 – 2022)

Growth rates and Instability Indices in oil palm fruit output (tons) in Nigeria

The analysis indicates that the coefficient of variation (COV) and the compound growth rate (CGR) for oil palm fruit production from 1961 to 1970 were recorded at 10.53% and -2.80%, respectively as presented in Table 1. This decade was characterized by a significant reduction in the output of oil palm fruit within the country. The CGR value indicates that oil palm fruit production declined during this timeframe with an annual decline rate of 2.80%. Furthermore, the coefficient of variation reflects low annual fluctuations in oil palm output during this period, while the negative skewness denotes a persistent trend of marginal growth that was unfavorable.

Table 1: Growth rates and Instability Indices in oil palm fruit output (tons) in Nigeria
Çizelge 1. Nijerya'da palmiye yağı meyvesi üretimindeki büyüme oranları ve İstikrarsızlık Endeksleri (ton)

	1961-1970	1971 - 1980	1981-1990	1991-2000	2001-2010	2011-2022	1961-2022
Mean	6062500	5312500	5625000	7507000	8463200	9383891.80	7134011.32
Std. dev.	638058.04	277951.34	533983.98	549283.37	192608.30	1639091.57	1749761.01
COV (%)	10.5247	5.2320	9.4930	7.3169	2.2758	17.4671	24.5270
Skewness	-0.3012	0.4155	-0.52442	-0.6072	-1.3988	1.1431	0.8669
CGR (%)	-2.8001	0.9656	2.9733	2.3573	-4.3315	4.2165	1.1061
				Instability indices			
CDI	6.2920	4.3011	3.7374	2.3631	1.8734	7.8115	13.4631
COI	40.9601	38.7508	40.5671	39.6581	37.6522	43.2741	46.5696

Note: Prepared by authors.

The CDI and the COI indices demonstrate that the instability in oil palm fruit output from 1961 to 1970 was relatively low in Nigeria, suggesting minimal activity within this agricultural sub-sector during the specified period. Between 1971 and 1980, the coefficient of variation in oil palm fruit output suggests that the production of oil palm fruit during this decade experienced a modest annual positive growth rate of 0.96% per annum. The CDI was determined to be 4.30, with a COI of 38.75. Notably, this period exhibited lower instability in oil palm output compared to the preceding decade.

In a similar vein, the periods from 1981 to 1990 and from 1991 to 2000 recorded CGRs of 2.97% and 2.36%, respectively. The findings indicate that the production of oil palm fruit experienced a significant increase during these time frames in Nigeria. The coefficients of variation rose significantly compared to the earlier decade, reaching 9.49% and 7.32%, respectively. The CDI and COI for the 1981-1990 period were 3.74 and 40.57 respectively, while for 1991-2000, they were 2.36 and 39.75 respectively. However, there was still low instability in output in these periods.

Conversely, the period from 2001 to 2010 experienced a negative CGR of -4.33%, indicating a detrimental relationship between oil palm fruit output and the time during this decade. The finding suggests that the oil palm fruit production declined significantly during this period. The decline coincided with significant encroachments on oil palm estates due to rising urbanization and other economic activities. Additionally, increased volatility in critical macroeconomic indicators, such as inflation and exchange rates, adversely affected the sub-sector's performance. Nevertheless, the CDI and COI values during this period still suggested a significant low instability

within the subsector.

The years 2011 to 2022 marked a pivotal transformation in the oil palm fruit production landscape of Nigeria. During this timeframe, the sector experienced a remarkable compound growth rate (CGR) of 4.14% alongside a coefficient of variation (COV) of 17.52%. The result implies that the oil palm fruit production increased at the rate of 4.14% per annum during this period. Numerous policies were enacted to enhance the production of oil palm fruits. The CBN and the Federal Government introduced various initiatives, including a ban on the importation of oil palm derivatives, the establishment of the Anchored Borrower Program (ABP) Program aimed at providing financial support to oil palm farmers, and the intensified operations of the Agricultural Credit Guarantee Scheme Fund focused on oil palm production, among others. This period was characterized by a notable increase in oil palm fruit production. Despite this progress, the instability indices, as indicated by the values of CDI of 7.81 and the COI of 43.27 remained relatively low, although they showed improvement compared to previous decades. The instability indices during this period reflected a significant enhancement relative to the other decades examined.

Analyzing pooled data from 1961 to 2022 revealed a COV of 24.53% and a CGR of 1.11%. These findings suggest that, on average, oil palm fruit production experienced an increase or positive growth with a low coefficient of variation from 1961 to 2022 in Nigeria. Nonetheless, the CDI of 13.46 and COI of 46.57 reflected low instability in oil palm fruit output and indicated that activities in the sub-sector were relatively minimal.

Growth rates and Instability Indices in harvested area (ha) of oil palm fruit in Nigeria

The distribution pattern of indicators related to the harvested area exhibits similarities to those of output indicators. Notably, the compound growth rate was negative during the periods of 1961 to 1970 and 2001 to 2010. Throughout all examined periods, the coefficient of variation remained below 30.00%. The instability indices, specifically the CDI and COI, were consistently low across all periods under review. This indicates that the variability in harvested land area during the analyzed decades was minimal. For example, the coefficients of variation for the harvested area of oil palm fruit were recorded at 10.53%, 7.37%, 17.52%, and 23.71% for the periods 1961 to 1970, 1991 to 2000, 2011 to 2022, and 1961 to 2022, respectively. This suggests that the fluctuations in harvested land during these specified periods were relatively low. Furthermore, the observed negative skewness values imply that the harvested land areas decreased persistently during the periods of 1961 to 1970, 1981 to 1990, and 1991 to 2000.

Table 2: Growth rates and Instability Indices in oil palm fruit harvested area (ha) in Nigeria
Çizelge 2. Nijerya'da palmiye yağı meyvesi hasat edilen alanda (ha) büyüme oranları ve İstikrarsızlık Endeksleri

	1961-1970	1971 - 1980	1981-1990	1991-2000	2001-2010	2011-2022	1961-2022
Mean	2425000	2125000	2163000	2831800	3215500	3651037.17	2764765.26
Std. dev.	255223.21	111180.54	142520.95	208787.93	83946.08	639664.23	655466.82
COV (%)	10.5247	5.2320	6.5890	7.3730	2.6107	17.5201	23.7079
Skewness	-0.30117	0.41546	-0.80319	-0.7035	0.20244	1.1147	1.0584
CGR (%)	-2.8001	0.9656	1.8978	2.3676	-0.1802	4.1435	1.0252
				Instability indices			
CDI	6.2919	4.3011	3.4872	2.5402	2.5519	8.4042	13.8706
COI	40.9601	38.7508	39.3707	39.6909	37.7629	43.3150	46.0158

Note: Prepared by authors.

The timeframe from 2011 to 2022 exhibited superior performance regarding the compound growth rate of 4.14% and instability indices including CDI of 8.40 and COI of 43.32 compared to the previous periods. The underlying factors contributing to this improvement are akin to those affecting the output variable. An analysis of the pooled data revealed a COV of 23.71% and a CGR of 1.025% for the harvested area spanning from 1961 to 2022. Furthermore, the overall instability index, as assessed by the CDI (13.87) and COI (46.02), indicates a low level of instability in the harvested area throughout this period.

Growth rates and Instability Indices in the Yields (ton/ha) oil palm fruit in Nigeria

The findings indicated that the COV, CGR, and CDI, for oil palm fruit yield were recorded as zero during the two decades from 1961 to 1970 and 1971 to 1980. This phenomenon can be attributed to the constancy of yield (ton/ha) throughout these periods. Such stability suggests that both the output and the harvested area of land remained unchanged during these times. These decades coincided with a phase of significant oil exploitation driven by heightened global demand, civil war, and the onset of Dutch disease in Nigeria in 1977, among other factors (Otaha, 2012). During this time, the agricultural sector was largely neglected as a source of foreign exchange by all levels of government, which instead became heavily dependent on the crude oil industry. This era is frequently marked by substantial government investment in agriculture through import substitution policies, which were marred by

corruption and a lack of genuine commitment. Consequently, the agricultural sector faced a decline in private sector investment due to an obnoxious policy mandating government participation in agricultural investments within the country.

Between 1981 and 1990, the oil palm fruit sub-sector yield experienced a revival, characterized by a coefficient of COV of 3.38% and a positive compound growth rate (CGR) of 1.06%. Despite the yield variation being minimal and exhibiting a negative skew, it experienced a marginal positive increase during this period in Nigeria. This period aligned with the introduction of several intervention policies, including the Economic Stabilization Act of 1985 and the Structural Adjustment Program policies of 1986, aimed at mitigating economic volatility and fostering private investment in agriculture among others. Nevertheless, the compound growth rate for oil palm fruit yield during this time was not particularly remarkable. The low instability indices, with a CDI of 1.13 and COI of 38.06, indicate that despite the ambitious policies implemented by the federal government, the oil palm fruit yield instability remained persistently low, reflecting limited activity and slow advancement in the sub-sector throughout this period.

Table 3: Growth rates and Instability Indices in oil palm fruit yields (ton/ha) in Nigeria
Çizelge 3. Nijerya'da palmiye yağı meyve verimlerinde (ton/ha) büyüme oranları ve istikrarsızlık endeksleri

	1961-1970	1971 - 1980	1981-1990	1991-2000	2001-2010	2011-2022	1961-2022
Mean	2.5000	2.5000	2.5962	2.6511	2.6329	2.5709	2.5751
Std. dev.	0.0000	0.0000	0.0878	0.0166	0.0669	0.0469	0.0752
COV (%)	0.0000	0.0000	3.3819	0.6262	2.5409	1.8243	2.9203
Skewness	0.0000	0.0000	-0.1269	-0.3721	-0.9828	0.2520	0.2582
CGR (%)	0.0000	0.0000	1.0555	0.0062	-0.2537	0.0752	0.0828
				Instability indices			
CDI	0.0000	0.0000	1.1289	0.6254	2.4263	1.8099	2.5148
COI	36.7918	36.7918	38.0606	37.0229	37.7498	37.4689	37.8786

Note: Prepared by authors.

In contrast, the subsequent decades of 1991 to 2000 and 2001 to 2010 marked a return to a phase of significant decline in productivity within the sub-sector. For these periods, the COV was recorded at 0.62% and 2.42%, respectively, indicating marginal variability. The Compound Growth Rates of 0.0062% and -0.25% for the two respective periods underscore the declining yields within the sector. Additionally, the CDI and COI values of 0.63 and 37.02 for the period from 1991 to 2000, and 2.43 and 37.75 for the period from 2001 to 2010, indicate a notable low instability in yields and a continuous absence of significant activity in the sub-sector. This era of oil palm fruit production was characterized by extensive encroachment on oil palm estates due to rising urbanization and other economic activities necessitating land expansion. Additionally, as noted by PIND (2011) and Shehu et al. (2021), factors such as aging plantations, deteriorating infrastructure, and high labor costs significantly contributed to the poor performance of the sub-sector during these decades.

The swift response of the federal government to rescue the oil palm sub-sector's fortunes from 2011 to 2022 resulted in only a modest improvement in productivity. Notably, initiatives such as the anchored borrowers' program and the Central Bank of Nigeria's (CBN) financial allocations to the sub-sector in 2015 contributed to a slight increase in productivity levels. During this timeframe, the COV and CGR were recorded at 1.82% and 0.075%, respectively. However, this marginal improvement was insignificant as evidenced by the relatively low CDI of 1.81 and COI of 37.47, which still indicated limited activity and low instability within the subsector.

The analysis of pooled data (1961 – 2022) revealed overall figures for COV, CGR, CDI, and COI at 2.92%, 0.083%, 2.52 and 37.88, respectively. These findings suggest that the annual growth rate of the oil palm fruit yield was merely 0.083%, a figure that is insufficient to satisfy domestic annual demand or to enable competitive positioning of the country in the global market. However, during the same time frame, Nigeria's oil palm fruit yield compound growth rate outperformed Côte d'Ivoire (-0.768%) and Ghana (-0.00283%), but fell significantly short of Indonesia (0.508%), Malaysia (0.583%), and Thailand (2.313%) (FAO, 2024).

Decomposition of Output of oil palm fruit in Nigeria

The analysis of the decomposition of oil palm fruit output is presented in Table 4. The findings showed the various components contributing to the total effect, which encompasses the area effect, yield effect, and interaction effect. It was observed that during the periods of 1961 to 1970 and 1971 to 1980, the entirety of the total effect on oil palm fruit output in the country was solely due to the area effect. These periods were marked by significant neglect of the subsector, civil war, and the phenomenon of Dutch disease, which arose from the sudden influx of revenue from crude oil extraction (Otaha, 2012). In contrast, the decade from 1981 to 1990 saw approximately 62.50% of the total

effect attributed to the area effect, 32.61% to the yield effect, and 4.89% to the interaction effect. This era coincided with the Structural Adjustment Program (SAP), which fostered private investment in agriculture. During this time, there was a notable encouragement for farmers and private investors to adopt innovative practices within the subsector.

Table 4. Percentage decompositions of area, yield, and their interaction effects on oil palm fruit production in Nigeria

Çizelge 4. Nijerya'da yağ palmiyesi meyvesi üretimi üzerindeki alan, verim ve bunların etkileşim etkilerinin yüzdesel ayrışımı

Components	1961-1970	1971 - 1980	1981-1990	1991-2000	2001-2010	2011-2022	1961-2022
Area effect	100.00	100.00	62.50	97.18	-10.69	92.62	94.20
Yield effect	0.00	0.00	32.61	2.24	110.00	4.81	3.19
Interaction effect	0.00	0.00	4.89	0.58	0.69	2.57	2.61
Total effect	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Source: computed by authors.

In the subsequent period from 1991 to 2000, the area effect accounted for over 97.18% of oil palm fruit production, while yield and interaction effects contributed 2.25% and 0.58%, respectively. However, in the decade from 2001 to 2010, the yield effect remarkably contributed 110.00% to the output, while area effect exhibited a negative contribution of -10.69%, with the interaction effect contributing 0.69%. During this period, the yield effect compensated for the negative effects of the area of harvested land (-10.69%) and the marginal contribution of the interaction effect. This period's output can be attributed to the expansion of private oil palm estates and the revitalization of existing estates. Additionally, numerous State governments in the South-South region implemented various programs aimed at enhancing oil palm fruit production, integrating these initiatives into their agricultural policy frameworks. The influence of area effect on output was revitalized during the period from 2011 to 2022. In this period, the area effect contributed about 92.62%, while yield and interaction effects were responsible for 4.81% and 2.57% respectively. The introduction of the Anchor Borrower Programme (ABP) in 2015, along with the initiatives of the Nigerian Institute for Oil Palm Research (NIFOR), may have played a significant role in this outcome. A similar observation was noted in the combined data spanning from 1961 to 2022. The contributions of area, yield and their interaction effects accounted for 94.20%, 3.19%, and 2.61% respectively of the overall output effect. Overall, these results indicate that area effect is a key or major factor in explaining the total variation in oil palm fruit production within Nigeria. The results differ from those observed in Malaysia, Indonesia, and Thailand, where the growth of oil palm fruit production is driven by increased yield due to the adoption of advanced technologies rather than expanding land area (FAO, 2024; Hassan et al., 2024).

Summary and Recommendations

The study examined the trends, growth rates, and instability index of oil palm fruit production, harvested area, and yield in Nigeria from 1961 to 2022. The decomposition of the total effect of output into area effect, yield effect and interaction effect were carried out. The analysis was segmented into distinct sub-periods: 1961–1970, 1971–1980, 1981–1990, 1991–2000, 2001–2010, 2011–2022, and the overall period from 1961 to 2022. The trend analysis revealed fluctuations in the output, harvested area, and yield of oil palm fruit across all sub-periods as well as in the aggregated data. The CGR for oil palm output and harvested land area were negative during the periods of 1961–1970 and 2001–2010, while positive growth rates were observed in the remaining sub-periods and the pooled data. The CGR for yield was recorded as zero for the periods of 1961 to 1970 and 1971 to 1980, negative during 2001–2010, and a marginal 0.08% for the overall period from 1961 to 2022. These findings indicate that only a slight positive compound growth rate (below 1.50%) was evident in the output, harvested area, and yield of oil palm fruit in Nigeria from 1961 to 2022. The coefficient of variation across all specified periods was relatively low (under 30.00%), indicating limited variability in the output, harvested area, and yield of oil palm fruit within the country. Furthermore, the CDI and COI for the pooled data concerning output (13.46 and 46.57, respectively), harvested area (13.87 and 46.02, respectively), and yield (2.52 and 37.88, respectively) were also low. Similarly low values were observed for each of the other specified periods regarding output, harvested area, and yield. The result suggests that there was a lack of significant activity in the sub-sector from 1961 to 2022.

Decomposition analyses indicate that during the periods of 1961 to 1970 and 1971 to 1980, the entirety of the growth in output was exclusively due to area effects. This finding suggests that the increases in output during these periods were derived solely from area effect components, with both yield and interaction effects contributing nothing to the total effect. In contrast, the periods from 1981 to 1990 and 1991 to 2000 showed that area effects

accounted for 62.50% and 97.18% of the total growth effect, respectively. During these same periods, yield effects contributed only 32.61% and 2.25% to the total effect, while interaction effects contributed 4.89% and 0.58%, respectively. This indicates that area effects were the primary drivers of output growth during the analyzed periods. In the decade from 2001 to 2010, the area effect was recorded at -10.69%, with interaction effects at 0.69%, and yield effect at a substantial 110.00%, effectively offsetting the negative contribution of the area effect. The periods from 2011 to 2022 and the overall period from 1961 to 2022 further underscored the significant influence of area effects on total output growth, contributing 92.62% and 94.19% to the total effect, respectively, while yield effects accounted for 4.81% and 3.19% respectively.

In conclusion, the oil palm fruit sub-sector witnessed limited annual activities within the time frame under review. This observation implies that the fluctuations in output, harvested area, and yield were relatively low. Specifically, the coefficient of variation for oil palm output, harvested area, and yield remains below 30.00%. Furthermore, the average compound growth rate (CGR) for these parameters across all specified periods was less than 5.00% per annum. Such a growth rate magnitude implies that oil palm fruit production currently lacks the necessary impetus to satisfy domestic demand and significantly enhance export supply. The Cuddy-Della Valle instability index for output, harvested area, and yield is recorded at less than 15.00 units, indicating that the activities within this sub-sector were insufficient to instigate substantial change. Likewise, the COI remains low for output, harvested area, and yield throughout all examined periods. In light of these findings, it is crucial to implement additional programs such as the small grower scheme, oil palm farmers' cooperative, off-takers scheme, and the strengthening of the marketing chain within the sub-sector to stimulate increased activity, production, and yield. To further enhance, oil palm production and yield, the sub-sector must also adopt improved technologies in addition to empowering oil palm farmers through easy access to quality seeds, financial resources, and land. Only in this case can production increases be achieved through high yield in Nigeria as is the case in Malaysia, Indonesia, and Thailand.

Conflict of Interest Declaration

The authors of the article declare that they do not have any conflict of interest.

Contribution of the Authors

Authors contributed equally

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A Research on Growth and Meat Quality Parameters and Economic Conversion Rates of Different Feeding Regimes Applied to Cultured Large Rainbow Trout (*Oncorhynchus mykiss*) in Net Cages in the Black Sea

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ABSTRACT
This study aimed to determine the effects of different feeding regimes applied to large commercial rainbow trout (*Oncorhynchus mykiss*) with an initial weight of 1045.12±43.51 g in the Black Sea on growth, meat quality performances, and economic conversion rates. The study was conducted in a commercial fish farm in the Sinop district of the Southern Black Sea (Turkey). Fish were grouped according to three different feeding regimes (R group fed according to feeding table (1% fish weight); D group fed 1 day/fasted 1 day; E group fed 6 days/fasted 1 day) and fed twice a day for five months. At the end of the 150-day study, it was found that the R and E groups had the best growth parameters (weight gain, specific growth rate, and thermal growth rate) and these results were statistically different from the D group ($p<0.05$). The best feed conversion rates (FCR) were determined to be in the E (1.57±0.04) and R (1.59±0.01) groups. Depending on the FCR of the groups, the economic conversion rate (ECR) of the E group was better than the other groups. In terms of meat quality, the biochemical, fatty acid, and amino acid compositions of the large rainbow trout fillets commercially grown in the Black Sea were found to be of good quality, nutritious, and safe for human consumption.

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Karadeniz'de Ağ Kafeslerde Yetiştirilen Büyük Gökkuşağı Alabalıklarına (*Oncorhynchus mykiss*) Uygulanan Farklı Besleme Rejimlerinin Büyüme ve Et Kalite Parametreleri ile Ekonomik Dönüşüm Oranları Üzerine Bir Araştırma

ÖZET
Bu çalışmada, Karadeniz'de ticari olarak üretilen ve başlangıç ağırlıkları 1045.12±43.51 g olan gökkuşağı alabalıklarına (*Oncorhynchus mykiss*) uygulanan farklı besleme rejimlerinin büyüme, et kalitesi performansları ve ekonomik dönüşüm oranları üzerindeki etkilerinin belirlenmesi amaçlamıştır. Çalışma, Güney Karadeniz'in (Türkiye) Sinop ilçesindeki ticari bir balık çiftliğinde yürütülmüştür. Balıklar üç farklı besleme rejimine göre gruplandırılmış (besleme tablosuna göre beslenen R grubu (% 1 balık ağırlığı); 1 gün beslenen/1 gün aç bırakılan D grubu; 6 gün beslenen/1 gün aç bırakılan E grubu) ve beş ay boyunca günde iki kez beslenmiştir. 150 günlük çalışma sonunda, R ve E grupları en iyi büyüme parametrelerine (ağırlık artışı, spesifik büyüme oranı ve termal büyüme oranı) sahip olduğu ve bu sonuçların istatistiksel olarak D grubundan farklı olduğu bulunmuştur ($p<,05$). En iyi yem dönüşüm oranlarının (YDO) E (1,57±0,04) ve R (1,59±0,01) gruplarının olduğu belirlenmiştir. Grupların YDO'larına bağlı olarak da E grubunun ekonomik dönüşüm oranının (EDO) diğer gruplardan daha iyi bulunmuştur. Et kalitesi bakımından ise, Karadeniz'de ticari olarak yetiştirilen büyük gökkuşağı alabalığı filetolarının biyokimyasal, yağ asidi ve aminoasit kompozisyonları iyi kalitede, besleyici ve insan tüketimi için güvenli bulunmuştur.

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INTRODUCTION

Nutrition is the most crucial concern in aquaculture, similar to other culture systems. Because nutrition, which determines all vital activities of every living thing, is effective in the production period and costs as well as the biological activity of the living thing. Therefore, feeding activities are important for the blue economic sustainability of cultural systems. Fish nutrition aims to develop production and feeding procedures that are both blue economically viable and environmentally friendly, with minimal feed and total consumption costs. They are still researching fish nutrition studies nowadays to ensure blue economic sustainability by determining suitable feeding models for fish development performance (Martínez-Llorens et al. 2007; Silva et al. 2007; Eroldoğan et al. 2008; Ofor & Ukpabi 2013; Adaklı & Taşbozan 2015; Nagar & Patidar 2015; Hvas et al. 2022).

In natural habitats, fish can starve for short or long periods under unsuitable environmental conditions (Dempster et al. 2016; Stehfest et al. 2017; Wade et al. 2019). In culture conditions, this situation occurs when feeding cannot be done under adverse environmental conditions, before harvest, or during the transfer processes of fish (Remen et al. 2014; Hvas et al. 2017; Hvas et al. 2021). Fish have been observed to exhibit compensatory growth after being subjected to complete or restricted starvation (Ali et al. 2003). Even though changes in body biochemistry during starvation (Adaklı & Taşbozan 2015; Dong et al. 2017; Ashouri et al. 2020; Altaf et al. 2021), fish have been shown to have high growth efficiency (Ali et al. 2003). Many starvation treatments were administered to fish in various investigations, and their growth performance, infection risks, flesh quality characteristics, stock density in the transporting and stress enzymes were assessed (Känkänen et al. 2009; Peres et al. 2011; Stefansson et al. 2009; Pérez-Jiménez et al. 2012; Dong et al. 2017; Ashouri et al. 2020; Torfi Mozanzadeh et al. 2021; Sakyi et al. 2020; Tamadoni et al. 2020; Yanar et al. 2020; Cai et al. 2021; Altaf et al. 2021; Hasanpour et al. 2021; Hvas et al. 2021; Hvas et al. 2022; Messina et al. 2023; Xavier et al. 2023). Rainbow trout (*Oncorhynchus mykiss*) is an inland water fish that is farmed all over the world and is produced (191130 t, Anonymous 2023). Even though it has been produced for a long time in Türkiye, it is now sold on the worldwide market as "Turkish salmon" and competes with Atlantic salmon (*Salmo salar*) in terms of both meat quality and price. So much so that, for 10 years, producers produced solely large rainbow trout / Turkish salmon in net cages in the Black Sea, with (45454 tons of production in 2022), 86.7 % of this production exported (Anonymous 2023).

As long as aquaculture development continues, companies use feed most efficiently reduce to feed and overall consumption expenditures in their operations, as mentioned above. Considering sustainable blue economics and fish growth performance, studies on starvation, feed restriction, compensation feeding, and different feeding regimes are still gaining importance. For the first time, three different feeding regimes were administered to large rainbow trout in this study, which was conducted in collaboration with large rainbow trout producers in the Black Sea. The study's objective is to determine how three different feeding regimens for large rainbow trout affect the fish's growth performance, biochemical, fatty acid, and amino acid compositions, and rates of economic conversion.

MATERIAL and METOD

The large rainbow trout (*Oncorhynchus mykiss*, 1045.12±43.51 g body mass) was obtained from the Altınkaya Dam Lake in Samsun-Bafra. Fish (SAGUN Aqua) were produced in Sinop, Turkey's southern Black Sea (Demirciköy site; 35°10′55,92″E–41°54′44,15″N; 35°11′03,42″E–41°54′33,92″N; 35°10′60,00″E–41°54′32,52″N; 35°10′52,50″E–41°54′) in nine open sea cages (⌀=30 m) under natural photoperiod. The study was carried out between 15 December 2018 and 15 May in a sea cage in 2019.

Water temperature, salinity, and oxygen were measured using the HANNA (HI9829) multiparameter device and during the study, water temperature, salinity, and O2 value were 11.02±0.94 ºC, 16.86±0.74 ppt, and 11.76±0.40 mg L-1. Each cage contained approximately 16000 fish that were fed commercial diets (4.5–6 mm pellets, BioMar-SAGUN, Aydın-Turkey). The diet manufacturer uses a closed diet formula for large rainbow trout. (In pursuant to the manufacturer's diet label, the biochemical composition of the diet is shown in Table 1). The feeds used in the study were in two different sizes, and the fish were given 4.5 mm feed in the dam lakes and 6 mm in the sea. The biochemical, amino, and fatty acid compositions of the diets are given in Table 2.

The feeding regimes of the fish were determined by the operating protocols. Three different feeding regimes were

tested on the fish. According to this, three treatment groups were fed two times a day: according to the feeding table, everyday feeding (1 % of fish weight) (R), 1 day feeding/1 day fasted (D), 6 days feeding/1 day fasted (E).

Table 1. Biochemical compositions of the diets used in the study
Çizelge 1. Çalışmada kullanılan yemlerin biyokimyasal kompozisyonları

Biochemical composition	*Initial diet (4.5 mm*)*	*Final diet (6.0 mm**)*
Crude Protein, %	42.60	41.80
Crude Fat, %	26.80	28.50
Crude Ash %	7.10	5.90
Crude cellulose, %	2.30	5.90
Phosphorus, %	1.07	0.90
Calcium, %	1.18	0.90
Sodium, %	0.28	0.22
Astaxanthin, mg/kg	50.00	50.00
Copper (II) sulfate pentahydrate, mg/kg	1.10	1.00
Manganese (II) sulfate monohydrate, mg/kg	9.00	8.00
Zinc (II) sulfate monohydrate, mg/kg	57.00	50.00
Calcium iodate, mg/kg	1.40	1.20
*Antioxidant (BHA)****	88.00	84.00

Raw materials:
*Fish Meal, fish oil, chicken meal, sunflower meal, guar protein, wheat, wheat flour, blood meal, hydrolyzed feather meal, soy concentrate, soy meal (made from genetically modified soy), astaxanthin, mineral substance
**Fish meal, fish oil, pea proteins, sunflower meal, blood meal, wheat, wheat flour, guar protein, chicken meal, wheat gluten, soy flour (genetically modified soy), astaxanthin, mineral substance
*** BHA Butylated hydroxyanisole

Growth Performance, Chemical Analysis, Amino and Fatty Acids Analysis

Farming personnel killed fish with a high dose of anesthetic (MS-222, 25–50 mg L^{-1}, Ortuno et al. 2002) and randomly sampled 30 fish at the start and end of the study. Therefore, no ethical approval is required for this manuscript. Fish and feed samples taken from the farm were transported to the Faculty of Fisheries and Aquaculture's Scientific and Technological Research Center under cold chain conditions (University of Sinop). For the length measurement of fish, a 1 mm precision height measurement ruler, fish, internal organs, etc. weight Kern brand balance with 0.1g precision was use According to Jobling (2003), Abdel-Tawwab et al. (2015), and Lu et al. (2020), growth and feed efficiency parameters, and biometric data were calculated:

SGR: specific growth rate (%) = ((ln BW_f – ln BW_i) / t) × 100, where t is experimental period = 150 days;
WG: weight gain (%) = ((BW_f – BW_i)/BW_i) × 100;
SR: survival (%) = number of fish in each group remaining on day 150/initial number of fish) × 100;
TGR: Thermal growth rate=((BW_f)$^{1/3}$-(BW_i)$^{1/3}$)/((Temperature x experimental days))
FCR: feed conversion ratio = (feed intake (g) / weight gain (g));
HSI: hepatosomatic index (%) = (liver weight (g) / BW_f (g)) × 100;
VSI: viscerosomatic index (%) = (visceral weight (g) / BW_f (g)) × 100;
K: Fulton's condition factor = (BW_f (g)/ standard length (cm)^3) × 100;

in which BW_i and BW_f are initial body weight and final body weight, respectively.

Economic indices were calculated using formulas reported by Martínez-Llorens et al. (2007):

Economic conversion ratio (€ kg^{-1}) (ECR)=feed offered (kg) × feed cost (€ kg^{-1})/Weight gain (kg)

Economic profit index (€ $fish^{-1}$) (EPI)=final weight (kg $fish^{-1}$) × fish sale price (€ kg^{-1})–ECR (€ kg $fish^{-1}$) × weight increase (kg).

The economic conversion rate and economic profit ındex calculated used a price of 2 euros per kilogram of feed and an 8 euros per kilogram pricing for fish sales.

Fish were filleted into boneless fillets in the laboratory after their internal organs and skins were separated, and they were maintained in a deep freezer (WiseCryo/WUFD500 80 °C) until analysis. Association of Official Agricultural Chemists (AOAC 1995) approved techniques were used for the biochemical analyses of the diet and fillet samples. All biochemical analyses in fillets were done in triplicate and on a wet basis. Amino acid and fatty acid analyses of diets and fillets were made by the Sinop University Scientific Research and Application Center (SUBITAM).

Table 2. The biochemical (%), amino acid (g 100g^{-1} protein) and fatty acid compositions of the diets
Çizelge 2. Yemlerin biyokimyasal (%), amino asit (g 100g^{-1} protein*) ve yağ asidi (%) kompozisyonları*

	Initial diet	***Final diet***		***Initial diet***	***Final diet***
Crude Protein	46.28±0.22^{b}	41.71±0.56^{a}	*C12:0*	0.11±0.01^{a}	0.08±0.01^{a}
Crude Fat	19.47±0.05^{a}	23.96±0.68^{b}	*C13:0*	0.02±0.01^{a}	0.02±0.01^{a}
Crude Ash	8.33±0.17^{a}	9.57±0.33^{b}	*C14:0*	3.56±0.02^{a}	3.78±0.01^{b}
Dry Matter	91.36±0.05^{a}	92.64±0.24^{b}	*C15:0*	0.34±0.01^{a}	0.38±0.01^{a}
Alanine	2.43±0.01^{b}	1.97±0.01^{a}	*C16:0*	11.16±0.08^{b}	10.61±0.09^{a}
Aspartic acid	4.95±0.01^{b}	4.04±0.01^{a}	*C17:0*	0.34±0.01^{a}	0.34±0.01^{a}
Methionine	0.91±0.01^{a}	1.03±0.01^{b}	*C18:0*	4.65±0.07^{b}	3.96±0.01^{a}
Glutamic acid	5.89±0.01^{b}	5.10±0.01^{a}	*C20:0*	0.88±0.01^{a}	1.05±0.01^{b}
Phenilalanine	1.85±0.01^{b}	1.65±0.01^{a}	*C21:0*	0.04±0.01^{a}	0.02±0.01^{a}
Lysine	3.79±0.01^{b}	2.67±0.01^{a}	*C22:0*	0.42±0.01^{a}	1.12±0.01^{b}
Histidine	0.96±0.01^{b}	0.91±0.01^{a}	*C23:0*	0.07±0.01^{a}	0.07±0.01^{a}
Tyrosine	1.07±0.04^{b}	0.91±0.01^{a}	*C24:0*	0.40±0.01^{a}	0.48±0.01^{b}
Glysine	2.34±0.01^{b}	1.98±0.01^{a}	*C14:1*	0.18±0.01^{a}	0.19±0.01^{a}
Valine	1.95±0.01^{b}	1.58±0.01^{a}	*C15:1*	0.05±0.01^{a}	0.06±0.01^{a}
Leucine	2.97±0.01^{b}	2.69±0.02^{a}	*C16:1*	0.29±0.01^{a}	0.33±0.01^{a}
Isoleucine	1.26±0.01^{b}	1.03±0.01^{a}	*C17:1*	0.27±0.01^{a}	0.32±0.01^{a}
Threonine	1.79±0.01^{b}	1.52±0.01^{a}	*C18:1n-9c*	25.16±0.12^{b}	23.91±0.11^{a}
Serine	2.40±0.02^{b}	1.95±0.01^{a}	*C18:1n-9t*	4.15±0.02^{b}	2.83±0.57^{a}
Proline	2.32±0.01^{b}	2.00±0.01^{a}	*C20:1n-9c*	5.85±0.01^{a}	6.26±0.05^{b}
Ornitine	0.02±0.01^{a}	0.02±0.01a	*C22:1n-9*	4.93±0.01^{a}	5.32±0.04^{b}
Cystine	0.19±0.01^{a}	0.17±0.01^{a}	*C24:1*	1.01±0.03^{a}	1.18±0.02^{a}
Arginine	2.69±0.01^{b}	2.24±0.01a	*C18:2n-6t*	0.29±0.01^{a}	0.35±0.01^{b}
ΣEAA	18.15±0.03^{b}	15.30±0.02^{a}	*C18:2n-6c*	13.84±0.06^{b}	13.45±0.09^{a}
ΣSEAA	3.65±0.01^{b}	3.15±0.01^{a}	*C18:3n-3*	7.59±0.01^{a}	8.71±0.09^{b}
ΣNEAA	21.60±0.02^{b}	18.12±0.02^{a}	*C18:3n-6*	0.25±0.01^{a}	0.28±0.01^{b}
∑SFA=C12:0+C13:0+C14:0+C15:0+C16:0+C17:0+C18:0+ C20:0+C21:0+C22:0+C23:0+C24:0			*C20:2*	1.99±0.01^{a}	2.25±0.02^{b}
			C20:3n-3	0.01±0.01^{a}	0.02±0.01^{a}
∑MUFA=C14:1+C15:1+C16:1+C17:1+C18:1n-9c+C18:1n-9t+C20:1n-9c+C22:1n-9+C24:1			*C20:3n-6*	0.44±0.01^{a}	0.51±0.01^{b}
			C20:4n:6	0.53±0.01^{a}	0.62±0.03^{b}
∑PUFA=C18:2n-6t+C18:2n-6c+C18:3n-3+C18:3n-6+C20:2+C22:2+C20:3n-6+C20:5n-3+C20:4n-6+C22:6n-3			*C20:5n-3*	4.85±0.02^{a}	5.05±0.01^{b}
			C22:2	0.21±0.01^{a}	0.25±0.01^{a}
Essential Amino Acids (EAA)= Histidine + Lysine+ Phenylalanine+ Methitonine+ Threonine+ Leucine+ Isoleucine+Valine+ Arginine			*C22:6n-3*	6.09±0.01^{a}	6.17±0.06^{b}
			ΣSFA	21.97±0.18^{a}	21.91±0.12^{a}
Semi-Essential Amino Acids (SEAA)= Histidine + Arginine			*ΣMUFA*	41.88±0.13^{b}	40.40±0.47^{a}
			ΣPUFA	36.10±0.08^{a}	37.65±0.38^{b}
Non-Essential Amino Acids (NEAA)= Alanine+ Aspartic acit+ Glutamic acit+ Tyrosine+ Glicine+ Serine+ Proline					

Each value means mean±standard error. Values in rows marked with different letters are significantly different ($p < 0.05$).

The fillet and diet samples were converted to methyl esters by derivatization of fat samples in a gas chromatography device (Thermo Scientific Trace 1310) for fatty acid analyses. For this purpose, 0.25 g of the extracted oil was removed, and 4 ml of heptane and 0.4 ml of 2 N KOH were added. The mixture was stirred in a vortex for 2 min and then centrifuged at 5000 rpm for 5 min. After centrifugation, 1.5–2 ml of the heptane phase was collected and transferred to glass tubes for GC/MS analysis. The injection of samples into the device was carried out with an automatic sampler (Autosampler AI 1310). Samples were analyzed by Thermo Scientific ISQ LT model GC/MS. For this analysis, Trace Gold TG-WaxMS capillary column (Thermo Scientific code: 26088-1540) with a film thickness of 0.25 µm and 60 m length was used. The injection block temperature was set to 240 °C, and the column temperature was increased from 100 °C to 240 °C in the temperature program. Helium gas (1ml/min) was used as a carrier gas at constant flow, and a 1:20 split ratio was applied. The MS unit (ISQ LT) was used in electron ionization mode. Fatty acids were defined by comparing the standard FAME mixture of 37 components based on the arrival times. Once fatty acid compositions were determined, total fatty acids and fatty acid quality assessments were calculated according to Ulbricht and Southgate, (1991) and Santos-Silva et al. (2002).

Atherogenicity Index (AI)= [(C12:0+(4 x C14:0)+C16:0)] / (MUFA+Omega-3+Omega-6);
Thrombogenicity Index (TI)=(C14:0+C16:0+C18:0)/[(0.5 x MUFA)+(0.5xOmega-6) +(3xOmega-3)+(Omega-3/Omega-6)];
Hypocholesterolemic/Hypercholesterolemic ratio ratio (HH)= (C18:1n-9+C18:2n-6+C18:3n-3+C20:4n-6+C20:5n-3+C22:6n-3) /(C14:0+C16:0)

Amino acid analyses of diet and fish fillets were performed using the Jasem LC-MS/MS amino acid assay kit. The concentration of the target amino acids was measured using the electrospray ionization (ESI)-based multiple reaction monitoring (MRM) mode. 0.5 g sample was taken into a glass vial with a screw cap and 4 ml of reagent-2 was added, and then, a hydrolysis reaction was performed at 110 °C for 24 hr. The hydrolysate was centrifuged for 5 min at 4000 rpm when it reached room temperature. Then, 100 µl of the supernatant was transferred to a vial and completed to 1 ml with distilled water. This dilution procedure was repeated to yield 800-fold diluted hydrolysate of the sample. 50 µl of the diluted hydrolysate was transferred to a sample vial and 50 µl of internal standard mixture with isotope-labeled and 700 µl of reagent-1 was added, respectively, and then, the mixture was vortexed for 5 s. All samples were prepared according to the above procedures and injected into the LC-MS/MS system, where the amounts of amino acids were read. According to the obtained amino acid data, total amino acids and the quality of amino acids were calculated according to Li et al. (2009).

Color Analysis of Fish Skin and Meat

white plate as a reference before each measurement (standard values for white plate L*=91.97; a*=−1.4; b*=2.0, Standard C2-22326). L*, a*, and b* values represent lightness, redness, and yellowness, respectively. Color measurement of fillets of fish groups was done from three locations: 1st location: between the behind of the operculum; 2nd location: under the dorsal fin; and 3rd location: front of the caudal fin. The hue is a descriptor of what is generally understood to be the true color, and the chroma (C*) is the intensity or degree of saturation of the color. The angle of Hue and C* was calculated using a* and b* values (Hernández et al. 2009):

$C^*=\sqrt{(a^{*2}+b^{*2})}$ and $Hue=\arctan(b^*/a^*)$.

Statistical Analysis

The data were reported as average values with standard error (average±SE). The IBM SPSS 21 statistics package application was used for statistical analysis. The significance of the differences in the data was determined using one-way ANOVA, followed by Tukey's procedure for multiple comparisons.

RESULTS

Growth Performance, Economic Parameters, Biochemical Composition and Biometric Index of Large Rainbow Trout

The growth parameters, biometric indices, and biochemical composition of fish in the study are provided in Table 3; Table 4 lists the fish's feed conversion and economic conversion rates. After the study, the R and E groups had the best growth outcomes [weight gain (p= .043), specific growth rate (p= .048), and thermal growth rates (p= .438), which were statistically distinct from the D group. Fish carcass yield (CY) was in the following order: R> D> E and the CY of group E was statistically different (p= .037).

In comparison to the initial study, the crude protein (CP) values of the R and E group fillets increased, whereas those of the D group fillets fell. Additionally, group D fillet had the lowest level of crude protein at the end of the study (p= .026). After 150 days, all group fillet's crude lipid (CL) ratios of fillets, with the R and E group fillets having the highest CL values and the CL values of E group fillets were significantly different (p= .046)

At the end of the 150-day study, there was no statistical difference between the feed conversion rates of the R and E groups, while the D group was statistically different (p= .043). In the study, the best feed conversion rates were in the groups fed every day according to the feeding table (R) and fed for 6 days starved for 1 day (E). The E group had the highest economic conversion rate, whereas the D group had the highest economic profit index.

Amino and Fatty Acid Composition and Color Analysis of Large Rainbow Trout Fillets

Table 5 lists the amino acid compositions of large rainbow trout fillets with different feeding regimens. The total amino acid values of all group fillets increased from the start of the study to the completion of the 150-day research. In particular, the total amino acid (TAA), essential amino acid (EAA), total branched-chain amino acid (BcAA), total sulfur-containing amino acid (SAA), total aromatic amino acid (ArAA), total basic amino acid (BAA) and total acidic amino acid (AAA) values of the R group fillets were higher than the other two group fillets (D and E groups). The D group fillets that had been fed one day and then fasted had high levels of total non-essential amino acids, and there was a statistically significant difference between the groups for all groups (p= .033). The order of the EAA/NEAA ratio and essential amino acid index (EAAI) values was R>E>D. Group D's fillets' EAA/NEAA and EAAI values were statistically different from those of the other two groups (respectively p= .048 and p= .046).

Table 3. Growth performance (weight gains, specific growth rate, thermal growth rate), biometric indices (condition factor, viscerosomatic index, hepatosomatic index, and carcass yield), and biochemical composition (crude protein, crude fat, crude ash, and dry matter) of the groups during the study.

Çizelge 3. Çalışma süresince grupların büyüme performansı (ağırlık kazançları, spesifik büyüme oranı, termal büyüme oranı) biyometrik indeksleri (kondisyon faktörü, viserosomatik indeks, hepatosomatik indeks ve karkas randımanı) ve biyokimyasal kompozisyonu(ham protein, ham yağ, ham kül ve kuru madde)

Parameters	*Initial*	*Final*			*p value*
		R	*D*	*E*	
Weight (g)	1045.12±43.51	3769.80±226.89^{b}	3445.03±102.12^{a}	3770.60±127.51^{b}	.049
CF[1]	1.55±0.04	1.46±0.07^{a}	1.54±0.03^{b}	1.51±0.03^{b}	.030
VSI (%)[2]	16.21±0.81	13.42±0.51^{a}	14.67±1.06^{a}	14.25±0.70^{a}	.875
HSI (%)[3]	1.50±0.07	0.88±0.05^{a}	0.91±0.10^{a}	1.09±0.05^{b}	.049
CY (%)[5]	49.31±0.05	55.61±0.78^{b}	54.58±0.50^{b}	51.39±0.68^{a}	.037
SR (%)[6]		87.93±0.04^{a}	86.00±0.01^{a}	98.20±0.06^{b}	.047
Weight gain (g)		2721.89±226.89^{b}	2397.12±102.12^{a}	2722.69±127.51^{b}	.043
Weight gain (%)		259.74±21.65^{b}	228.75±9.75^{a}	259.82±12.17^{b}	.025
SGR (%)[7]		0.85±0.03^{b}	0.79±0.02^{a}	0.84±0.04^{b}	.048
TGR (%)[8]		0.33±0.02^{a}	0.30±0.01^{a}	0.32±0.01^{a}	.438
CP[9]	19.97±0.34	20.56±0.22^{b}	15.92±0.15^{a}	20.00±0.61^{b}	.026
CL[10]	10.52±1.36	27.67±0.30^{b}	27.92±1.20^{b}	17.79±1.75^{a}	.046
CA[11]	2.98±0.25	2.96±0.11^{a}	2.85±0.17^{a}	3.21±0.20^{b}	.041
DM[12]	31.04±0.69	49.12±0.20^{b}	50.21±1.02^{b}	41.99±1.60^{a}	.023

Each value means mean±standard error. Values in rows marked with different letters are significantly different ($p < 0.05$).
[1]CF= condition factor, [2]VSI= vicerosomatic index, [3]HSI = hepatosomatic index, [4]GSI= gonadosomatic index, [5]CY= carcass yield, [6]SR= survival rate, [7]SGR = specific growth rate, [8]TGR= thermal growth rate, [9]CP= crude protein, [10]CF= crude lipid, [11] CA= crude ash, [12]DM= dry matter

Table 4. The feed and economic conversion ratio and economic profitability indices of large rainbow trout fed with different feeding regimes at the end of the study

Çizelge 4. Farklı besleme rejimleri ile beslenen büyük gökkuşağı alabalıklarının deneme sonundaki yem ve ekonomik dönüşüm oranı ile ekonomik karlılık indeksleri

Parameters	*R*	*D*	*E*	*p value*
FCR[1]	1.59±0.01^{a}	1.66±0.02^{b}	1.57±0.04^{a}	.043
ECR[2] *(€ kg^{-1})*	3.18	3.32	2.54	-
EPI[3] *(€ fish^{-1})*	21.50	19.60	23.25	-

Each value means mean±standard error. Values in rows marked with different letters are significantly different ($p < 0.05$).
[1]FCR = feed conversion ratio; [2]ECR=Economic conversion ratio; [3]EPI= Economic profit index

The fatty acid compositions of large rainbow trout fillets with different feeding regimens are shown in Table 6. The C16:0 was the most prevalent saturated fatty acid found in all group fillets.

At the start of the study, the C16:0 value was 11.60±0.36 %; however, at the end of the study, it had dropped in all groups, and the R group fillets were found to have the highest C16:0 value. The C16:0 value of Group E fillets was statistically different from the C16:0 values of other groups' fillets (p= .046). The fillets' total saturated fatty acid (ΣSFA) levels were in the following order: E>R>D, and there was a statistically significant difference between the groups (p= .019). The most prominent representative of all total monounsaturated fatty acids (ΣMUFA), C18:1n-9c, rose in the R and E group fillets as compared to the initial fillets while declining in the D group fillets. All groups' C18:1n-9c levels showed a statistically significant difference (p= .023). Total monounsaturated fatty acids of fillets showed parallelism with C18:1n-9c. The C12:2n-6c, C22:6n-3 (DHA), C18:3n-3, C20:5n-3 (EPA), and C20:2 were the polyunsaturated fatty acids (PUFA) most commonly found in fillets in this study. Among these fatty acids, C20:2 was found at the highest values in the R group, C18:2n-6c, C18:3n-3, and C22:6n-3 in the E group, and C20:5n-3 in the D group. While the statistical difference between the C20:5n-3 (EPA) values of large rainbow trout fillets was significant in all groups (p= .010), the difference between the fillets' C22:6n-3 (DHA) values was significant only in group E (p= .040).

In group E fillets, total polyunsaturated fatty acids were prominent. Total omega-3 and omega-6 values of fillets were high in group E, which was fed for 6 days/fasted for 1 day, and the statistical difference between these values was significant (respectively, p=.048 and p= .040). Group D fillets had a high total omega-3/omega-6 value and

omega-6/omega-3 value. In fatty acid quality values, the atherogenicity index (AI)value was high in group D fillets, the thrombogenicity index (TI) value was high in R and D groups, and the hypocholesterolemic/hypercholesterolemic ratio (HH) value was high in E and R groups. The results of the study showed that there was no statistically significant difference between the fatty acid quality values (AI, TI, and HH) found in the fillets of the experimental groups.

Table 5. Amino acid compositions of large rainbow trout fillets at the initial and end of the study (g $100g^{-1}$ protein)
Çizelge 5. Deneme başı ve deneme sonunda büyük gökkuşağı alabalığı filetolarının amino asit kompozisyonları (g $100g^{-1}$ protein)

Amino Acids	*Initial*	*Final*			*p value*
		R	*D*	*E*	
Alanine	1.08±0.05	1.09±0.01^{a}	1.24±0.01^{b}	1.11±0.05^{a}	.040
Aspartic Acid	1.93±0.20	1.88±0.01^{b}	2.12±0.01^{c}	1.64±0.01^{a}	.023
Methinonine	0.53±0.01	0.52±0.01^{b}	0.48±0.01^{a}	0.52±0.01^{b}	.039
Glutamic Acid	2.48±0.04	2.60±0.01^{c}	2.31±0.01^{a}	2.52±0.12^{b}	.037
Phenylalanine	0.73±0.01	0.75±0.01^{b}	0.68±0.01^{a}	0.73±0.04^{b}	.046
Lysine	1.84±0.03	2.23±0.01^{b}	1.96±0.01^{a}	2.00±0.15^{b}	.040
Histidine	0.49±0.02	0.47±0.01^{a}	0.58±0.01^{b}	0.49±0.02^{a}	.036
Tyrosine	0.56±0.01	0.65±0.01^{b}	0.49±0.01^{a}	0.62±0.04^{b}	.038
Glysine	0.70±0.13	0.19±0.01^{a}	0.75±0.01^{c}	0.60±0.08^{b}	.045
Valine	0.68±0.02	0.90±0.01^{c}	0.60±0.01^{a}	0.80±0.06^{b}	.044
Leucine	1.28±0.02	1.43±0.01^{c}	1.23±0.01^{a}	1.35±0.07^{b}	.035
Isoleucine	0.40±0.01	0.56±0.01^{c}	0.34±0.01^{a}	0.50±0.05^{b}	.045
Threonine	0.77±0.01	0.89±0.01^{b}	0.69±0.01^{a}	0.86±0.08^{b}	.043
Serine	0.85±0.02	0.92±0.01^{b}	0.82±0.01^{a}	0.92±0.03^{b}	.048
Proline	0.70±0.01	0.78±0.01^{b}	0.68±0.01^{a}	0.75±0.04^{b}	.046
Ornithine	0.19±0.02	0.29±0.01^{b}	0.19±0.01^{a}	0.24±0.03^{b}	.039
Cystine	0.14±0.01	0.17±0.01^{a}	0.14±0.01^{a}	0.15±0.01^{a}	.865
Arginine	0.98±0.01	1.13±0.01^{b}	0.99±0.01^{a}	1.12±0.07^{b}	.047
TAA	15.99±0.28	17.43±0.01^{b}	16.27±0.01^{a}	16.71±0.02^{a}	.045
ΣEAA	7.71±0.01	8.87±0.01^{c}	7.54±0.01^{a}	8.36±0.52^{b}	.040
ΣSEAA	1.47±0.02	1.60±0.01^{a}	1.57±0.01^{a}	1.60±0.01^{a}	.678
ΣNEAA	8.28±0.29	8.57±0.01^{b}	8.74±0.01^{c}	8.35±0.01^{a}	.033
EAA/NEAA	0.93±0.03	1.04±0.01^{b}	0.86±0.01^{a}	1.00±0.04^{b}	.048
ΣBcAA	2.39±0.02	2.89±0.01^{b}	2.17±0.01^{a}	2.65±0.01^{b}	.048
ΣSAA	0.67±0.01	0.69±0.01^{b}	0.62±0.01^{a}	0.67±0.02^{b}	.043
ΣArAA	1.29±0.01	1.40±0.01^{b}	1.17±0.01^{a}	1.35±0.01^{b}	.040
ΣBAA	3.33±0.02	3.83±0.01^{b}	3.53±0.01^{a}	3.60±0.01^{a}	.048
ΣAAA	4.16±0.25	4.48±0.01^{b}	4.43±0.01^{b}	4.15±0.07^{a}	.047
EAAI	0.89±0.01	0.95±0.01^{b}	0.88±0.01^{a}	0.92±0.03^{b}	.046

The each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).
Branched-chain amino acid (BcAA)= Leucine+ Isoleucine+ Valine; Sulfur-containing amino acids (SAA)= Cystine+ Methinonine; Aromatic amino acids (ArAA)= Phenylalanine+ Tyrosine; Basic (alcaline) amino acids (BAA)= Lysine+ Arginine+ Histidine; Acidic amino acids (AAA)= Aspartic acid+ Glutamic acid

The table 7 lists the L*, a*, b*, C*, and Hue values found in the skins and fillets of large rainbow trout fed on three different feeding regimes. At the conclusion of the study, all groups' fillets' lightness (L*) values decreased, and the L* values of the R group's fillets fed consistently in accordance with the feeding table were statistically different (p= .040). The fillets' redness (a*) value was approximately twice the initial, with the highest value occurring in the E group, which was fed 1 day/fasted for 6 days (p= .035). The yellowness (b*) and C* values of group E fillets were statistically different and, higher than the b* and C* values of the other two groups (respectively, p= .017 and p= .0.41). Despite the high Hue values of the fillets in the R and D groups, there was no statistically significant difference between the groups (p= .538).

Table 6. Fatty acid compositions of large rainbow trout fillets at the initial and end of the study (% of fatty acids)
Çizelge 6. Deneme başı ve deneme sonunda büyük gökkuşağı alabalığı filetolarının yağ asitleri kompozisyonları (% yağ asitleri)

Fatty acid	*Initial*	*Final*			p value
		R	*D*	*E*	
C12:0	0.06±0.01	0.08±0.01[a]	0.07±0.01[a]	0.09±0.01[a]	.636
C13:0	0.02±0.01	0.02±0.01[a]	0.02±0.01[a]	0.02±0.01[a]	.405
C14:0	2.88±0.14	2.99±0.02[a]	3.26±0.04[b]	3.03±0.08[a]	.019
C15:0	0.41±0.01	0.39±0.01[a]	0.39±0.01[a]	0.44±0.02[b]	.035
C16:0	11.60±0.36	11.48±0.06[b]	11.36±0.15[b]	11.04±0.12[a]	.046
C17:0	0.52±0.04	0.45±0.01[b]	0.39±0.01[a]	0.51±0.04[b]	.019
C18:0	6.94±0.09	7.69±0.05[b]	7.08±0.11[a]	8.00±0.12[c]	.006
C20:0	0.98±0.06	1.01±0.01[b]	0.86±0.01[a]	1.09±0.10[b]	.038
C21:0	0.02±0.01	0.05±0.01[a]	0.02±0.01[a]	0.03±0.01[a]	.486
C22:0	0.57±0.03	0.49±0.01[a]	0.51±0.01[a]	1.62±0.37[b]	.010
C23:0	0.11±0.06	0.14±0.02[b]	0.07±0.01[a]	0.10±0.02[ab]	.047
C24:0	0.51±0.06	0.60±0.01[b]	0.32±0.01[a]	0.60±0.10[b]	.047
ΣSFA	24.61±0.46	25.27±0.10[b]	24.49±0.25[a]	26.56±0.57[c]	.019
C14:1	0.17±0.02	0.19±0.01[a]	0.17±0.01[a]	0.22±0.01[b]	.045
C15:1	0.06±0.01	0.06±0.01[a]	0.06±0.01[a]	0.07±0.01[a]	.189
C16:1	0.43±0.06	0.41±0.01[a]	0.40±0.01[a]	0.55±0.04[b]	.045
C17:1	0.47±0.06	0.45±0.01[a]	0.45±0.01[a]	0.63±0.04[b]	.046
C18:1n-9c	20.83±0.72	25.88±0.09[c]	17.33±0.35[a]	23.45±0.38[b]	.023
C18:1n-9t	2.59±0.28	2.69±0.02[b]	2.88±0.19[c]	1.41±0.28[a]	.009
C20:1n-9c	3.74±0.80	1.18±0.01[a]	2.29±1.71[b]	3.44±0.70[c]	.048
C22:1n-9	2.02±0.54	4.55±0.02[c]	0.09±0.01[a]	2.09±0.78[b]	.020
C24:1	0.88±0.11	1.29±0.01[c]	0.65±0.04[a]	0.80±0.01[b]	.005
ΣMUFA	31.19±0.73	36.70±0.08[c]	24.31±1.13[a]	32.65±0.72[b]	.011
C18:2n-6t	0.46±0.01	0.51±0.01[b]	0.43±0.02[a]	0.57±0.04[b]	.047
C18:2n-6c	15.06±0.03	13.15±0.18[a]	13.21±0.03[a]	13.48±0.29[a]	.360
C18:3n-3	6.72±0.07	6.74±0.10[a]	6.82±0.02[a]	6.99±0.01[a]	.871
C18:3n-6	0.63±0.01	0.62±0.10[a]	0.69±0.01[b]	0.73±0.04[b]	.037
C20:2	3.54±0.03	3.42±0.05[b]	3.31±0.02[a]	3.39±0.03[b]	.032
C20:3n-3	1.69±0.04	2.00±0.02[b]	1.47±0.01[a]	1.94±0.09[b]	.047
C20:3n-6	1.13±0.03	1.45±0.12[c]	0.39±0.02[a]	1.02±0.22[b]	.024
C20:4n-6	1.53±0.01	1.21±0.01[a]	1.41±0.34[ab]	1.67±0.11[b]	.040
C20:5n-3	3.95±0.03	3.40±0.04[a]	3.93±0.04[c]	3.73±0.06[b]	.010
C22:2	0.065±0.01	0.04±0.01[a]	0.06±0.03[b]	0.03±0.01[a]	.048
C22:6n-3	9.27±0.08	6.86±0.09[a]	7.06±0.02[a]	7.40±0.22[b]	.040
ΣPUFA	44.02±0.51	39.38±0.62[ab]	38.79±0.30[a]	40.93±0.21[b]	.042
Σn-3	21.63±0.04	19.00±0.25[a]	19.28±0.07[ab]	20.05±0.20[b]	.048
Σn-6	18.79±0.46	16.94±0.32[ab]	16.13±0.30[a]	17.47±0.13[b]	.040
Σn-9	29.18±0.63	33.49±0.81[b]	34.30±0.07[b]	30.38±0.72[a]	.036
n3/n6	1.15±0.03	1.12±0.01[a]	1.20±0.02[b]	1.15±0.02[a]	.040
n6/n3	0.87±0.01	0.89±0.01[b]	0.84±0.02[a]	0.87±0.01[a]	.042
EPA/DHA	0.43±0.01	0.50±0.01[a]	0.56±0.01[b]	0.51±0.02[a]	.049
EPA+DHA	13.22±0.09	10.25±0.13[a]	10.99±0.06[b]	11.12±0.18[b]	.035
AI	0.32±0.01	0.33±0.01[a]	0.34±0.01[a]	0.30±0.03[a]	.599
TI	0.24±0.01	0.26±0.01[a]	0.26±0.01[a]	0.25±0.01[a]	.801
PUFA/SFA	1.79±0.02	1.56±0.03[a]	1.58±0.03[a]	1.54±0.03[a]	.763
HH	3.54±0.02	3.57±0.06[a]	3.55±0.06[a]	3.57±0.03[a]	.947

Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).
∑Omega-3 (n-3) = C18:3n-3+C20:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; ∑Omega-6 (n-6)= C18:2n-6t + C18:2n-6c+ C18:3n-6+ C20:4n-6+ C20:3n-6; ∑Omega-9 (n-9)= C18:1n-9c+ C18:1n-9t+ C20:1n-9c+ C22:1n-9;

Table 7. The average L*, a*, b*, C*, and Hue values of large rainbow trout fillets and skins at the initial and end of the study

Çizelge 7. Deneme başı ve deneme sonunda büyük gökkuşağı alabalığı filetolarının ve derilerinin ortalama L, a*, b*, C* ve Hue değerleri*

		Initial	*Final*			*p value*
			R	*D*	*E*	
Skin	*L**	72.01±0.95	74.57±4.13^{a}	93.15±0.97^{b}	79.27±1.71^{a}	0.040
	*a**	0.19±0.10	1.29±0.71^{c}	-0.10±0.19^{a}	0.76±0.22^{b}	0.005
	*b**	3.66±0.25	5.07±0.56^{b}	3.29±0.41^{a}	3.81±0.32^{a}	0.041
	*C**	7.32±1.19	5.71±0.48^{b}	3.35±0.40^{a}	8.97±0.32^{c}	0.032
	Hue	0.03±0.09	0.87±0.27^{b}	-0.44±0.44^{a}	0.17±0.16^{a}	0.039
Fillet	*L**	54.53±0.41	50.54±1.45^{b}	48.33±1.24^{a}	47.38±0.52^{a}	0.040
	*a**	8.57±0.22	15.49±0.61^{a}	15.16±0.68^{a}	17.09±0.42^{b}	0.035
	*b**	10.66±0.24	18.04±1.20^{a}	17.66±0.99^{a}	20.84±2.14^{b}	0.017
	*C**	13.98±0.39	23.89±1.23^{a}	23.36±1.11^{a}	27.69±2.13^{b}	0.041
	Hue	0.89±0.01	0.85±0.02^{a}	0.85±0.02^{a}	0.82±0.02^{a}	0.538

Each value represents the mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).

DISCUSSION

In aquaculture, feeding techniques are crucial because feeds and feeding account for approximately 60 % of production costs, and both underfeeding and overfeeding can have a negative impact on production (Ntantali et al. 2023). Thus, effective farming is heavily reliant on feed management (Chatzifotis et al. 2011). Many feeding approaches, such as ad libitum feeding, restricted feeding, and intermittent feeding, are used to find the ideal eating plan for each species and developmental stage (Da Silva et al. 2016) This study used various feeding regimens to investigate the impact of large rainbow trout (*Onchorchynus mykiss*) raised in net cages in the Black Sea on growth performance, fillet color and meat quality ratings, and economic conversion rates.

Fish nutrition is critical to the production cycle since it is the most essential growth component and the largest operating cost in aquaculture. Aquaculture has long sought to improve growth; for example, fasting and refeeding regimes, which have been used to increase growth, have been well evaluated. Compensatory growth within a certain period is thought to be much faster than the growth rate of fish that have not been subjected to feed deprivation. Although the large rainbow trout had an average weight of 1045.12±43.51 g at the beginning of the study and were of similar weight in the groups fed every day (R) and fed for 6 days (E) at the end of the study (3769.80±226.89 and 3770.60±127.51 g, respectively, p= .049), they were fed for one day. It was determined to be less in the one-day fasting (D) group (3445.03±102.12 g, p= .049). The trial end weights of all three groups are in accordance with the harvest policy of the enterprise. The majority of the overall production costs were made up of feed costs. In aquaculture, feeding expenses are crucial as they account for 40% to 50% of overall production costs (Abowei & Ekubo 2011). In this study, when calculating economic conversion rates, fixed costs (cost depreciation, labor, electricity, equipment, building, etc.), which represent a small part of total costs, were counted equally for each group. The only income for the enterprise includes the sale of fish. Intermittent fasting has been proposed to achieve compensatory growth in a variety of economically important fish species in different studies, including Atlantic salmon (*Salmo salar*) (Stefansson et al. 2009), rainbow trout (*Oncorhynchus mykiss*) (Nikki et al. 2004), Nile tilapia (*Oreochromis niloticus*) (Ali et al. 2016), European seabass (*Dicentrarchus labrax*) (Chatzifotis et al. 2011; Adaklı et al. 2015) and gilthead seabream (*Sparus aurata*) (Bavčević et al. 2010; Peres et al. 2011). The current study found that Group E, which was fed for 6 days and fasted for 1, was the best group when all costs were held constant, and the economic conversion rate (ECR) and economic profit index (EPI) were taken into consideration. The first thing that comes to mind here is the feed conversion rate (FCR), which comes into play in calculating economic transformation and is known to be directly proportional to the economic conversion rate. When the FCR was examined, it was found that the groups with the best rate were the E group (1.57±0.04), which was fed for 6 days fasting for 1 day, and the R group (1.59±0.01), which was fed every day. These rates were very close to each other in these groups, the ECR of the E group was also better at the same rate depending on the FCR value.

In the current study, the crude protein (CP) ratios of the groups fed every day (R) and every other day (D) increased compared to the beginning of the experiment, while the protein ratios of the fish fed for 6 days and fasted for one day (E) decreased (Table 3). The crude lipid (CL) ratio of fillets rose in all groups compared to the beginning of the study, however, the CL ratio of the fillets in every other day fed group (D) reduced compared to the other groups (p= .046). Most restricted feeding or starvation studies have suggested that during fasting lipids and glycogen are

mobilized primarily to provide energy, while muscle protein is largely spared (Jørgensen et al. 2013, Barreto-Curiel et al. 2017; Shirvan et al. 2020; Xu et al. 2022). Bowzer et al. (2011) reported that *Morone chrysops × M. saxatilis* fillets protein was depleted faster than lipid at the end of the 14-day fasting phase. In the current study, although the decrease in the CL ratio of fish fillets belonging to group E is supported by the mentioned literature, it is thought that the reduction of the CP ratio of group D is due to the increase in the body water content of the fish.

The study determined that the total amino acid values of fish fillets applied to different feeding regimes increased compared to the total amino acid values of the initial fish fillets. The daily fed group (R) had greater total amino acid levels, essential amino acid values, and EAA/NEAA ratios than the other groups (D and E). Fillets from group D had the highest non-essential amino acid levels. According to McCarthy and Brown (2016), amino acids contribute to protein metabolism as well as tissue protein synthesis. In addition to this literature, it reported that other amino acids such as non-essential ones are also used as energy substrates to maintain metabolic activity in fish (Duan et al. 2016). As a result, animals require amino acids not only for development but also for energy supply (Kasozi et al. 2019). Different studies reported that it can use amino acids as an energy source (Moughan 2003; Cui et al. 2006), and some fish prefer to use glutamic acid and alanine instead of lysine and arginine as energy sources (Rønnestad et al. 2001; Conceiçao et al. 2002). At the end of the current study, the glutamic acid, lysine, and arginine values of groups D and E decreased compared to group R, and this result was similar to the mentioned literature, except for the alanine value. The most significant source of essential amino acids found in muscle proteins are the branched-chain amino acids (BCAAs), which include leucine, isoleucine, and valine, and these amino acids make up an average of 30–40 % of total amino acids in muscle proteins (Nie et al. 2018). The BcAA values of fish fillets in this study, except for group D (28.78 %), group R (32.58 %), and group E (31.70 %), were in line with the values reported by Nie et al. (2018).

Fillet fatty acids influence fillet quality. Previous investigations have shown that starving and re-feeding have a considerable impact on fatty acid composition (Arslan et al. 2021; Yang et al. 2021). Additionally, fatty acids in fish are known as essential energy sources, which are vital for their growth, survival, and several physiological mechanisms (Tocher et al. 2019). Although the fatty acid content of fish fillets is often reflected in the profile of fatty acids found in diets (Matani Bour et al. 2018; Roohani et al. 2019), in our study, feed restriction had an impact on the fatty acid composition of large rainbow trout fillets. This research found a total of 32 fatty acids, with C18:1n9c having the greatest value across all groups. Additionally, there were statistically significant variations between the groups in terms of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Although there was a statistical difference, the numerical values of total fatty acids were close to each other. Different feeding regimes effectively increased the fillet n-3 and n-6 polyunsaturated (PUFA) levels (ARA, EPA, and DHA), and Σ n-3/Σ n-6 ratio of large rainbow trout. There have also been reports of other teleosts' fish preservation of PUFAs during periods of dietary restriction (Luo et al. 2009; Ölmez et al. 2015; Barreto-Curiel et al. 2017; Ding et al. 2017). Asadi et al. (2021) reported that different protein restrictions in the diet had no negative effects on fatty acid profiles in fish fillets. Considering the literature and at the end of the analyses of fatty acids, it was shown that large rainbow trout fed a restricted diet were able to maintain a balanced metabolism of fatty acids. Indicators of the relationship between saturated and unsaturated fatty acids that are employed in the evaluation of cardiovascular disorders include atherogenicity (AI) and thrombogenicity index (TI) (Ghaeni et al. 2013). According to Łuczynska et al. (2017), AI and TI values for human health shouldn't be higher than 1.00. The hypocholesterolemic/hypercholesterolemic index indicated the fatty acid ratio based on cholesterol metabolism and foods with high H/H ratios (>3) have been reported as more beneficial to human health. (Fernandes et al. 2014). The AI, TI, and H/H values of large rainbow trout fed with varying feeding strategies were within the appropriate range for human health and comparable to those found by Devadawsaon et al. (2016) and particularly to those found in studies on large rainbow trout in the Black Sea (Kaya Öztürk et al. 2019; Kaya Öztürk 2024).

According to Ocaño-Higuera et al. (2009), color is one of the most crucial factors taken into account when assessing the caliber of fishing goods. The distinct coloration of large rainbow trout, characterized by red, orange, yellow, green, and blue hues, is highly beneficial in characterizing their skin tone. Customers love the worldwide look of red-pink fillet of rainbow trout. Color values (L*, a*, b*, C*, and Hue) of skin and fillets of large rainbow trout applied to different feeding regimes are shown in Table 7. Throughout the study, fish were fed the same amount of feed containing astaxanthin (50 mg/kg) (Table 1) however, at the end of the trial, there were differences, in the color parameters of the fillets. Regarding sensory analysis of fillet colors, Einen and Thomassen (1998a; 1998b) found no definite advantages or disadvantages in their starvation study with Atlantic salmon. According to research by Montero et al. (2005), Rørå et al. (2005) and Rincon et al. (2016), lipid concentrations in feed and fillet have an impact on L*, a*, and b* values. After the study, diet and fillet lipid rates had an indirect effect on color parameters, whereas other feeding tactics had a direct impact. When evaluated in terms of consumer satisfaction, it was concluded that the E group—which was fed for six days and fasted for one day—had higher redness and yellowness ratings

CONCLUSION

The study assessed the growth performance, meat quality, and economic conversion rates of various feeding regimens administered to large rainbow trout cultivated in the Black Sea under identical conditions (same environmental parameters, diets, and ages). When the enterprise's harvest policy was considered, the study found that the feeding limitation had no detrimental effects on fish weight (<3kg) and growth performance. The study's most important emphasis is on the FCR and ECR rates of the E group, which was fed for 6 days after fasting for 1 day. By using this feeding regimen, businesses that produce huge rainbow trout might lower their feed expenditures. Furthermore, this article has demonstrated that large rainbow trout raised commercially in the Black Sea and fed on a variety of diets are healthy, nutrient-dense, and of high quality for human consumption. It has also demonstrated the existence of a "Turkish salmon" that is competitive with Atlantic salmon on the domestic or international market

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Authors' Contributions

DKO: Supervisor, Writing – review and editing; RD: data collection, methodology, sampling, and writing. All authors read and approved the final manuscript.

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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Prediction of Beef Production Using Linear Regression, Random Forest and k-Nearest Neighbors Algorithms

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ABSTRACT
The rapid increase in the global population and evolving dietary habits have significantly heightened the demand for high-quality protein sources. Beef, as a vital protein source, plays a crucial role in meeting this growing demand. This study aims to develop and evaluate a machine-learning model to predict beef production using meteorological, agricultural, and economic data. To achieve this, three different machine learning algorithms—Linear Regression, Random Forest, and k-Nearest Neighbors—were employed. The results indicate that the Random Forest algorithm outperformed the other methods in terms of R^2 and error metrics, demonstrating superior predictive accuracy. The study highlights the potential of machine learning techniques in predicting beef production, offering valuable insights for stakeholders involved in strategic decision-making to meet nutritional needs. As the global demand for protein continues to rise, the importance of such predictive models becomes increasingly significant, emphasizing the distinct advantages that machine learning approaches provide in this context.

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ÖZET
Küresel nüfusun hızla artması ve değişen beslenme alışkanlıkları, yüksek kaliteli protein kaynaklarına olan talebi önemli ölçüde artırmıştır. Önemli bir protein kaynağı olan sığır eti, bu artan talebin karşılanmasında kritik bir rol oynamaktadır. Bu çalışma, meteorolojik, tarımsal ve ekonomik veriler kullanarak sığır eti üretimini tahmin etmek için bir makine öğrenimi modeli geliştirmeyi ve değerlendirmeyi amaçlamaktadır. Bu amacı gerçekleştirmek için, üç farklı makine öğrenmesi algoritması—Doğrusal Regresyon, Rastgele Orman ve k-En Yakın Komşu—kullanılmıştır. Sonuçlar, Rastgele Orman algoritmasının R^2 ve hata metrikleri açısından diğer yöntemlerden daha iyi performans gösterdiğini ve üstün tahmin doğruluğu sağladığını göstermektedir. Çalışma, sığır eti üretiminin tahmin edilmesinde makine öğrenimi tekniklerinin potansiyelini vurgulamakta ve beslenme ihtiyaçlarını karşılamak için stratejik karar alma süreçlerine dahil olan paydaşlar için değerli bilgiler sunmaktadır. Küresel protein talebinin artmaya devam etmesiyle, bu tür tahmin modellerinin önemi giderek daha belirgin hale gelmekte ve makine öğrenmesi yaklaşımlarının bu bağlamda sunduğu belirgin avantajları öne çıkarmaktadır.

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INTRODUCTION

The accelerating growth of the global population, coupled with evolving dietary preferences, has substantially increased the demand for high-quality protein sources (Henchion et al., 2017). Beef, with its rich protein content, plays a pivotal role in satisfying this demand and serves as a crucial source of essential micronutrients such as iron, zinc, and vitamin A (Li, 2017; Ruxton & Gordon, 2024). However, recent years have witnessed a deceleration in the growth rates of agricultural production and crop yields, raising concerns about the world's capacity to adequately feed its future population (FAO, 2024a). The increasing demand for meat has already posed significant challenges to the meat industry, further exacerbated by the fact that meat production is considerably more resource- and energy-intensive compared to other food sources, prompting the exploration of lab-grown artificial meats as potential alternatives (Marshall et al., 2011; Rout Srutee et al., 2021; Ching et al., 2022).

Accurate prediction of agricultural production data is crucial for both enhancing understanding of production processes and supporting the achievement of sustainable development goals (Bharadiya et al., 2023). Traditionally, production predictions have relied on robust statistical methods, including multivariate statistical techniques. However, these conventional approaches often fall short when dealing with complex, high-dimensional data characterized by intricate, nonlinear relationships, thereby limiting their predictive accuracy and flexibility (Yıldız et al., 2024). In response to these limitations, recent advancements in production prediction have increasingly turned towards artificial intelligence (AI) applications, particularly those based on machine learning (ML) techniques. Machine learning algorithms excel at analyzing large datasets and modeling complex interdependencies, often outperforming traditional statistical methods. In particular, supervised learning algorithms exhibit superior performance in utilizing historical data to capture the dynamic relationships within production processes (Kononenko, 2001; Ahmed & Hussain, 2022).

Numerous studies have highlighted the advantages of machine learning algorithms in agricultural production analysis. For instance, Nosratabadi et al. (2021) demonstrated that high accuracy in predicting animal food production could be achieved using machine learning algorithms such as the Adaptive Network-Based Fuzzy Inference System (ANFIS) and Multilayer Perceptron (MLP). Similarly, Alonso et al. (2013) employed Support Vector Regression (SVR) to predict the carcass weight of Asturiana de los Valles cattle, showing that carcass weight could be estimated 150 days before slaughter. Coşkun et al. (2023) successfully utilized eXtreme Gradient Boosting (XGB), Random Forest (RF), and Bayesian Regularized Neural Network (BRNN) data mining algorithms to predict the live weight of Anatolian Merinos lambs. Furthermore, Rahman et al. (2021) developed a machine learning-based prediction model for marine fish and aquaculture production by integrating Linear Regression (LR), Gradient Boosting Regression (GB), and Random Forest Regression (RFR) into an ensemble approach known as Voting Regression (VR), achieving high-performance outcomes. In another study, Yıldız et al. (2024) developed a model for predicting honey production in Turkey using various machine learning algorithms, including k-Nearest Neighbor (k-NN), Random Forest (RF), Linear Regression (LR), and Gaussian Naive Bayes (GNB). However, to date, there has been a noticeable gap in the literature specifically focusing on the application of machine learning algorithms to predict beef production.

The primary aim of this study is to develop and evaluate a machine-learning model for predicting beef production. This research seeks to compare the performance of LR, RF, and k-NN algorithms to identify the most effective predictive method. By leveraging the accuracy and reliability of machine learning, the study aims to facilitate more precise predictions of beef production, ultimately contributing to the optimization of production strategies and planning.

MATERIAL and METHOD

Material

The attributes were selected based on factors influencing beef production, as identified through a comprehensive review of the existing literature (FAO, 2024a; Van Kernebeek et al., 2016; Humer & Zebeli, 2017; Godfray et al., 2018; Nosratabadi et al., 2021; Çakan & Tipi, 2023). The study utilized a dataset comprising 62 annual average data points for each of the 18 attributes, collected between 1961 and 2022, as this period was chosen due to the availability of the most comprehensive and reliable data. These attributes include beef production, cattle population, beef price, total population, rural population, urban population, agricultural land area, pasture and meadow area, food price inflation, temperature, precipitation, Gross Domestic Product (GDP), Gross National Product (GNP), per capita GNP, barley production, corn production, barley price, and corn price. All attributes were treated as continuous variables, and no subgroup analyses were performed.

Agricultural data, such as beef production, cattle numbers, agricultural land area, pasture and meadow area, barley production, and corn production, were sourced from the Turkish Ministry of Agriculture and Forestry's official website (TMAF, 2024). Meteorological data, including temperature and precipitation, were obtained from

the General Directorate of Meteorology under the Turkish Ministry of Environment, Urbanization, and Climate Change (GDM, 2024). Population and economic data—including total population, rural and urban population, food price inflation, GDP, GNP, per capita GNP, barley price, and corn price—were acquired from the Food and Agriculture Organization (FAO) of the United Nations website (FAO, 2024b). All available data from the mentioned sources between 1961 and 2022 were included in the analysis, without the use of any specific sampling technique. To address missing data within the dataset, mean imputation was employed, which involved filling the missing values with the average values calculated from the available data. The statistical properties of the attributes, including the mean, standard deviation, maximum, and minimum values, are summarized respectively in Table 1.

Table 1. Statistical properties for attributes
Çizelge 1. Özniteliklere ilişkin istatistiksel özellikler

Attributes	Mean	Standard deviation	Minimum	Maximum
Beef production (tonne)	432920.6950	378035.02030	90634.00	1572747.15
Cattle population (units)	13028781.8100	1971869.20700	9788102.00	17965482.00
Beef price (tonne/$)	6700.2241	1501.80565	2715.20	12251.40
Population (units)	56014.3943	17363.82823	28255.00	85279.55
Rural population (units)	21823.5306	1349.22945	19121.82	24723.16
Urban population (units)	33622.5860	17580.66390	9025.07	65453.23
Agricultural area (thousand ha)	38753.7032	1176.67056	36517.00	41223.00
Meadow and pasture area (thousand ha)	12415.6229	1797.07352	10000.00	14617.00
Food price inflation (%)	19.3578	12.19365	3.90	77.87
Temperature (°C)	12.0503	1.53099	9.58	15.10
Precipitation (mm)	606.1389	70.49718	460.69	793.80
GNP (million $)	349669.3695	283621.07450	904.19	941689.70
GDP (million $)	369093.8850	294729.94470	23609.87	957799.00
GDP per capita ($)	5286.1638	3465.49856	649.34	12507.80
Barley production (tonne)	6258980.6450	1868969.44400	2900000.00	9551000.00
Corn production (tonne)	2727806.4520	1921057.94700	800000.00	8500000.00
Barley price (tonne/$)	202.8625	49.39973	100.10	370.30
Corn price (tonne/$)	228.0594	44.37527	138.00	375.30

Method

In this study, commonly used machine learning algorithms—Linear Regression (LR), Random Forest (RF), and k-Nearest Neighbors (k-NN)—were selected to predict beef production. The analyses were conducted using the Python programming language, version 3.12.2 (Python Software Foundation, 2024), leveraging libraries such as Pandas (1.3.0) for data manipulation, Numpy (version 1.21.0) for numerical operations, Matplotlib (version 3.4.2) for data visualization, and Scipy (version 1.10.0) for scientific computations (Yıldız et al. 2024). The dataset, after addressing missing values through mean imputation, was split into training and test sets, with 70% of the data allocated for training the algorithms and the remaining 30% reserved for testing the predictive accuracy of the models. To ensure consistency and enhance the performance of the machine learning models, the data were standardized to balance the value differences among all attributes. This standardization process involved scaling the data to have a mean of zero and a standard deviation of one, thereby aligning the varying scales of different features and improving the models' convergence during training.

In addition, a hyperparameter optimization process was implemented using the Grid Search technique to further enhance the performance of the machine learning models. This approach entailed a systematic exploration of a range of hyperparameters for each algorithm, facilitating the discovery of the most effective combinations aimed at enhancing predictive accuracy. The selected hyperparameters for LR, RF and k-NN algorithms are detailed in Table 2.

The predictive performance of the algorithms was evaluated separately on both the training and test sets. Predictions generated by the models were compared against the actual values in the test set, and the performance was assessed using the following metrics: Coefficient of Determination (R^2), Mean Absolute Error (MAE), Mean Squared Error (MSE), and Root Mean Square Error (RMSE). R^2 (Coefficient of Determination) indicates the proportion of variance in the dependent variable explained by the independent variables, with values ranging from 0 to 1. The closer R^2 is to 1, the better the model explains the data. MAE (Mean Absolute Error) represents the average of the absolute differences between predicted and actual values, measuring how close the predictions are to the true values. A lower MAE indicates better model performance. MSE (Mean Squared Error) calculates the

average of the squared differences between predicted and actual values, penalizing larger errors more heavily. It provides a measure of the model's overall error. Finally, RMSE (Root Mean Squared Error) is the square root of MSE and expresses the magnitude of the errors directly in the original data units. RMSE gives a precise measure of the model's overall accuracy. Each of these metrics provides a different perspective on model performance, offering a more comprehensive evaluation.

Table 2. Hyperparameter settings for the selected algorithms
Çizelge 2. Kullanılan algoritmalar için hiperparametre ayarları

Algorithm	Hiperparameter	Value
Linear Regression	C	1.0
	Solver	'lbfgs'
Random Forest	n_estimators	100
	max_depth	None
	min_samples_split	2
k-Nearest Neighbor	n_neighbors	5
	weights	'uniform'
	algorithm	'auto'

These error metrics were computed using the formulas outlined below:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (1)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y}_i| \quad (2)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2 \quad (3)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \quad (4)$$

$(n) = Total\ number\ of\ observations$; $(yi) = (i) - th\ observation\ of\ true\ values$; $(\hat{y}_i) = (i) - th\ observation\ of\ predicted\ values$

To visually assess the alignment between the model predictions and the actual observed values, appropriate plots and graphs were generated. These visualizations facilitated a clearer understanding of how well the models performed in predicting beef production, highlighting areas of strong correlation as well as potential discrepancies.

Furthermore, a feature importance analysis was conducted using the RF algorithm. This analysis aimed to enhance the interpretability of the model by identifying and quantifying the relative impact of each attribute on beef production predictions. By assessing the contribution of each feature, the feature importance score analysis provided valuable insights into which factors most significantly influence the predictive outcomes, thus aiding in the understanding of underlying patterns and relationships within the data. This approach not only improves the model's transparency but also helps prioritize key variables that could be targeted in strategic interventions or policy formulations.

RESULTS and DISCUSSION

In this study, Linear Regression (LR), Random Forest (RF), and k-Nearest Neighbors (k-NN) algorithms were employed to predict beef production. The performance evaluations were conducted using key metrics, including the Coefficient of Determination (R^2), Mean Absolute Error (MAE), Mean Squared Error (MSE), and Root Mean Square Error (RMSE). An R^2 value close to 1 indicates a strong alignment between the model's predictions and the actual data, reflecting a high level of accuracy in capturing the relationships among the variables. Conversely, error metrics such as MAE, MSE, and RMSE being close to zero suggest that the model's predictions have minimal error margins, demonstrating a high degree of concordance with the observed values. Based on these criteria, the performance of the algorithms was assessed, and the most successful models were identified by their high R^2 values and low error metrics, which indicate superior predictive accuracy and reliability (Gültepe, 2019; Rahman et al., 2021; Yıldız et al., 2024).

To comprehensively evaluate the performance of the algorithms, results from both the training and test datasets

were meticulously analyzed. The training set results reflect the model's performance on data it was trained on, while the test set results provide an evaluation of the model's predictive capability on unseen, general data. High-performance values on the training set indicate that the model fits the training data well, suggesting that the model has effectively learned the underlying patterns within the data (Table 3).

This analysis ensures that the models not only excel in terms of training data but also maintain robustness and generalizability when applied to new datasets. Such thorough evaluation is crucial in establishing the models' utility in real-world applications, where the ability to generalize from past data to predict future outcomes is of paramount importance.

Table 3. Performance metrics of algorithms on the train set
Çizelge 3. Eğitim setinde kullanılan algoritmaların performans metrikleri

Algorithms	R^2	MAE	MSE	RMSE
Linear Regression	0.996	13103.153218	1249431000.0	35341.421005
Random Forest	0.997	8201.004167	663951600.0	25767.542022
k-Nearest Neighbor	0.96	47185.176091	19511660000.0	139666.572517

R^2, coefficient of determination; MAE, Mean Absolute Error; MSE, Mean Squared Error; RMSE, Root Mean Square Error

The results from the test set reflect the model's generalization ability and its performance on new data (Table 4). The minimal differences between the training and test results indicate that the model performs well on both the training data and general data, demonstrating its capability to make accurate predictions. Upon examining the test performances, the R^2 values for LR, RF, and k-NN were calculated as 0.98, 0.98, and 0.93, respectively. The highest R^2 value and the lowest error metrics (MAE, MSE, RMSE) were obtained with the RF algorithm, indicating higher accuracy. However, it is also observed that the LR algorithm exhibits a performance very close to this level of accuracy.

Table 4. Performance metrics of algorithms on the test set
Çizelge 4. Test setinde kullanılan algoritmaların performans metrikleri

Algorithms	R^2	MAE	MSE	RMSE
Linear Regression	0.98	46152.704444	4103773000.0	64060.696764
Random Forest	0.98	40239.739185	3867224000.0	62187.010216
k-Nearest Neighbor	0.93	84995.350154	13417670000.0	115834.650608

R^2, coefficient of determination; MAE, Mean Absolute Error; MSE, Mean Squared Error; RMSE, Root Mean Square Error

The alignment analysis between the actual and predicted values, as visualized in the fit plot, indicates that the predictions from the RF algorithm are closely aligned with the y = x line at a 45-degree angle (Figure 1). This strong alignment demonstrates a high level of accuracy in the RF predictions. Notably, while the RF algorithm consistently outperformed the other models in terms of overall accuracy and flexibility, it is important to highlight that the LR algorithm also exhibited commendable performance, with R^2 values being very close to that of RF. This proximity in results underscores the effectiveness of LR as a reliable alternative in predicting beef production.

The effectiveness of the machine learning algorithms used in the current research is consistent with findings from previous studies conducted in agricultural contexts. Li et al. (2018) evaluated the performance of three different machine learning algorithms—Random Forest (RF), Gradient Boosting Machine (GBM), and eXtreme Gradient Boosting (XGB)—in predicting genomic breeding values using SNP markers and body weight phenotypes in Brahman cattle. Among the three methods, RF and GBM were reported to consistently outperform XGB in terms of genomic prediction accuracy. Similarly, Maya Gopal and Bhargavi (2019) employed machine learning techniques to accurately predict crop yields. In their study, Artificial Neural Network (ANN), Support Vector Regression (SVR), k-Nearest Neighbors (k-NN), and RF algorithms were selected. Their results indicated that the RF algorithm achieved the highest accuracy, as determined by error analysis values.

Mishra et al. (2021) aimed to predict the most suitable agricultural crop to be grown in a specific region by using k-NN and RF machine learning algorithms along with data on soil quality, NPK values, moisture, and expected rainfall. They reported that the RF algorithm demonstrated superior accuracy. In another study, Bhardwaj et al. (2024) used machine learning algorithms such as Logistic Regression (LogR), XGB, CatBoost (CB), Gradient Boosting (GB), RF, and Support Vector Machine (SVM) for the prediction and classification of livestock diseases. Their findings indicated that RF and CB outperformed the other algorithms, with the RF algorithm achieving 83.56% accuracy, a precision and recall score of 0.84, and an F1 score of 0.82.

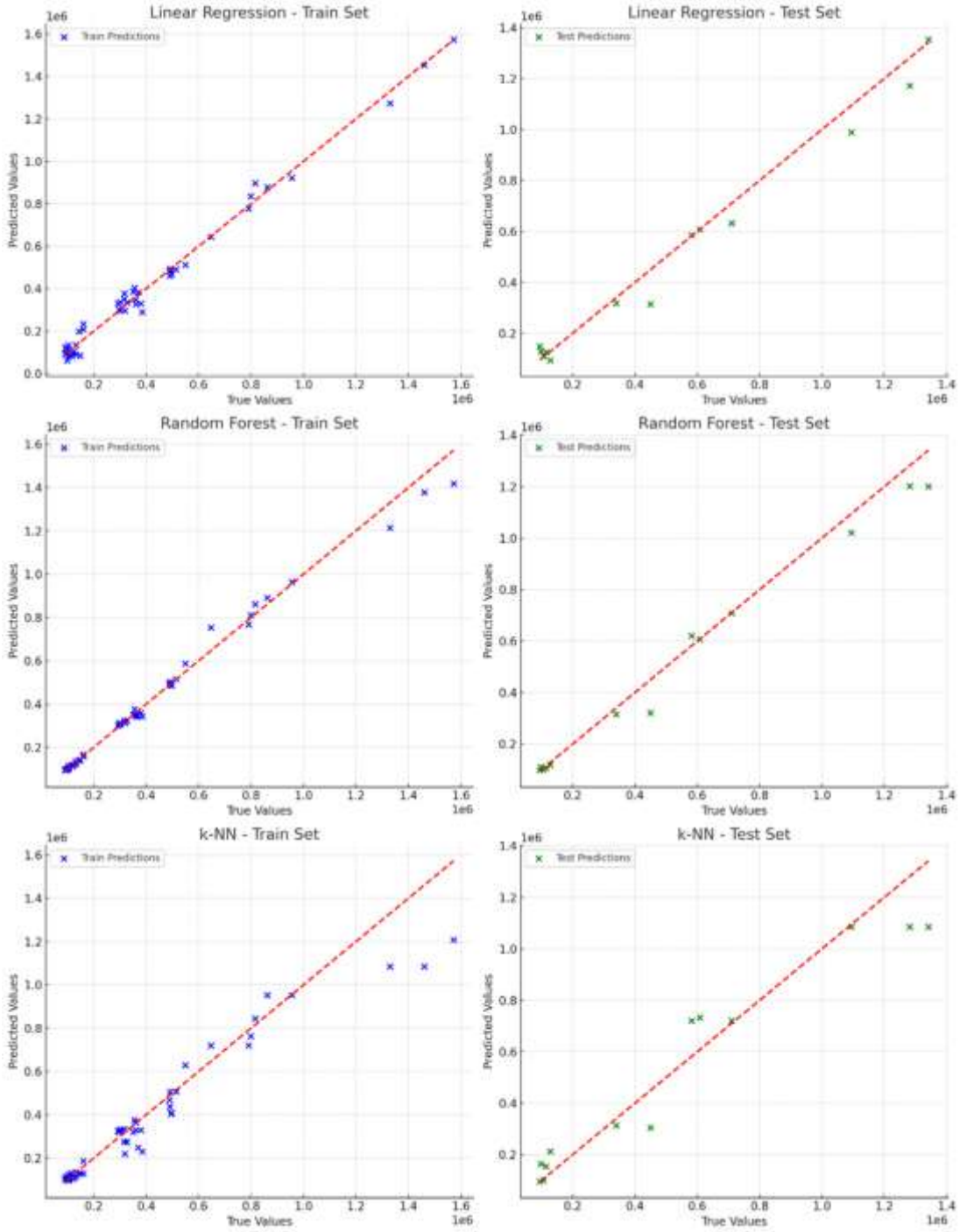

Figure 1. Alignment analysis between actual and predicted values
Şekil 1. Gerçek ve tahmin edilen değerler arasındaki uyum analizi

However, the performance of algorithms can vary across different studies. Yıldız et al. (2024) developed a model to predict honey production in Turkey using machine learning algorithms such as k-NN, RF, LR, and Gaussian Naive Bayes (GNB), based on data including honey production volume, number of farms, colony count, amount of pesticides used, agricultural and forest area, temperature, precipitation, and wind speed. Their results indicated that, among the four algorithms tested, LR was the most effective method for predicting honey production levels, with an R^2 value of 0.97. Similarly, Patel and Patel (2021) aimed to assist farmers in making informed crop selection decisions by developing a model to predict suitable crops for specific lands based on seasonal and soil parameters using popular supervised machine learning algorithms like SVM, k-NN, RF, and ANN. They found that the k-NN algorithm had superior performance metrics compared to the other approaches.

One possible reason for the poorer performance of the RF algorithm in these two studies could be its inherent

random operational mechanism. As a tree-based method, RF trains each tree on a specific random subset, causing the model's performance to vary depending on certain characteristics of the data (Breiman, 2001). This randomness can sometimes lead to suboptimal results, particularly when the relationships among the dataset features are linear.

In studies that typically employ boosting algorithms, the RF algorithm has often lagged in terms of performance. For instance, Alsahaf et al. (2018) used four different machine learning algorithms—RF, Extremely Randomized Trees (ET), GBM, and XGB—to predict the age at which pigs reach a slaughter weight of 120 kg, and they reported that GBM and XGB, which are sequential ensemble methods, achieved lower error metrics than RF and ET. Similarly, Luo et al. (2021) applied three machine learning algorithms—Random Forest Regression (RFR), XGB, and CatBoost (CB)—to predict forest above-ground biomass (AGB). Their results showed that the CB algorithm outperformed both XGB and RFR in predicting AGB across all forest types. Additionally, it was noted that CB, unlike XGB, includes an algorithm to calculate leaf nodes when selecting a tree structure, which can help prevent overfitting.

In another study aimed at developing a machine learning-based prediction model for marine fish and aquaculture production, Rahman et al. (2021) found that the RFR algorithm performed worse than the Gradient Boosting Regression (GBR) algorithm. Ultimately, an ensemble approach called Voting Regression (VR) was used to combine these three machine learning algorithms, and the best performance metrics were achieved by VR ($R^2 = 0.81$). In a separate study, Srivastava et al. (2021) evaluated the performance of RF, EGB, and SVM algorithms in predicting carcass weight (CWT), marbling score (MS), backfat thickness (BFT), and eye muscle area (EMA) in Hanwoo cattle. They reported that EGB provided the lowest MSE for CWT and MS, while SVM yielded the lowest MSE for BFT and EMA.

Additionally, Coşkun et al. (2023) compared the performance of EGB, RF, and Bayesian Regularized Neural Network (BRNN) data mining algorithms in predicting the live weights of Anatolian Merinos lambs using body trait data collected at the onset of the fattening period. Their findings indicated that the XGB algorithm produced better results than the RF and BRNN algorithms across several performance metrics, including RMSE, standard deviation ratio (SDR), mean absolute percentage error (MAPE), and adjusted coefficient of determination (R^2).

To enhance the interpretability of the model and aid in the optimization of beef production strategies, a feature importance analysis was conducted. The analysis results are presented in Figure 2, where each feature is represented by columns that indicate a specific level of importance based on its contribution to the model. The height of the columns reflects the impact of these features on the model's performance; thus, taller columns indicate that the corresponding feature contributes more significantly to predictive power. The analysis revealed that the attributes with the highest impact were population, corn production, and urban population, each with importance scores exceeding 0.15. These high-importance scores indicate that these are the most critical factors influencing beef production. Notably, population had the highest impact on the model with a score above 0.25, highlighting its central role. Increasing population necessitates a direct increase in beef production due to higher demand (FAO, 2024a; Godfray et al., 2018). This underscores the significance of population as a key driver in beef production, suggesting that production strategies should be aligned with population growth considerations.

Corn production was also identified as a significant factor due to its role as a primary feed source in cattle nutrition (Klopfenstein et al., 2013). The high impact of corn on the model indicates a direct influence on the amount of beef production. Therefore, increasing corn production could potentially enhance cattle nutrition, thereby boosting beef output. Urban population was another attribute with a substantial impact, reflecting the changing dietary habits and increased demand for high-quality protein sources associated with urbanization (FAO, 2024a). This trend correlates with the rising demand for beef, which is a primary source of high-quality protein.

These findings illustrate the complex interrelationships among various factors affecting beef production, emphasizing the need for strategic planning that considers these influential attributes.

CONCLUSION

In this study, the effectiveness of LR, k-NN, and RF algorithms in predicting beef production in Türkiye was examined. The findings indicate that the RF algorithm outperformed the other algorithms, demonstrating higher performance with superior R^2 values and lower error rates. The RF algorithm was also effective in evaluating the relative importance of features, enhancing model interpretability, and providing more accurate and reliable predictions for future beef production.

Ensuring food security and planning agricultural policies sustainably will become increasingly important in the coming years. In this context, the high accuracy provided by the RF algorithm could play a critical role in predicting beef production outcomes and addressing potential food security challenges. Given factors such as population growth, climate change, and the reduction of agricultural lands, such predictions can serve as strategic tools in

shaping agricultural policies and preventing future food deficits. The results of this study demonstrate the applicability of the RF algorithm in agricultural data analysis and production prediction, offering forward-looking solutions for agricultural sustainability.

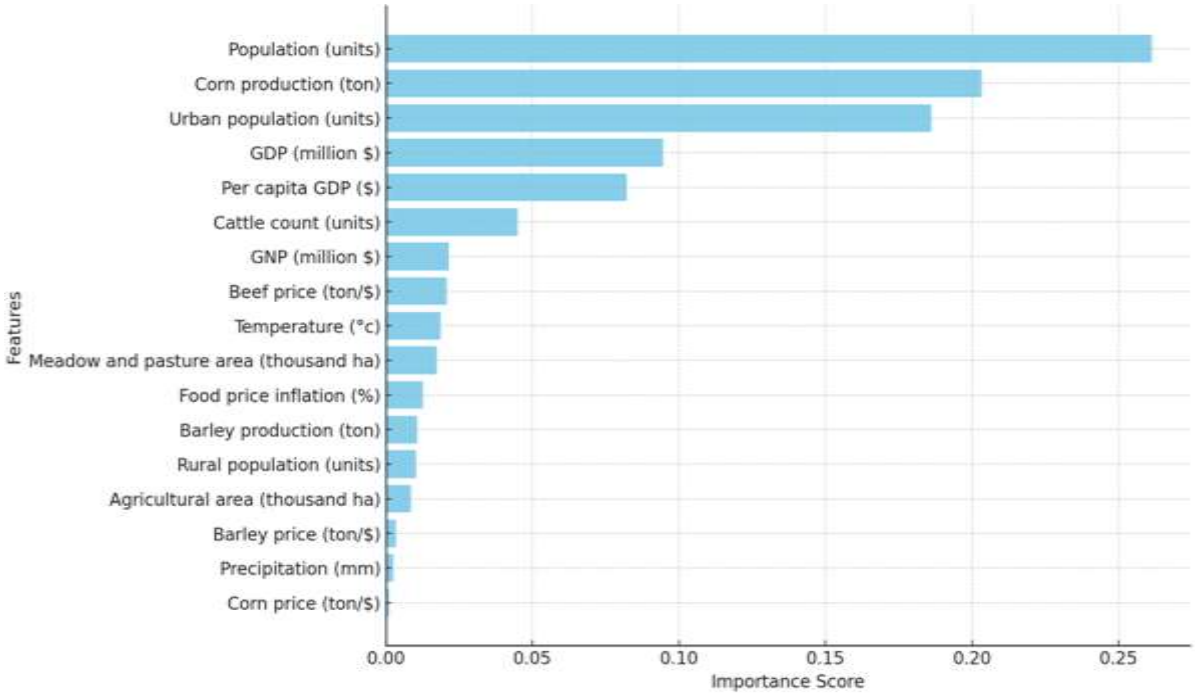

Figure 2. Feature importance analysis for predicting beef production

Şekil 2. Sığır eti üretimini tahmin etmek için özellik önem analizi

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors have declared no conflict of interest.

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Aspir Genotiplerinin Sap Verimi ve Samanlarının Bazı Yem Değerlerinin Belirlenmesi

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ÖZET

Bu çalışma, aspir (*Carthamus tinctorius* L.) genotiplerinin sap verimi ve bazı yem değerlerinin belirlenmesi amacıyla yapılmıştır. Araştırma, 2022-2023 yıllarında Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü'nde Tesadüf Blokları deneme desenine göre dört tekerrürlü olarak gerçekleştirilmiştir. Materyal olarak melezleme yoluyla geliştirilen 10 aspir hattı, 5 tescilli aspir çeşidi (Koç42, Dinçer, Balcı, Linas, Göktürk) ve Taner ekmeklik buğday çeşidi kullanılmıştır. Sonuçlar, aspir çeşitleri arasında en yüksek ortalama sap veriminin 308 kg da^{-1} ile Koç 42 çeşidinden elde edildiğini, bunu 237 kg da^{-1} ile Göktürk ve 224 kg da^{-1} ile Linas çeşitlerinin izlediğini göstermiştir. Taner ekmeklik buğday çeşidinin sap verimi 241 kg da^{-1} ile altıncı sırada yer almıştır. Sap verimi açısından Taner çeşidinden daha yüksek verim sağlayan 4 aspir hattı ve 1 aspir çeşidi bulunmaktadır. Aspir genotiplerinin sap verimlerinin buğday samanı ile rekabet edebilir düzeyde olduğunu ve bazı durumlarda daha yüksek verim sağladığı tespit edilmiştir. Araştırmada kullanılan materyallerin Asit çözücülerde çözünmeyen lif (ADF), Nötral çözücülerde çözünmeyen lif (NDF), ham selüloz (HS) ve ham protein (HP) içerikleri analiz edildiğinde, aspir samanının genel olarak ham protein ve selüloz değerleri buğday samanına yakın veya daha yüksek olarak tespit edilmiştir. Bulgular aspir samanının genel olarak buğdaydan daha düşük ADF ve NDF içeriğine sahip olduğunu, bu nedenle hayvan yemi olarak daha iyi besin değerine sahip olduğunu ortaya koymuştur.

Zootekni

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 03.07.2024
Kabul Tarihi : 26.12.2024

Anahtar Kelimeler
Aspir
ADF
NDF
Ham Protein
Selüloz

Determination of Stalk Yield and Some Feed Values of Straw from Safflower Genotypes

ABSTRACT

This study was conducted to determine the stem yield and some forage values of safflower (Carthamus tinctorius L.) genotypes. The research was carried out at Bahri Dağdaş International Agricultural Research Institute in 2022-2023 with four replications according to the random block design. Ten safflower lines developed by crossbreeding, five registered safflower varieties (Koç42, Dinçer, Balcı, Linas, Göktürk) and bread wheat variety Taner were used as materials. The results showed that the highest average stem yield among safflower varieties was obtained from Koç 42 variety with 308 kg da-1, followed by Göktürk variety with 237 kg da-1 and Linas variety with 224 kg da-1. The stem yield of bread wheat cultivar Taner ranked sixth with 241 kg da-1. There are 4 safflower lines and 1 safflower cultivar with a higher stem yield than Taner cultivar. The stem yields of the safflower genotypes were found to be competitive with wheat straw and in some cases higher. When the acid-soluble fibre (ADF), neutral soluble fibre (NDF), crude cellulose (HS), and crude protein (HP) contents of the materials used in the study were analyzed, it was found that the crude protein and cellulose values of safflower straw were generally close to or higher than those of wheat straw. The results showed that safflower straw generally had lower ADF and NDF contents than wheat straw and therefore had better nutritional value as animal feed.

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GİRİŞ

Aspir (*Carthamus tinctorius* L.), çok yönlü bir yağlı tohum bitkisidir. Soğuğa, kuraklığa, tuzluluğa dayanıklı olup, üretim maliyeti de düşüktür (Ebrahimi ve ark., 2017). Aspir, çoklu doymamış (linoleik) ve tekli doymamış (oleik) yağ asitlerinden oluşan yüksek kaliteli yemeklik yağı yanında birçok alanda kullanılabilmektedir. Aspir, gıda, tıbbi, endüstriyel, hayvan yemi ve çiçekçilik amacıyla kullanılır (Dinçel, 2024). Soğuğa, kuraklığa ve tuzluluğa toleranslı olduğu için kurak ve yarı kurak iklimlerde yetiştirilebilen bir bitkidir (Öztürk ve ark., 2008; Janmohammadi, 2015).

Doğal çayır mera alanları ve yetiştirilen yem bitkileri üretimi Türkiye'de kaliteli yem kaynaklarının temelini oluşturmaktadır. Bu çayır ve meraların otlatma yönetiminde amenajman ilkelerine uyulmaması sebebiyle bu alanların yem üretim potansiyeli büyük ölçüde kaybolmayla yüz yüzedir. Bunun yanında tüm tarla bitkileri içerisinde yem bitkileri üretiminin payı düşüktür. Bu faktörlerden dolayı mevcut hayvan varlığının kaba yem ihtiyacı karşılanamamaktadır (Özkan & Şahin Demirbağ, 2016; Bıçakçı & Açıkbaş, 2018). Bu yüzden, serin iklim tahıllarının hasatından sonra elde edilen sap ve saman artıkları, çayır-mera ve yem bitkilerinden elde edilen kaba yemlerin yanında büyük ölçüde kullanılmaktadır (Açıkbaş & Özyazıcı, 2019).

Buğday samanı, Türkiye'de üretilen samanların büyük bir kısmını oluşturur. Fakat samanlar genellikle düşük protein ve yüksek ham selüloz, lignin, hemiselüloz ihtiva ettikleri için sindirilebirlikleri düşüktür (Abdi & Kılıç, 2018). Bu durumda saman tüketiminin hayvanlara sadece tokluk hissi verdiği kanaati oluşmaktadır. Uygun ve dengeli besleme için hayvanlara yedirilen yemlerin içerdikleri besin maddelerinin miktarının bilinmesi büyük önem taşımaktadır (Açıkbaş & Özyazıcı, 2019).

Aspir bitkisi tohum ve yağı dışında, kaba yem kaynağı olarak da ideal bir bitkidir. Aspir bitkisinin sapları, yaprakları ve çiçekleri, yaş veya kuru olarak, silaj yapılarak hayvan beslenmesinde kaliteli kaba yem olarak kullanılabilir. Bunun yanında aspir yem olarak kullanıldığında antimetanolojik etkisi düşük olduğu için metan (CH_4) salınımını azaltma potansiyeli bulunmaktadır. (Gümüş & Küçükersan, 2016; Selçuk ve ark., 2023).

Aspir, sığırlar tarafından otlatılarak ya da saman ve silaj olarak kullanılabileceği vurgulanmaktadır. (Weinberg ve ark., 2002; Peiretti, 2017). Ancak, aspir yaprakları ve çiçek salkımları dikenler ürettiğinden, hayvanlar tarafından tüketimi sınırlanmaktadır. (Landau ve ark., 2004). Bu yem tüketimi sorunu, bitki büyüdükçe ve olgunlaştıkça daha da kötüleşmektedir. Dikenlerin varlığından kaçınmak için aspir bitkisinin tomurcuklanma aşamasında, dikenler henüz tam olarak gelişmemişken hasat etmek, pratik bir stratejidir. Ancak yapılan araştırmalar, aspirin tomurcuklanma başlangıç aşamasında kuru madde potansiyel veriminin düşük olduğunu göstermiştir (Reta ve ark., 2017). Geç tomurcuklanma aşamasında hasat edilen dikensiz aspir samanı, gebe Holstein inekleri için tek yem olarak başarıyla kullanılmıştır (Leshem ve ark.,2001). Koyunlarda aspir samanının bazı özellikleri nedeniyle yonca- samanından daha üstün olduğunu ve doğurganlıklarını artırdığını bildirilmiştir (Stanford ve ark., 2001).

Bitkinin dikenli yapısı doğal olarak hayvanlar tarafından tüketimini sınırlamaktadır. Bu nedenle saman olarak kullanımı önerilmektedir (Mündel ve ark., 2004). Bu çalışma ile aspir genotiplerinin sap verimi ve samanın bazı yem değeri özelliklerinin belirlenmesi ve Türkiye'de yoğun olarak kullanılan buğday samanı ile karşılaştırılması amaçlanmıştır.

MATERYAL VE METOT

Bu çalışma, Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü deneme alanlarında, aspir ıslah çalışmaları kapsamında kurulan verim denemeleri çerçevesinde gerçekleştirilmiştir. 2022 ve 2023 yıllarında melezleme ile geliştirilen 10 aspir hattı, 5 tescilli aspir çeşidi (Koç42, Dinçer, Balcı, Linas, Göktürk) ve bölgede yaygın olarak üretimi yapılan Taner ekmeklik buğday çeşidi kullanılmıştır. Denemeler, tesadüf blokları deneme desenine göre dört tekerrürlü olarak düzenlenmiştir. Deneme parselleri, 1,2 m x 5 m boyutlarında olup, her bir parselin alanı 6,0 m² olarak belirlenmiş ve ekimler gerçekleştirilmiştir.

Ekim işlemi her iki yılda da Nisan ayının ilk haftasında yapılmış olup, sıra arası mesafe 20 cm olarak ayarlanmıştır. Hasat işlemi ise her iki yılda da ağustos ayının son haftasında, parsel alanının tamamında (6,0 m²) parsel biçerdöveri kullanılarak gerçekleştirilmiştir.

Hasattan sonra, her parselde kalan saplar tartılarak sap verimi hesaplanmış, daha sonra parsellerden alınan sap parçaları ot değirmeninde öğütülerek saman haline getirilmiş ve yem değerini saptamak üzere enstitü laboratuvarlarına getirilmiştir. Saman numunelerinin Asit çözücülerde çözünmeyen lif (ADF), Nötral çözücülerde

çözünmeyen lif (NDF), ham protein (HP) ve ham selüloz (HS) analizleri gerçekleştirilmiştir. Hasat, hasattan sonraki deneme tarlasındaki saplar ve öğütülmüş, analize hazır hale getirilmiş numuneler Resim 1 ve Resim 2'de gösterilmiştir.

Şekil 1 . Parseller hasat edildikten sonra geriye kalan saplar
Figure 1. Stalks remaining after plots were harvested

Şekil 2. Öğütülerek kalite analizleri için hazır hale gelmiş saman numuneleri
Figure 2. Straw samples prepared for quality analysis after milling

Ham protein oranı (%KM): Dumas yöntemine göre, AOAC 992. 23 metodu kullanılarak (azot oranı * 6.25) azot tayin cihazı LECO FP 528 ile belirlenmiştir (Anonymous, 2009).

ADF), NDF (%) ve selüloz (%) analizleri, Van Soest ve ark. (1991) metoduna göre A 200I model marka cihazla belirlenmiştir. Nem Oranı (%): Örnekler etüvde 105 ^{o}C' de 4 saat kurutularak saptanmıştır (Elgün ve ark., 2005). Elde edilen verilerin varyans analizi, JUMP (JMP) istatistik programı kullanılarak yapılmıştır. Ortalamalar arasındaki farklılıklar ise aynı programda Least Significant Difference (LSD) testi ile incelenmiştir.

BULGULAR ve TARTIŞMA

Araştırmada kullanılan aspir genotiplerinin 2022-2023 yılı birleştirilmiş sap verimlerine ait varyans analiz sonuçları saptanmış ve Çizelge1' de verilmiştir. Birleştirilmiş varyans analiz sonuçlarına göre sap verimi değerleri bakımından yıllar, genotipler ve genotip-yıl interaksiyonu önemli bulunmuştur ($p<.01$). Genotip-yıl etkileşiminin önemli bulunması, çevresel faktörlerin yıldan yıla farklılık göstermesinden ve bu genotiplerin değişik çevre koşullarına farklı tepkiler vermesinden kaynaklanmaktadır.

Çizelge 1. Araştırmada kullanılan genotiplerin sap verimlerine ait yılların birleştirilmiş varyans analiz sonuçları

Table 1 Combined analysis of variance results of the genotypes used in the study for stalk yields

Varyasyon Kaynağı *Source of Variation*	**SD** *DF*	**KO** *MS*	***Pr>F***
Genel *(Total)*	127	4000.7	<.0001**
Yıllar *(Years)*	1	60179.7	<.0001**
Tekrarlar *(Replication)*	3	944.6	0.1442
Genotipler *(Genotypes)*	15	12634.3	<.0001**
Genotip x Yıl *(GenotypesxYear)*	15	13865.3	<.0001**
Hata *(Error)*	93	511.7	

**p < .01:Önemli (%1); *p < .05: Önemli (%5); SD: Serbestlik Derecesi, KO: Kareler Ortalaması
***p<.01: Significant (1%), *p<.05: Significant (5%) DF: Degrees of Freedom, MS: Mean squares*

Araştırmada kullanılan aspir genotiplerin yıllara göre sap verimleri saptanmış Çizelge 2'de verilmiştir. Çizelge 2'de görüldüğü gibi aspir çeşitleri arasında en yüksek ortalama sap verimi 308 kg da^{-1} ile Koç 42 çeşidinden elde edilmiştir. Bu verimi, 237 kg da^{-1} ile Göktürk ve 224 kg da^{-1} ile Linas çeşitleri takip etmiştir. Taner ekmeklik buğday çeşidi ise 241 kg da^{-1} ile altıncı sırada yer almıştır. Sap verimi açısından Taner çeşidinden daha yüksek verim sağlayan 4 aspir hattı ve 1 aspir çeşidi bulunmaktadır. En düşük sap verimi ise 160 kg da^{-1} ile Dinçer çeşidinden elde edilmiştir.

Çizelge 2. Araştırmada kullanılan genotiplerin sap verimi değerleri
Table 2. Stalk yield values of the genotypes used in the study

		Sap Verimi (kg da^{-1}) *Stalk yield (kg da^{-1})*		
Hat No *Line No*	**Genotipler** *Genotypes*	**2022**	**2023**	**Ortalamalar** *Means*
1	Koç42	313±6 a	303 ±13 abc	308±6 a
2	BD13	312±6 a	277±4 bcd	295±7 ab
3	BD15	230±13 cd	329±4 a	279±19 bc
4	BD20	232±15 c	293±21 abc	262±16 cd
5	BD12	274±4 b	242±4 de	258±6 cde
6	**Buğday (Taner)**	**304**±13 **a**	**179** ±4 **gh**	**241**±8 **def**
7	BD16	171±17 fg	311±19 ab	241±29 def
8	Göktürk	202±15 e	272 ±8 cd	237±15 ef
9	BD17	144±6 h	304±10 abc	224±30 fg
10	Linas	243±9 c	205±20 fg	224±12 fgh
11	BD14	206±3 de	241±1 de	223±6 fgh
12	BD24	117±4 ı	299±16 abc	208±35 ghı
13	BD19	147±12 gh	256±19 de	202±23 hı
14	Balcı	162±11 fgh	228±13 ef	195±15 ı
15	BD18	184 ±11 ef	197±4 fg	191±6 ı
16	Dinçer	162±7 fgh	159±5 h	160±4 j
LSD(%5)		25	36	22
CV(%)		8	9	9

Çalışkan ve Yüksel (2022), farklı gelişme dönemlerinde aspirin kuru ot verimlerini 303 kg da^{-1} ile 585 kg da^{-1} arasında, Strasil ve Vorlicek (2002) 369 kg da^{-1} ile 756 kg da^{-1}, Yau (2007) 331 ile 519 kg da^{-1} arasında bulmuşlardır. Fakat bu çalışmalarda farklı olarak aspirin danesini hasat ettikten sonra kalan sapının değil, tohum

olgunlaşmadan bitki biçilerek tartılmıştır. Araştırıcılar, bitkinin dikenli olması sebebiyle daha erken devrede hayvanlara yedirilebileceğini belirtmişlerdir. Hasat daha olgun zamanda gerçekleştiğinde lif oranında artma ve toplam kuru madde içeriğinde azalma olmaktadır. Bunun nedeni bu dönemde bitkinin daha az yapraklı ve büyük ve kalın gövdesinin olmasıdır (Corleto ve ark.,2005). Kuru madde içeriğindeki azalmanın ve lif oranındaki artışın, hasatların daha olgun gelişme aşamalarda gerçekleştirilmesinde bitkinin biokütlesinde daha az yaprak oranı ve daha büyük gövde ile ilişkili olduğunu belirtmiştir. Başka bir çalışma, daha olgun gelişme aşamalarda aspir yeminde lif artışının, yapraklar ve gövdelerden tohumlara çözünebilir hücresel içeriğin taşınmasından kaynaklandığını ifade etmektedir (Peiretti, 2009). Yau (2009) yaptığı çalışmada hasattan sonra aspirde ortalama sap verimini 269 kg da^{-1} bulmuştur. Bulduğumuz sonuçlar bu değere yakındır. Aspirin dikenli olması doğal olarak endişe yaratmaktadır. Türkiye'de çiftçiler aspir hasadı esnasında biçerdöverim arkasına saman makinesi takarak bitki artıklarını aspir sapını saman haline getirdikten sonra hayvanlarına yedirdikleri, tohumlarını da yemeklik yağ için kullandıkları bilinmektedir. Çalışmada elde edilen bulgular; aspirin sap veriminin, samanı yoğun olarak kullanılan buğdayla karşılaştırıldığında tatminkâr düzeyde olduğunu göstermektedir.

Çalışmada kullanılan hat ve çeşitlerin ADF, NDF, selüloz, ham protein oranlarına ait değerler Çizelge 3'de verilmiştir. Aspir samanlarının ADF değerleri iki yıllık ortalamalara göre %39.8 ile %49.1 arasında değişmektedir. Buğdayda samanında ise bu değer %45.4 olarak tespit edilmiştir. Toplam 13 adet genotipin ADF değeri buğday samanından daha düşük saptanmıştır (Çizelge3).

Çizelge 3. Araştırmada kullanılan genotiplerin samanlarının bazı yem kalite değerleri (%KM)
Table 3. Some feed quality values of straw of the genotypes used in the study (DM %)

	ADF (%)			NDF (%)			Selüloz (*Cellulose)(%)*			Ham protein *(Crude Protein)(%)*		
Genotipler *Genotypes*	2022	2023	Ort.	2022	2023	Ort. Mean	2022	2023	Ort.	2022	2023	Ort. Mean
Koç42	40.1	43.2	41.6±1.5	54.2	52.4	53.3±0.8	37.6	35.9	36.7±0.8	4.4	4.6	4.5±0.10
Göktürk	45.1	42.8	43.9±0.5	55.9	52.3	54.1±1.7	39.0	35.4	37.2±1.7	3.1	5.3	4.2±1.08
Balcı	42.3	41.0	41.6±0.6	55.4	50.2	52.8±2.5	37.7	33.9	35.8±1.9	3.7	5.7	4.7±0.96
Linas	43.6	41.8	42.7±0.8	55.5	49.9	52.7±2.7	38.5	34.0	36.2±2.2	4.0	5.4	4.7±0.68
Dinçer	44.7	41.7	43.2±1.5	57.3	50.6	53.9±3.3	40.6	34.3	37.4±3.1	3.8	5.9	4.8±1.02
BD12	44.1	43.0	43.5±0.5	58.0	51.6	54.8±3.2	39.6	34.5	37.0±2.5	4.5	5.0	4.7±0.28
BD13	39.1	41.3	40.2±1.1	50.3	50.1	50.2±0.1	34.5	33.6	34.0±0.4	4.5	5.4	4.9±0.45
BD14	51.5	46.8	49.1±2.3	65.6	50.7	58.1±7.4	44.3	38.3	41.3±3.0	3.3	5.9	4.6±1.28
BD15	41.3	42.2	41.7±0.5	52.9	51.0	51.9±0.9	36.1	34.4	35.2±0.8	4.9	5.2	5.0±0.15
BD16	50.2	44.0	47.1±3.0	62.9	55.2	59.0±3.8	43.1	38.7	40.9±2.2	3.6	4.6	4.1±0.50
BD17	40.0	41.9	40.9±0.9	53.3	49.5	51.4±1.8	35.9	33.6	34.7±1.1	5.4	5.5	5.4±0.06
BD18	39.6	42.2	40.9±1.3	52.0	50.8	51.4±0.6	34.0	34.3	34.1±0.1	4.3	5.2	4.7±0.46
BD19	44.3	40.5	42.4±1.8	57.5	50.3	53.9±3.5	38.8	33.9	36.3±2.5	4.7	5.8	5.2±0.53
BD20	45.1	41.8	43.5±1.7	58.7	51.3	55.0±3.6	41.7	34.6	38.1±3.5	3.4	5.3	4.3±0.97
BD24	37.1	42.4	39.8±2.6	48.6	50.5	49.5±1.0	33.4	34.1	33.7±0.3	4.1	5.4	4.7±0.62
Buğday (Taner)	44.2	46.6	45.4±1.5	60.6	59.4	60.0±7.0	38.4	39.4	38.9±1.0	4.8	5.0	4.9±0.07

ADF: Asit çözücülerde çözünmeyen lif, NDF: Nötral çözücülerde çözünmeyen lif
ADF: Fibre insoluble in acid solvents, NDF: Fibre insoluble in neutral solvents

Hayvan sağlığı ve ekonomiklik açıdan optimum ADF miktarını bilinmesi ve uygun miktarda verilmesi gerekir. Asit çözücülerde çözünmeyen lif içeriği yüksek yemlerin aşırı miktarda verilmesi sonucu yem alımı düşer ve hayvanda beklenen verimlilik oluşmaz. Yemlerin ADF içeriğinin düşük olması ise ilk olarak asidoz olmak üzere abomosum deplasmanı, laminitis, süt yağı oranının düşmesi gibi sonuçlar ortaya çıkarır (Avellaneda ve ark., 2009). Asit çözücülerde çözünmeyen lif oranı kuru madde bazında %25-30 civarında olmalıdır (Tekçe & Gül, 2014). Bu çalışmada ADF değerleri optimum düzeyden daha yüksek olmakla birlikte BD24 hattında %39.8, BD13 hattında %40.2, BD17 hattında %40.8 ve BD18 hattında %40.9, olarak belirlenmiştir (Çizelge 3).

Aspirde ADF oranları da hasat dönemlerine göre değişiklik göstermiş ve ilerleyen hasat döneminde ADF oranlarının arttığı gözlemlenmiştir. En düşük ADF oranı tabla çıkarma döneminde %27.61 olarak belirlenmiştir (Çalışkan & Yüksel,2022).

Aspir bitkisi tabla çıkarma devresinde optimum ADF değerine sahip olmakla birlikte Türkiye'de yemeklik yağ ihtiyacını karşılamak için aspirin erken dönemde hasadı ve hayvanlara yedirilmesi imkânı yoktur.

Aspirde ADF değerlerini Bar-Tal ve ark. (2008) %30.9 ile % 43.9 arasında, Peiretti (2009) %41.5, Arslan ve ark. (2012) %36.4, Selçuk ve ark. (2023) %34.79 ile %39.73 arasında bulmuşlardır. Araştırmacılar arasındaki bu farklılıklar kullanılan genotiplerin farklı olmasından ve farklı olgunlaşma dönemlerinde hasat edilmesinden kaynaklandığı söylenebilir. Nitekim yapılan çalışmada da aspir genotipleri arasında ADF bakımından geniş bir varyasyon olduğu saptanmıştır.

Kaba yemlerin kalitesi açısından ADF içeriğinin düşük olması tercih edilir. Yemin sindirilebilirliğinin yüksek olması, yemin hücre duvarı bileşenlerinin düşük olmasına bağlıdır (Van Soest, 1994; Kaya, 2008). Yapılan çalışmalarda, buğday samanının ADF içeriğinin %46.8 ile %47.5 arasında değiştiği bildirilmiştir (Eser, 2016; Abdi & Kılıç, 2018). Bu çalışmada Taner ekmeklik buğday çeşidinin samanının ADF değeri %45.4 ile bu değerlere yakın bulunmuştur. Yapılan diğer çalışmalarda da buğdayın ADF değeri istenen düzeyden daha yüksek olması esasında buğday samanının besleyicilik özelliğini düşürmektedir. Nitekim,Yavuz (2005) tarafından yapılan çalışmada buğdayın samanının nispi yem değeri, diğer bitki samanlarına göre oldukça düşük bulunmuştur.Bu çalışmada aspir genotiplerinin NDF değerleri iki yıllık ortalamalara göre %49.5 ile %59 arasında değişmektedir. Buğday samanında ise bu değer % 60 olarak tespit edilmiştir. Aspir genotiplerinin NDF değerlerinin tamamı buğday samanına göre daha düşük olmuştur (Çizelge 3).

Ruminantlarda maksimum verimi sağlamak ve sürü sağlığını korumak için her zaman NDF'ye ihtiyaç vardır. Yüksek verimli ruminant rasyonları, en iyi şekilde çiğneme kolaylığı, uygun rumen fermentasyonu, yeterli partikül boyutuna sahip, NDF içeriği optimal olan kaba yemlerden oluşmalıdır (Lean ve ark., 2007). Ruminant beslenmesinde, NDF oranının kuru madde bazında %25-32 arasında olması, en uygun verim elde edilmesini sağlar (Tekçe & Gül, 2014).

Aspir samanında yapılan çalışmalarda, NDF içeriğini %44.8 ile %54.8 arasında bildirmişlerdir (Bar-Tal ve ark., 2008; Arslan ve ark.,2012; Selçuk ve ark., 2023). Bu çalışmada ise aspir genotiplerinin NDF değerleri %49.5 ile %59 arasında değişmiş olup bu değerler diğer araştırmacılar tarafından bulunan değerlere yakındır. Gerek bu çalışmada bulunan sonuçlar gerekse diğer araştırmacılar tarafından bulunan sonuçlar, aspir samanının NDF değeri açısından yem değerinin buğday samanından daha iyi olduğunu göstermektedir. Aspir genotiplerinde NDF içeriği bakımından geniş bir varyasyon mevcut olup çalışmada iki yıl ortalaması olarak BD24 hattından %49.5, BD17 v1 BD18 hatlarından %51.4 değerleri tespit edilmiştir. Bu değerler %60 olan buğday samanı NDF içeriğinin altındadır. Fakat %25-32 olan optimum değerlerden uzaktır. Bu optimum değerlere ancak aspir hasat olgunluğuna gelmeden önceki dönemlerde ulaşılabiceği düşünülmektedir Nitekim araştırıcılar tarafından Aspirde farklı hasat dönemlerinde yemlik kalite değerleri belirlenmiştir. NDF değeri hasat dönemlerinden önemli ölçüde etkilenmiş olup, en düşük NDF oranı %31.30 ile tabla çıkarma döneminde yapılan biçimlerde belirlenmiştir (Çalışkan & Yüksel, 2022). Bu dönemdeki hasat üretici gelirinde kayba neden olacağı için samanı değerlendirmeye alınmıştır.

Buğday samanının NDF oranının ADF'de olduğu gibi düşük olması istenir (Kaya, 2008). Buğday samanı üzerine yapılan çalışmalarda NDF içeriğini %54.4 ile 89.3 arasında değiştiği bildirilmiştir (Can ve ark., 2004; Kalkan & Filya, 2011; Eser, 2016; Abdi & Kılıç, 2018; Açıkbaş & Özyazıcı, 2019). Bu çalışmada buğday samanı için NDF değeri ortalama %60 olarak tespit edilmiştir. Araştırmalar arasındaki bu farklılıklar kullanılan çeşit ve iklim koşullarına bağlanabilir.

Aspir genotiplerinin selüloz değerleri iki yıllık ortalamalara göre %33.7 ile %41.3 arasında değişmektedir. Buğday samanında ise bu değer %38.9 olarak tespit edilmiştir. Aspir genotiplerinin samanlarının selüloz değerleri buğdayın değerlerine yakındır (Çizelge 3).

Rumende selülozun parçalanması sonucu, asetik asit, bütirik asit ve propiyonik gibi uçucu asit yağ asitleri oluşur. Ayrıca, selülozun varlığı, tükürük üretimini artırarak rumen pH'sının optimum seviyede kalmasına yardımcı olur ve bu sayede ruminantları bazı metabolik hastalıklardan korur (Argov-Argaman ve ark., 2012; Kaur ve ark., 2013).

Aspir genotiplerinin ham protein değerleri iki yıllık ortalamalara göre %4.1 ile %5.4 arasında değişmektedir. Buğday samanında ise bu değer %4.9 olarak tespit edilmiştir. Aspir genotiplerinin samanlarının protein değerleri Taner buğday çeşidinin değerlerine yakındır (Çizelge 3). Araştırmada kullanılan aspir genotiplerinden BD17 %5.5, BD19 %5.2, BD15 %5.0 ile daha yüksek protein elde edilmiştir. Yaygın olarak ekilen aspir çeşitlerinin (Koç42, Göktürk, Linas, Balcı ve Dinçer) protein içeriği ise %4.2-4.8 arasında değişmiştir.

Aspir samanında protein oranını; Bar-Tal ve ark. (2008) %8.1-12.8, Çağrı ve Kara (2018) %3.74, Selçuk ve ark. (2023) %6.4-%10.5 arasında olduğunu bildirmişlerdir. Çeşitler arasındaki farklılıkların, çeşitlerin kendisinden, hasat zamanından ve yetiştirildikleri bölgenin iklim özelliklerinden kaynaklandığı ifade edilebilir. Nitekim aspir bitkisinin farklı hasat dönemlerinde yapılan çalışmada, ham protein oranları %8.36 ile %12.29 arasında değişim göstermiştir. Hasat dönemi ilerledikçe, protein oranlarının azaldığı gözlemlenmiştir. En yüksek ham protein oranı

%12.2 ile tabla çıkarma döneminde kaydedilmiştir (Çalışkan & Yüksel, 2022).

Araştırmalarda buğday samanı için protein içeriğinin %2.42 ile %5 arasında değiştiği bildirilmiştir (Güngör ve ark.,2008; Eser, 2016; Abdi & Kılıç, 2018; Açıkbaş & Özyazıcı, 2019). Çalışmamızda buğday samanı için tespit edilen %4.9 değeri bu sınırlar içerisindedir. Yapılan çalışmalarla tespit edildiği gibi aspir ve buğday samanının protein değerleri birbirine yakın olup düşüktür.

SONUÇ

Bu çalışma, aspir genotiplerinin sap verimi ve samanlarının yem değerlerinin belirlenmesi ve Türkiye'de yaygın olarak kullanılan buğday samanı ile karşılaştırılması amacıyla gerçekleştirilmiştir. Araştırma sonuçları, aspir genotiplerinin sap verimlerinin buğday samanı ile rekabet edebilir düzeyde olduğunu, bazı genotiplerin ise daha yüksek verim değerlerine ulaşabildiğini göstermiştir. Bu durum, aspir bitkisinin samanının tarımsal üretimde alternatif bir yem kaynağı olarak değerlendirilebileceğine işaret etmektedir.

Yapılan analizlerde, aspir samanının ADF (asit deterjan lif), NDF (nötral deterjan lif), ham selüloz (HS) ve ham protein (HP) içerikleri incelenmiştir. Bulgular, aspir samanının buğday samanına kıyasla genellikle daha düşük ADF ve NDF değerlerine sahip olduğunu ve buna bağlı olarak daha yüksek sindirilebilirliğe sahip olduğunu göstermiştir. Ayrıca, aspir genotiplerinin ham protein içeriklerinin buğday samanına yakın veya daha yüksek olduğu tespit edilmiştir. Bu sonuçlar, aspir samanının hayvan beslenmesinde daha kaliteli bir kaba yem kaynağı olabileceğini ortaya koymaktadır.

Aspir bitkisinin dikenli yapısı nedeniyle doğrudan tüketimi zor olsa da saman haline getirilerek hayvan yemi olarak kullanılması daha uygun ve verimli bir yöntem olarak öne çıkmaktadır. Özellikle Türkiye gibi kurak ve yarı kurak iklim kuşaklarında aspir bitkisi, hayvancılık sektörünün kaba yem ihtiyacını karşılayabilecek uygun bir alternatif olarak değerlendirilebilir. Aspir samanının hayvan beslenmesinde kullanılması, yem maliyetlerini düşürebileceği gibi, besin değerlerini artırarak ruminant hayvanların verimliliğine katkıda bulunabilir.

Sonuç olarak, bu araştırma aspir bitkisinin samanlarının, Türkiye'de yaygın olarak kullanılan buğday samanı ile karşılaştırıldığında hem kalite hem de verim açısından avantajlı bir kaba yem kaynağı olduğunu ortaya koymuştur. Bu bulgular doğrultusunda, aspir bitkisinin yaygın üretiminin teşvik edilmesi ve samanının hayvan yemi olarak kullanımının desteklenmesi önerilmektedir. Aynı zamanda, yaygın olarak ekilen diğer yağlı tohumlu bitkilerin samanlarının da yem olarak değerlendirilmesi, hayvancılık sektörünün kaba yem ihtiyacının karşılanmasında önemli bir potansiyel sunmaktadır.

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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Influence of Breed, Season, Sex, and Parity on Mortality of Holstein Friesian and Brown Swiss Calves

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ABSTRACT

This study aimed to investigate the effects of season, parity, sex, and breed on preweaning mortality in calves. Records of 1890 Brown Swiss and Holstein Friesian calves born and reared in Atatürk University Cattle Farm, Erzurum, were used in the study. The data of the study were analyzed using the Chi-Square test available in the SPSS statistical program. Study results showed that season had a statistically significant impact on calf mortality ($p < 0.05$). The highest mortality rate was observed in spring (11.5%) and winter (11.0%) born calves, while the mortality rate was significantly lower in summer (7.3%) and autumn (6.3%) born calves. There was no statistically significant effect of calf sex on mortality. Similarly, calves born to primiparous and multiparous dams did not differ significantly in terms of mortality. The mortality rate of Holstein Friesian calves was slightly lower than Brown Swiss calves. While 87.5% of the calves born in the spring died in the first month of life, only 51.9% of the calves born in the summer died in the first month after birth ($p < 0.01$). The mortality of female calves was significantly lower than that of male calves in the first month, but higher in the second month. The parity of the dam did not have a significant effect on the age of mortality. Similarly, there was no statistically significant difference in mortality age between Brown Swiss and Holstein Friesian calves. During the first month after birth, Holstein Friesian calves had a slightly higher mortality rate (78.1%) than Brown Swiss calves (75.2%).

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Siyah Alaca ve Esmer ırkı Buzağılarda Irk, Mevsim, Cinsiyet ve Paritenin Mortalite Üzerine Etkisi

ÖZET

Bu çalışmanın amacı, sütten kesim öncesi dönemde buzağı ölümleri üzerine mevsim, parite, ırk ve cinsiyetin etkilerini araştırmaktır. Çalışmada Erzurum'da Atatürk Üniversitesi Sığırcılık İşletmesinde doğan ve yetiştirilen 1890 Esmer ve Siyah Alaca ırkı buzağıya ait kayıtlar kullanılmıştır. Çalışmanın verileri SPSS istatistik programında bulunan Ki-Kare testi kullanılarak analiz edilmiştir. Araştırma sonuçları, mevsimin buzağı ölümleri üzerinde istatistiksel olarak önemli bir etkiye sahip olduğunu göstermiştir ($p < 0.05$). En yüksek ölüm oranının ilkbahar (%11,5) ve kış (%11,0) mevsimlerinde doğan buzağılarda gözlendiği, ölüm oranının yaz (%7,3) ve sonbahar (%6,3) mevsimlerinde doğan buzağılarda önemli ölçüde düşük olduğu belirlenmiştir. Buzağı cinsiyeti ile mortalite arasında istatistiksel olarak anlamlı bir ilişki olmadığı tespit edilmiştir. Benzer şekilde, sırasıyla primipar ve multipar analardan doğan buzağılar arasında da ölüm oranları açısından önemli bir fark gözlenmemiştir. Siyah Alaca buzağıların ölüm oranının Brown Swiss buzağılara göre daha düşük olduğu belirlenmiştir. İlkbaharda doğan buzağıların %87,5'i yaşamlarının ilk ayında ölürken, yazın doğan buzağıların yalnızca %51,9'u doğumdan sonraki ilk ayda öldüğü belirlenmiştir ($p < 0,01$). Dişi buzağıların ölüm oranının ilk bir aylık yaşta erkek buzağılardan önemli ölçüde düşük olduğu ancak ikinci ayda daha yüksek olduğu tespit edilmiştir. Paritenin buzağıların ölüm yaşı üzerinde önemli bir etkisi gözlenmemiştir. Benzer şekilde, Esmer ve

Zootekni

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Anahtar Kelimeler
Buzağı ölümleri
Esmer
Genetik olmayan faktörler
Siyah Alaca

Siyah Alaca ırkı buzağılar arasında ölüm yaşı bakımından istatistiksel olarak anlamlı bir fark olmadığı gözlenmiştir. Doğumdan sonraki bir aylık yaşa kadar, Siyah Alaca buzağıların ölüm oranının (%78,1) Esmer buzağılardan (%75,2) daha yüksek olduğu belirlenmiştir.

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INTRODUCTION

Livestock production is a major contributor to the global economy, accounting for nearly 40% of total agricultural production in developed countries and 20% in developing countries (Acosta and De los Santos-Montero, 2019). The cattle sector plays a significant role in meeting the world's demand for animal protein, producing over 81% of the world's milk and 19% of the world's meat (FAOSTAT, 2024). In this context, the health and productivity of dairy cattle, particularly calves, are central to sustaining the industry. However, calf mortality is a major cause of economic loss in the global livestock industry (Abebe et al., 2023; Özdemir & Yanar, 2024), and a major concern in all countries with extensive livestock production systems, and is exacerbated in developing countries by poor management practices (Ferede et al., 2014). The mortality rate in calves in the USA has been reported to be 6-8% (Jorgensen et al., 2017) and this rate is significantly higher in developing and underdeveloped countries. The future of cattle enterprises depends significantly on the calves (Medeiros et al., 2022), and calf rearing is highly critical for the success of cattle enterprises because the calves play a crucial role in ensuring a sustainable milk supply, replacing aging cows, and contributing to the genetic improvement of the herd (Kaygısız et al., 2022a; Gomes et al., 2021). The mortality in the calf-rearing period is considerably high (Gessess et al., 2021), and the majority of calf losses take place in the period before the weaning period (Zucali et al., 2013). High mortality rates in this period have a significant economic impact on farming systems, reducing the number of animals that can be sold, animal welfare, selection, and genetic improvement (Schmidek et al., 2013). Reducing calf mortality is therefore the first and foremost objective of cattle farms. A good understanding of the factors that may influence calf mortality is essential for the development of prevention strategies (Sedo et al., 2023). Several factors, including genetic, management, and environmental variables, can affect the survival of calves (Mee et al., 2019). Calf mortality has been the subject of a great deal of research and many factors that may have an influence have been the subject of detailed investigation. However, the effects of environmental and non-genetic factors on calf mortality have, to knowledge, not been studied extensively. This study aimed to investigate the effects of season, dams' parity, breed, and sex on calves' mortality before weaning on a dairy herd.

MATERIALS and METHOD

The data used in the study were the records from 18 years (1998 - 2016) for Brown Swiss and Holstein Friesian calves born and reared at the Atatürk University Cattle Farm in Erzurum (1821 m above sea level, 39°55'15.49' N, 41° 17'12.90 E). A total of 1890 records were used in the study. As this is a research and application farm, records from calves used as part of research studies were excluded from the study.

The breeding was practiced by artificial insemination. The newborn calves were allowed to suckle their dams for the first three days after birth for colostrum feeding. Subsequently, they were then taken into individual pens. Due to the harsh climatic conditions of the region, the calves are reared in an enclosed barn with heating. Individual pens were littered with straw. The straw bedding was removed and replaced daily. The calves were fed two feeds a day until weaning at 60 days of age. The milk amount offered to calves is kept constant at 10% of calves' birth weight. The starter and dry hay were fed to calves, once a day in the morning. Dry hay was harvested from natural pastures in the area. The calves had unlimited access to calf starter, dry hay, and clean water. They were weaned when they reached 60 days of age.

Records from 1134 Brown Swiss and 756 Holstein Friesian calves were used in the study. Only the records from the first 2 months of life until weaning were considered. The mortality data was first categorized as 1 (died) and 2 (did not die) to be able to analyze the effects of these factors on preweaning mortality. To analyze the effect of parity, we categorized parity as 1 (primiparous) and 2 (multiparous). The season was coded as 1 (spring), 2 (summer), 3 (autumn), and 4 (winter) based on the calves' birth dates. The sex of the calves was coded 1 (female) and 2 (male). Breed was coded as 1 (Brown Swiss) and 2 (Holstein Friesian). The influence of these factors on the mortality of calves was analyzed by the Chi-square test. Subsequently, the effects of these factors on the mortality age of calves in the first two months of age were analyzed. For this purpose, the data of 190 calves who died in the first two months of age were selected and categorized as 1 (died in 1-30 days of age) and 2 (died in 31-60 days of age). The numbers have been inserted into SPSS and the Chi-square test was applied to data to determine the

effects of season, sex, breed, and parity on the mortality age of caves (SPSS, 2020). To investigate the effects of season, sex, and parity on calf mortality across different breeds, mortality data was separated by breed. Using the frequencies module, we analyzed the data and calculated percentage values. The results were then presented in graphical format for clearer comparisons between breeds.

In the Chi-square test, the formula used to measure the deviation of observed and expected frequencies is as follows,

$$x^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \quad (1)$$

In this formula:

x^2 = Chi-Square

O_i = Observed value

E_i = Expected value

n = Number of observations

The study has been approved by Atatürk University, Agricultural Faculty Ethics Committee Chairmanship (Session No: 2024/26 Decision No: 2024/1).

RESULTS

The effect of season, sex, parity, and breed on the mortality of calves before weaning has been presented in Table 1. The season had a statistically significant effect ($p < 0.05$) on the mortality rate. Mortality was significantly less in the summer (7.3%) and autumn (6.3%) seasons. The mortality of female calves was slightly higher than that of male calves. However, the differences were not statistically significant. Similarly, parity had no statistically significant effect on the mortality of pre-weaned calves, with mortality rates of 10.8% and 9.8% for calves born to primiparous and multiparous dams, respectively. In comparison to Brown Swiss calves, Holstein Friesian calves had a slightly lower mortality rate. However, this difference did not reach a statistically significant level.

Table 1. Effects of season, sex, parity, and breed on the mortality of calves
Tablo 1. Mevsim, cinsiyet, parite ve ırkın buzağı ölümleri üzerindeki etkileri

		Mortality	Number of Calves Born	Percentage (%)	Chi-Square
Season	Spring	96	834	11.5	$p < 0.05$
	Summer	27	371	7.3	
	Autumn	11	175	6.3	
	Winter	56	510	11.0	
Sex	Female	89	872	10.2	$p = 0.448$
	Male	101	1018	9.9	
Parity	Primiparous	55	509	10.8	$p = 0.281$
	Multiparous	135	1381	9.8	
Breed	Brown Swiss	117	1134	10.3	$p = 0.349$
	Holstein Friesian	73	756	9.7	
	Total	190	1890	10.1	

Table 2 shows the effect of season, sex, parity, and breed on the age at which calves died in the period before weaning. Throughout the study, the first and second-month mortality rates were 76.3% and 23.6% respectively. Study findings showed that season had a statistically significant effect on age at death ($p < 0.01$). While 87.5% of spring calves died in the first month of their life, only 51.9% of summer-born calves died in the first month. Furthermore, autumn and winter calves had mortality rates of 72.7 % and 69.6 % respectively. Even though sex did not have a statistically significant influence on age at mortality ($p = 0.065$), female deaths were significantly lower than male deaths in the first month but higher in the second month. The parity of the dam had no significant effect on the age at which the calves died. There was a slight difference between the mortality of calves born to primiparous dams and those born to multiparous dams in the first month and the second month. Similarly,

between Brown Swiss and Holstein Friesian calves, there was no statistically significant difference in age at death. During the first month after birth, the mortality rate of Holstein Friesian calves was slightly higher (78.1 %) than that of Brown Swiss calves (75.2 %).

Table 2. Effects of Season, sex, parity, and breed on the mortality age of calves
Tablo 2. Mevsim, cinsiyet, parite ve ırkın buzağıların ölüm yaşı üzerindeki etkileri

		Mortality in 1-30 days	Mortality in 31-60 days	Total Mortality	Chi-Square
Season	Spring	84 (87.5%)	12 (12.5%)	96	$p < 0.01$
	Summer	14 (51.9%)	13 (48.1%)	27	
	Autumn	8 (72.7%)	3 (27.3%)	11	
	Winter	39 (69.6%)	17 (30.4%)	56	
Sex	Female	63 (70.8%)	26 (29.2%)	89	$p = 0.065$
	Male	82 (81.2%)	19 (18.8%)	101	
Parity	Primiparous	41 (74.5%)	14 (25.5%)	55	$p = 0.424$
	Multiparous	104 (77.0%)	31 (23.0%)	135	
Breed	Brown Swiss	88 (75.2%)	29 (24.8%)	117	$p = 0.394$
	Holstein Friesian	57 (78.1%)	16 (21.9%)	73	
Total		145 (76.3%)	45 (23.6%)	190	

Figure 1 shows the average mortality age of calves in the different seasons. The average age of calf mortality was determined to be 12.3 years in the spring season, 29.4 years in the summer season, 12.5 years in the autumn season, and 18.7 years in the winter season. The spring and autumn seasons were found to be the seasons in which the earliest calf deaths occurred, while the mortality age of the calves in the summer season was considerably high in comparison to the other seasons.

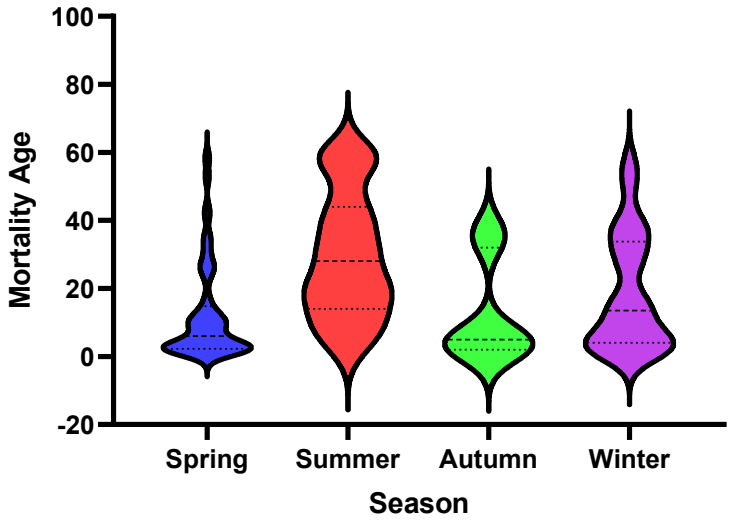

Figure 1. The distribution of the calf mortality ages in different seasons
Şekil 1. Buzağı ölüm yaşlarının farklı mevsimlere göre dağılımı

The mortality rates of pre-weaned Brown Swiss and Holstein Friesian calves born at different times of the year are presented in Figure 2. The findings of the study showed that the mortality rate of Brown Swiss calves born in the spring (12.1%) was significantly higher than the mortality rate of Holstein Friesian calves born in the summer (10.6%). Mortality was significantly higher in summer-born Holsteins Friesian (9.0 %) than in Brown Swiss calves (6.4 %). Mortality rates did not differ between autumn and winter calves. However, Brown Swiss calves born in winter (11.8%) had a higher mortality rate than Holstein Friesian calves (10.8%) in the pre-weaning period.

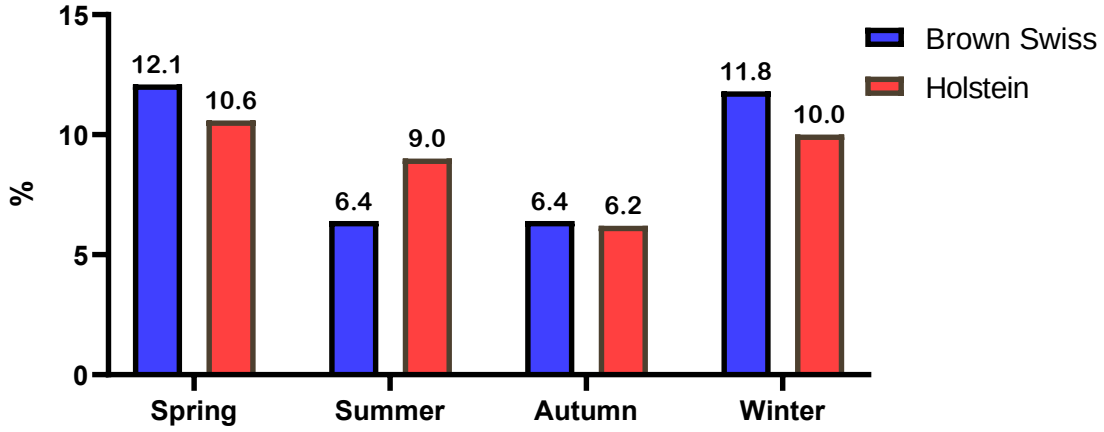

Figure 2. Mortality rates of pre-weaned Brown Swiss and Holstein Friesian calves born in different seasons
Şekil 2. Farklı mevsimlerde doğan sütten kesilmiş Esmer ve Siyah Alaca buzağıların ölüm oranları

The mortality rates of female and male calves of the Brown Swiss and Holstein Friesian breeds in the period before weaning are shown in Figure 3. Results showed that Brown Swiss female calf mortality (9.7%) was slightly lower than Holstein Friesian female calf mortality (10.8%). Male calf mortality differed significantly between the two breeds. There was no difference in mortality between autumn and winter-born calves. However, mortality in winter-born Brown Swiss calves (11.8%) was slightly higher than in Holstein Friesians (10.8%).

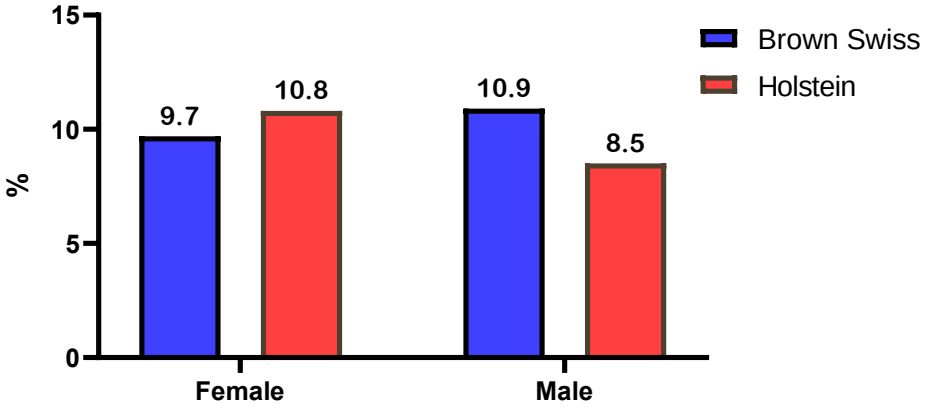

Figure 3. Mortality rates of Brown Swiss and Holstein Friesian female and male calves
Şekil 3 Esmer ve Siyah Alaca dişi ve erkek buzağıların ölüm oranları

Mortality rates of Brown Swiss and Holstein Friesian calves born from multiparous and primiparous dams in the pre-weaning period are shown in Figure 4. Brown Swiss calves having primiparous cows had a mortality rate of 10.3 percent, while Holstein Friesian calves had a mortality rate of 11.5 percent. Brown Swiss calves born to multiparity dams had a slightly higher pre-weaning mortality rate (10.3%) than Holstein Friesian calves (8.9%).

DISCUSSION

Mortality in calves in early life has mostly been attributed to infectious agents (Khan and Khan, 1991). However, some other factors may have an impact on the mortality and survival rate of calves such as season, sex, breed, and parity of the dam. To develop preventative strategies, it is essential to monitor all the factors that may influence the mortality of calves (Sedo et al., 2023). Within the first months of life, calves are highly susceptible to disease, and the mortality rate is considerably high in this period (Lora et al., 2018). Temperature extremes (hot and cold) can lead to stress in calves and increase their susceptibility to diseases. In certain seasons, certain diseases are more prevalent. In the autumn and winter seasons, the incidence of respiratory diseases increases considerably especially when animals are housed together (Gulliksen et al., 2009). In addition, most enteric pathogens are widespread during the winter season (Berber et al., 2021).

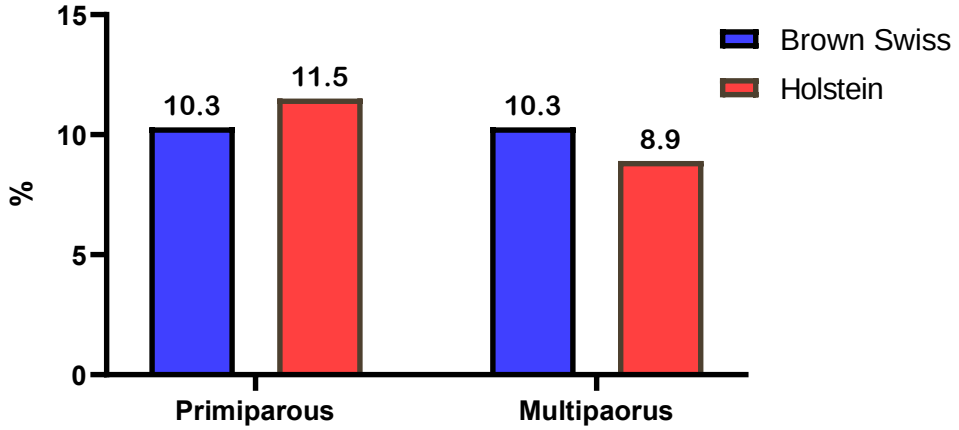

Figure 4. Mortality rates of Brown Swiss and Holstein Friesian calves born to primiparous and multiparous dams
Şekil 4. Primipar ve multipar analardan doğan Esmer ve Siyah Alaca ırkı buzağıların ölüm oranları

In the current study, the effect of season on calf mortality was significant ($p < 0.05$). The calf mortality was determined to be the highest in spring (11.5%) and winter (11.0%) seasons, whereas the mortality was considerably lower in summer (7.3%) and autumn (6.3%) born calves. Furthermore, the mortality of Brown Swiss calves was higher as compared to Holstein Friesian calves in winter, but lower in summer. Svensson & Liberg (2006), Pannwitz (2015), and Ismail & Muhaffel (2022) have also reported that the highest mortality rates were observed in winter seasons. During the winter months, calves experience cold stress and heat loss due to low temperatures. Thus, the mortality of calves is significantly high in the winter and early spring seasons due to exposure to harsh climatic conditions (Kozat, 2018). When newborn calves leave the warm environment of the uterus after birth and are exposed to the cold outside conditions, they experience dramatic changes in body temperature. Comparable results have also been reported by Ismail & Muhaffel (2022) and Ndung'u et al. (2024) who indicated that calf mortality was the highest in winter and spring months. The results of the study are consistent with the literature, especially considering that the study was conducted in harsh and cold Eastern Anatolian conditions. Moreover, the effect of season was also significant ($p < 0.01$) on the mortality age of calves. The above information is supported by this result. Newborn calves are highly susceptible to cold stress and heat loss due to low temperatures. Especially immediately after birth, calves' thermal protection systems are not fully developed, so lack of heat can increase mortality. Calf mortality was significantly high in the first month, in the spring season accounting for 87.5% of calf losses. However, this rate was drastically lower compared to other seasons with only 51.9% of calf mortality in summer-born calves. After this season, autumn and winter accounted for 72.7% and 69.6% of calves lost in the first 1 month of life respectively. Throughout the study, the mortality in the first and second months was determined to be 76.3% and 23.6% respectively. Comparably, Kaygısız et al. (2022b) reported that 97.6% of the calf losses occurred in the first month of life in Andırın District of Kahramanmaraş.

Calf sex is thought to influence perinatal calf mortality, with male calves being almost twice as likely as females (Mee et al., 2019). Mee et al. (2011) reported that male calves tend to have longer gestation periods than female calves, which increases the risk of perinatal loss. Although a slightly higher mortality rate was observed in female calves within two months in the current study, mortality of male calves in the perinatal period (first month) was found to be significantly higher (81.2%) than that of female calves (70.8%). However, after 1 month of age, the mortality rate of female calves was significantly higher than that of male calves. Compared to Holstein Friesian males, Brown Swiss male calves had a higher pre-weaning mortality rate. High male calf mortality rates at the beginning of their lives are often reported in the literature (Riley et al., 2004; Østerårs et al., 2007; Bleul, 2011; Baykan & Özcan, 2019). Schmidek et al. (2013) reported that a possible explanation for higher early mortality of male calves is the higher birth weight of male calves leading to calving difficulty, thus increasing mortality risk.

Dam parity is recognized as among the major factors influencing the survival of calves in the pre-weaning period (Bunter & Johnson, 2013; Gesseess et al., 2021). Mortality in calves is reported to be higher in calves born from primiparous dams than in multiparous dams (Mee et al., 2014). The reason for this may be the calving difficulty in young heifers which leads to higher mortality risk for the calves that are born. Lombard et al (2007) reported that calving assistance is required in more than half of the calvings (51.2%) in primiparous calvings, whereas the assisted calving rate for multiparous dams was 29.4%. The findings of the current study indicated that the mortality in the pre-weaning period was higher in calves having primiparous dams (10.8%) than in calves having

multiparous dams (9.8%), but the difference was not statistically significant. A higher pre-weaning mortality rate has also been reported for primiparous dams as compared to multiparous dams by Segura-Correa et al. (2018). In addition, Brown Swiss calves from multiparous dams had a higher mortality rate in comparison with the Holstein Friesian calves. Comparably, van Pelt et al. (2012) reported that calf survival increases in parallel with the increase in the dam parity. A higher mortality rate for primiparous dam calves has also been determined by Olsson et al. (1993). In the current study dam parity had no statistically significant effect on mortality age of calves.

Resilience, resistance to diseases, and adaptability to environmental conditions vary significantly based on breeds, all of these factors have a crucial role in the survival and mortality of calves. For example, Davis et al. (2020) noted that mortality rates of calves were 3.5%, 3.6%, and 5.3% in the first 1 month and 7.7%, 6.7%, and 9.1% between 1 month and 6 months of age in Red Holstein Friesian and Jersey calves raised in Denmark. In this study, pre-weaning mortalities were found to be 10.3% and 9.7% for Brown Swiss and Holstein Friesian calves, respectively. The first-week mortality rate of Holstein Friesian calves has been reported as 7.95% by Kaygısız et al. (2017). There was no significant effect of breed on the mortality ages of calves. In the first months of their life, the mortality rate of Brown Swiss calves was 75.2% and Holstein Friesian calves was 78.1% among all the calves that died in the pre-weaning period. Comparable results have also been reported by Koçak et al. (2008) who found that the first-month survival rate of Holstein Friesian calves was higher than Brown Swiss calves. In contrast, Koşum & Kaygısız (2019) found that the mortality of Brown Swiss calves was lower than Holstein Friesian calves in the first week of life. Baykan & Özcan (2019) reported a lower mortality rate for Brown Swiss calves in comparison with the Simmental calves.

CONCLUSION

High calf mortality rates in the pre-weaning period are among the major problems faced by the cattle sector. Among the factors influencing calf mortality are season, sex, breed, and parity of the dam. The findings of this study indicated that the effects of the season were significant on calf mortality, with the highest calf losses occurring in calves born in the spring and winter due to the cold stress and heat loss from harsh climatic conditions experienced during winter and early spring. Furthermore, mortality in Brown Swiss calves was higher than Holstein Friesian calf mortality in winter but lower in summer. The mortality of male calves at one month of age was significantly higher than that of female calves. Increased mortality of males in the first year of life may be due to increased birth weight of males leading to calving problems and increased risk of mortality. In addition, compared to Holstein Friesian males, Brown Swiss male calves had a higher mortality rate. Calves born to primiparous dams had a slightly higher mortality rate before weaning than calves born to multiparous dams. This may be due to calving difficulties in young heifers, resulting in a higher mortality risk for the calves born. Between Brown Swiss and Holstein Friesian calves, there was no statistically significant difference in pre-weaning mortality. In addition, the mortality rate of Brown Swiss calves born to multiparous dams was higher than the mortality rate of Holstein Friesian calves.

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Author Contributions

VFÖ: Collection of the data, design of the study, analysis of the data, interpretation of the results, and writing the manuscript.

Conflict of Interest

There is no conflict of interest.

Ethical Statement

The study has been approved by Atatürk University, Agricultural Faculty Ethics Committee Chairmanship in 2024 (Session No: 2024/26; Decision No: 2024/1).

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ÖZET

Yasal gereksinimler ve tüketici isteklerindeki değişimler hayvan refahını yükselen bir değer haline getirmiştir. Bu anket çalışması, 2020 yılında Aydın ili kamu kurumlarında görevli 139 veteriner hekimin hayvan refahı konusundaki algı ve tutumlarını belirlemek amacıyla gerçekleştirilmiştir. Ankete katılanların %97.1'i çiftlik hayvanlarında refahla ilgili sorunlar olduğunu bildirmiştir. Anket sonuçlarına göre önemli refah problemlerinin barınak koşulları (%75.4) ile bakım-besleme şartlarından (%44.6) kaynaklandığını, hayvan refahının hayvan sağlığı (%87.1) yanında ürün kalitesi (%92.1) ve insan sağlığı (%87.1) açısından da önemli olduğunu ortaya koymuştur. Hayvan refahının sağlanmasında en büyük sorumluluğun hayvan sahiplerine (%67.6), politikacılara (%15.8) ve hayvan bakıcılarına (%7.2) ait olduğu, refah problemlerinin hayvan sahipleri ile bakıcılarının bilgisizliği (%89.9), ekonomik sebepler (%55.4), yasal mevzuat (%51.1) ve kamuoyu duyarsızlığı (%46) sonucu oluştuğu saptanmıştır. Ankete katılanlar hayvansal ürünleri satın alırken hayvan refahını düşündüklerini (%59) ve bu ürünlerin tercih edilmesinin hayvan refahına bakışı olumlu yönde etkileyeceğini bildirmişlerdir. Katılımcılar, hayvansal ürünlerin refah koşullarında üretilip üretilmediklerini ürün etiketlerinde görmek istediklerini (%87.8) ve refaha uygun yetiştirilmişlerse ürüne daha fazla ücret ödemeyi kabul edebileceklerini (%79.1) ifade etmişlerdir. Sonuç olarak, hayvan refahını dikkate alan bir hayvansal üretimin başarılabilmesi için tüm paydaşların refah konusunda eğitilmesi ve uygulamalarının ilgili mevzuata uyumunun ise düzenli ve yeterli denetlenmesi gerekir.

Zootekni

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 26.08.2024
Kabul Tarihi : 26.12.2024

Anahtar Kelimeler
Hayvan refahı
Hayvan sağlığı
İnsan sağlığı
Mevzuat
Denetim

Animal Welfare Perception of Veterinarians in Aydin Province

ABSTRACT

Changes in legal requirements and consumer demands have made animal welfare a rising value. This survey study was conducted in 2020 to determine the perceptions and attitudes of 139 veterinarians working in public institutions in Aydin province regarding animal welfare. 97.1% of survey respondents reported welfare problems in farm animals. According to the survey results it was determined that important welfare problems were related to animal housing (75.4%), care and feeding (44.6%) and that animal welfare was important in terms of animal health (87.1%), product quality (92.1%) and human health (87.1%). It has been observed that the greatest responsibility in ensuring animal welfare belongs to animal owners (%67.6), politicians (%15.8), and animal breeders (%7.2), and welfare problems occur due to the lack of knowledge of animal owners and breeders (89.9%), economic reasons (55.4%), legal regulations (51.1%) and public insensitivity (46%). Participants in the survey reported that they consider animal welfare when purchasing animal products (%59), and that choosing these products would positively affect their view on animal welfare. Participants stated that they would like to see on the labels whether animal products are produced under welfare conditions (%87.8), and that they would be willing to pay more for the product (%79.1), if they were raised in accordance with welfare. As a result, to achieve animal production that takes animal welfare into

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account, all stakeholders must be trained on welfare and field practices must be regularly and adequately monitored for compliance with legislation.

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GİRİŞ

Hayvanların yaşam kalitesinin bir göstergesi olan hayvan refahı, hayvanların fizyolojik, biyolojik ve davranış yapısını bozmadan doğal yaşamlarındaki koşullarına uygun şekilde yetiştirilmesi, ihtiyaçlarının karşılanması ve sağlığının korunmasını hedefleyen bir hayvansal üretim anlayışıdır (Demirel & Çak, 2016). Bozkurt (2016) hayvan refahının değerlendirilmesinde ele alınan göstergeleri sıralamış, küresel ölçüde değerlendirme metotlarına ihtiyaç olduğunu bildirmiştir. Hayvan refahı, sadece çiftlik hayvanlarında değil tüm hayvanlarda düşünülmesi gereken bir olgudur. Bununla birlikte en çok refah problemi yaşayan hayvanların sırasıyla laboratuvar hayvanları, eğlence ve gösteri hayvanları ile çiftlik hayvanları olduğu bildirilmiştir (İzmirli, 2009). Aynı çalışmada çiftlik hayvanları arasında en fazla refah problemi yaşayan türlerin ise besi sığırları, yumurta tavukları ve atlar olduğu vurgulanmıştır.

Hayvan refahı olgusunun tarihçesine bakıldığında Osmanlı Devleti'nde özellikle yük taşıyan hayvanlara eziyet edilmemesi konusunda farklı dönemlerde fermanlar çıkarıldığı bilinmektedir (Menteş Gürler & Osmanoğlu, 2009; AA, 2021). Hayvanların kötü koşullarda yetiştirilmelerine karşı 1964'te yayınlanan Brambell Raporu bu konuda en kapsamlı ilk tepkidir. Brambell Raporu ile hayvanların temiz su ve sağlıklı yeme ulaşma, uygun dinlenme alanlarına sahip olma, ağrı, yara ve hastalıklardan korunma, korku ve sıkıntıdan uzak kendi türündeki hayvanlarla iletişim kurma hakları olduğu kabul edilmiştir.

Bir taraftan hayvanların refahını öncelemeyen üretim sistemlerine getirilen eleştiriler, diğer taraftan yaygınlaşan çevreci politikalar, üreticileri yeni hayvancılık yaklaşımlarına yöneltmiştir (Sert & Uzmay, 2017). Böylece 1990'larda hayvan refahı kavramı ön plana çıkmıştır. Gelişmiş ülkelerde hayvan refahı konusu belirli standartlara bağlanmış ve denetim altına alınmıştır. Avrupa Birliği ülkeleri 1997'de Amsterdam Antlaşması ile hayvanların "duygulara sahip canlılar" olduğunu kabul etmiş, 1998'de ise 'Çiftlik Hayvanlarının Korunmasına İlişkin Direktif (98/58/EC)' ile hayvan refahına yönelik en düşük standartlar saptanmış, bu konuda birçok yönerge çıkartılmıştır. Hayvan refahının dünyadaki tarihi gelişimi ve AB mevzuatı, Antalyalı (2007) tarafından ayrıntılı olarak sunulmuştur.

Türkiye'de hayvan refahı ile ilgili önemli düzenlemeler, 2004 yılında kabul edilerek yürürlüğe giren 5199 sayılı 'Hayvanları Koruma Kanunu' ile 2006'da yürürlüğe giren 'Hayvanların Korunmasına Dair Uygulama Yönetmeliği'dir. Bu Kanun'un amacı "hayvanların rahat yaşamalarını ve onlara iyi ve uygun muamele edilmesini temin etmek, acı, ızdırap ve eziyet çekmelerine karşı korunmalarını, her türlü mağduriyetlerinin önlenmesini sağlamaktır". Hayvan refahıyla ilgili bir diğer yasa, Gıda, Tarım ve Hayvancılık Bakanlığı tarafından 2010 yılında yayınlanan 5996 sayılı 'Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu'dur. Bu kanunda hayvan refahına vurgu yapılmış, "Hayvan Sağlığı, Hayvan Refahı ve Zootekni" başlığı altında ayrıntılar sıralanmıştır. Bu konudaki bir diğer mevzuat, 2011-2014 yılları arasında yürürlükte kalan 'Çiftlik Hayvanlarının Refahına İlişkin Yönetmelik'tir. Bu yönetmelik AB ile uyum sürecinde Avrupa Birliği'nin "Çiftlik Hayvanlarının Korunmasına İlişkin Direktifi (98/58/EC)", "Yumurta Tavuklarının Korunmasında Minimum Standartlara İlişkin Direktifi (1999/74/EC)" ve "Buzağıların Korunmasında Minimum Standartlara İlişkin Direktif (2008/119/EC)" hükümlerine uygun olarak hazırlanmıştır. Bu yönetmelik 2014 yılında yürürlükten kaldırılarak yerine Avrupa Birliği'nin "Çiftlik Hayvanlarının Korunmasına İlişkin Direktifine (98/58/EC)" uygun olarak hazırlanan 'Çiftlik Hayvanlarının Refahına İlişkin Genel Hükümler Hakkında Yönetmelik' kabul edilmiştir. Diğer taraftan 2014 yılında 'Yumurtacı Tavukların Korunması ile İlgili Asgari Standartlara İlişkin Yönetmelik' ile 'Buzağıların Korunması ile İlgili Asgari Standartlara İlişkin Yönetmelik'ler de kabul edilmiştir. Bu iki Yönetmelik AB'nin ilgili direktiflerine (1999/74/EC ve 2008/119/EC) uygun olarak hazırlanmış ve böylece ülkemizde ilk defa refahla ilgili yönetmelikler yayınlanmıştır.

Çiftlik hayvanlarında refahın sağlanması, bakım-besleme, nakliye ve kesim olmak üzere üç alanda koruma ile ön plana çıkmaktadır. Nitekim entansif yetiştirme uygulamaları, kötü barınak koşulları, yoğun ıslah çalışmaları, embriyo transferi vb. üreme uygulamaları, hastalıklar, yetersiz ve dengesiz besleme ile kötü koşullarda hayvanların nakli, sığır ve koyunların altlıksız, beton veya çamur-ıslak zeminde uzun süre tutulması gibi kötü yetiştirme koşulları hayvan refahını olumsuz etkilemektedir (Antalyalı, 2007; Sağmanlıgil ve ark., 2015; İzci ve ark., 2021). Bununla birlikte aşırı sıcak ve soğuk çevre koşulları, açlık, korku, kötü yetiştirme şartları, birim

alanda fazla hayvan bulundurma, kötü nakliye koşulları, hastalık vb. önemli stres faktörleri olup, bunların hayvan davranışlarında bozulmalara ve devamında hayvan kayıplarına neden olduğu bildirilmiştir (Altınçekiç & Koyuncu, 2012a, Bozkurt, 2016).

Çiftlik hayvanlarının refahı, sadece hayvan hakları açısından üzerinde durulması gereken bir konu olmayıp, hayvan sağlığı ve bu hayvanlardan elde edilen ürünlerin kalitesi açısından da önemlidir (Yıbar & Çetin, 2013; Sağmanlıgil ve ark., 2015). Tüketicilerin doğal hayvansal ürünlere olan yüksek talebinin bir sonucu olarak sağlık, çevre ve hayvan refahı açısından entansif hayvansal üretim sistemleri tartışılır hale gelmiştir (Özdemir & Singin, 2016). Bu nedenle hayvan refahı, sadece üretim verimliliğini arttırıcı bir faktör olarak ele alınmamış, gelişmiş ülkelerde hayvansal ürün tercihlerini ve uluslararası hayvan ve hayvansal ürün ticari koşullarını belirlemeye başlamıştır (Demirel & Çak, 2016). Bu nedenle gıda ekosisteminin ayrılmaz bir parçası olan hayvansal üretimin refah kriterlerine göre gerçekleştirilmesinin önemi artmış, hayvansal ürün tüketimleri, tüketicilerin eğitim ve gelir düzeyleri ile ilişkili olarak değişim göstermiştir. Bu nedenle tüketici tercihlerinde ürünün fiyatı, ambalajı ve satış yerinin önemi azalırken, ürün kalitesi ve üretim süreçleri ön plana çıkmıştır (Demirel & Çak, 2016).

Gıda sektörü, toplumların hayvanı refahı konusundaki bilinçlenmesi sonucu refah koşullarında yetiştirilmiş hayvanlardan elde edilen ürünlerin tercih edilmeye başlanmasını, yeni bir pazarlama aracı olarak ele almıştır (Bozkurt & Koçak, 2017). Ayrıca tüketiciler bu şekilde elde edilen ürünlerin tercih edilmesinin hayvan refahı olgusunun daha fazla benimsenmesine yol açacağını düşünmektedir. Amerika'da 2004 yılında gerçekleştirilen bir çalışmada (AAA, 2004), refah koşullarında yetiştirilmiş hayvanlardan elde edilmiş gıdalar için tüketicilerin %31'inin %5 daha fazla, %23'ünün ise %10 daha fazla ödeme esnekliği gösterdiğini ortaya koymuştur (Sert & Uzmay, 2017). Hayvan refahı dikkate alınarak üretilen organik somonlara Norveç'li tüketicilerin %15 daha fazla ödeme yapmayı kabul ettikleri bilinmektedir (Olesena ve ark., 2010). Benzer şekilde Cameron & Naald (2011) hayvan refahı olgusunun gelişmesi için müşterilerin daha fazla ödeme yapmayı kabul ettiklerini saptamıştır.

Türkiye'de de toplumun farklı kesimlerinin hayvan refahına bakışı üzerine çeşitli araştırmalar gerçekleştirilmiştir (İzmirli, 2009; Gökcam ve ark., 2012; Kılıç ve ark., 2013; Turan, 2018, Çelik ve ark., 2024). Veteriner hekimler, veteriner hekimliği öğrencileri, hayvan sahipleri ve hayvansal gıda tüketicilerinin hayvan refahı konusundaki tutumlarını inceleyen İzmirli (2009), bu gruplarda konuya genel bakışın hayvanların insan yararı için yetiştirilmesi ve yaşam koşullarının da refah kriterleri dikkate alınarak iyileştirilmesi yönünde olduğunu bildirmiştir. Gökcam ve ark. (2012) ise Süleyman Demirel Üniversitesi öğrencilerine yönelik gerçekleştirdiği anket çalışması ile öğrencilerin hayvan refahına bakış açısını değerlendirmiştir. Kılıç ve ark. (2013), Afyonkarahisar'daki koyunculuk işletmelerinde çalışanların hayvan refahını etkileyen faktörlere ilişkin algılarını belirlemeyi amaçlamıştır. Turan (2018) ise kırmızı et ve yumurta tavukçuluğunu dikkate alarak Türkiye'nin hayvan refahı konusunda ne durumda olduğunu belirlemeye, tüketicilerin hayvan refahını sağlama adına katlanabileceği parasal külfeti saptamaya çalışmıştır. Çelik ve ark. (2024) ise Bingöl ilinde yaşayan farklı yaş, cinsiyet, meslek ve gelir durumundaki 600 kişi ile yaptıkları anket çalışması ile tüketicilerin et tüketimi ve hayvan refahına ilişkin tutum ve davranışlarını analiz etmişlerdir. Ankete cevap verenlerin %26.8'i kesime gönderilen hayvanların refahına dikkat edilmesini, %53'ü hayvan refahına dikkat edilmesi durumunda fazladan ücret ödemeyi ve %66.2'si ise fiyat etiketlerinde hayvan refahı bilgisini görme konularında olumlu görüş bildirmişlerdir. Fiyat etiketinde hayvan refahı bilgisi görme durumu, yaş grupları ve cinsiyete göre, kesime gönderilen hayvanların refahına dikkat edilmesi durumu aylık gelire göre, hayvan refahına dikkat edilmesi durumunda fazladan ücret ödemeye razı olma durumu ise cinsiyet, aylık gelir ve mesleklere göre farklılıklar ($P<0.05$) göstermiştir. Bu çalışmanın amacı, Aydın ili kamu kurumlarında görevli veteriner hekimlerin hayvan refahı konusundaki algı ve tutumlarını belirlemek ve bu konuda gelinen son noktaya ait bir durum değerlendirmesi yapmaktır.

MATERYAL ve METOD

Bu araştırma, bir anket çalışmasıdır. Anketin hazırlanması ve sonuçlarının değerlendirilmesinde daha önce bu konuda yapılmış bazı anket çalışmalarından (İzmirli, 2009; Gökcam ve ark., 2012; Kılıç ve ark., 2013; Turan, 2018) yararlanılmıştır. Anket, araştırmanın gerçekleştirildiği 2020 yılında Aydın ilinin 17 ilçesinde kamuda görev yapan tüm veteriner hekimlere ulaştırılmış, biri hariç 139 veteriner hekim anketi doldurmuştur. Söz konusu 139 ankette katılımcıların genel özelliklerini ortaya koyan 7 adet demografik soru ile katılımcıların hayvan refahıyla ilgili genel tutumlarını saptayan 7 adet kapalı uçlu çoktan seçmeli ve 9 adet beşli likert ölçeğinde soru olmak üzere toplam 23 soru yer almıştır. Beşli Likert ölçekli sorularda 'katılıyorum, kısmen katılıyorum, kararsızım, kısmen katılmıyorum, katılmıyorum' şeklinde katılımcıların tutum ve eğilimleri belirlenmiştir.

Anketler, internet ortamında Google Formlar kullanılarak katılımcılara ulaştırılmış, katılımcılar tarafından doldurulan formlar otomatik olarak tek kaynakta toplanmış ve analiz edilmiştir.

İstatistik Analizler

Her soru için elde edilen sonuçlar, sorudaki seçenekleri tercih edenlerin adet ve yüzdeleri üzerinden değerlendirilmiştir. Araştırmada kullanılan sorulara ait güvenirlik için Cronbach's Alpha değerleri hesaplanmıştır. Çalışmada ele alınan kriterlerin ankete katılanların cinsiyeti, yaşı ve tecrübe grubu ile olan ikili ilişkileri çapraz tablolarla ve ki-kare analizi ile irdelenmiştir (İkiz ve ark., 2000). Söz konusu ilişkinin önem durumları ki-kare değeri (χ^2), serbestlik derecesi (sd) ve P-değeri verilerek raporlanmıştır. Ancak alt gruplarda beklenen frekansları beşten az olan gözlerin oranının %20'den fazla olduğu durumunda Fisher'in Exact Testi sonuçları üzerinden yorumlama yapılmıştır. Ele alınan kriterlerin cinsiyet, yaş ve tecrübe grubu ile olan ikili ilişkisi eğer önemsiz bulunmuş ise sadece ele alınan kriterin genel düzeylerine ait adet ve yüzde sonuçları verilmiştir. Fakat kriterin bu faktörlerle olan ilişkisi önemli bulunmuşsa çapraz tablolara göre değerlendirme yapılmıştır. Anket sorularının demografik özelliklere göre değişiminin incelenmesinde oranların ikili karşılaştırmalarında birden fazla karşılaştırma yapılması nedeniyle anlamlılık düzeylerine Bonferroni düzeltmesi uygulanmıştır. İstatistik analizler IBM SPSS V25 ile gerçekleştirilmiştir.

BULGULAR ve TARTIŞMA

Bu çalışmada refah algısını belirlemeye çalışan soruların Cronbach's Alpha güvenirlik değeri 0.522 olarak saptanmıştır. Yüksek olmasa da bu değer kabul edilebilir bir düzeydedir.

Aydın ili ve ilçelerindeki kamu kurumlarında çalışan ve ankete katılan veteriner hekimlerin yaş, cinsiyet, eğitim ve mesleki tecrübe düzeyleri Çizelge 1'de verilmiştir. Buna göre ankete katılan 139 veteriner hekimin 122'si erkek (%87.8) olup çoğunluğu (73+45=118 kişi, %85) 31-50 yaş aralığındadır. Veterinerlerin %74.8'inin (63+27+14=104 kişi) on yılın üzerinde bir tecrübeye sahip olduğu görülmektedir.

Ankete katılan veteriner hekimlerin %84.2'si çiftlik hayvanı yetiştiriciliği yapmasa da, %33.8'i evinde hayvan beslemekte ve yaklaşık %10'u ise hayvanları koruma amacıyla kurulmuş bir derneğe üyedir (Çizelge 1). Son iki oran, İzmirli (2009)'nin bildirdiği oranlara (sırasıyla %40, %8.9) oldukça yakındır. Bu durum, katılımcıların sadece profesyonel olarak hayvanlarla ilgilenmediklerini, aynı zamanda derin bir hayvan sevgisine sahip olduklarını göstermektedir.

Çizelge 1. Ankete katılanların bazı karakteristikleri
Table 1. Some characteristics of the respondents

Anket sorusu	Seçenekler	Adet	%
Cinsiyet?	Erkek	122	87.8
	Kadın	17	12.2
Yaş grubu?	21-30	5	3.6
	31-40	73	52.5
	41-50	45	32.4
	51-60	16	11.5
Eğitim Düzeyi?	Lisans	60	43.2
	Yüksek Lisans	75	54.0
	Doktora	4	2.9
Kaç yıldır veteriner hekim olarak çalışmaktasınız? (Mesleki Tecrübe)	0-9 yıl	35	25.2
	10-19 yıl	63	45.3
	20-29 yıl	27	19.4
	30 yıl ve üzeri	14	10.1
Evinde hayvan besliyor musunuz?	Evet	47	33.8
	Hayır	92	66.2
Çiftlik hayvanı yetiştiriyor musunuz?	Evet	22	15.8
	Hayır	117	84.2
Hayvan korumayla ilgili bir derneğe üye misiniz?	Evet	13	9.4
	Hayır	126	90.6

Başka bir bilimsel seçenek olmadığı durumlarda hayvan deneylerinde verilecek acı ve stresin kabul edilebilir olduğunu kısmen veya tamamen onaylayanların oranı (%49.6+%30.2=%79.8), bu görüşe kısmen veya tamamen katılmıyorum diyenlerden (%6.5+%9.4=%15.9) oldukça fazladır (Çizelge 2). İzmirli (2009) de bu ana gruplar için benzer oranları (sırasıyla %76.9 ve %16.5) saptadığını bildirmiştir. Bununla birlikte katılımcıların %48.9'u her türlü bilimsel araştırmada hayvanların kullanılabileceğini, %35.3 kısmen kullanılabileceğini, %4.3'ü ise bu görüşe hiç katılmadıklarını ve hayvanların bilimsel araştırmalarda kullanılmaması gerektiğini ifade etmişlerdir (Çizelge

3). Fakat bilimsel araştırmalarda hayvanların kullanılması konusunda katılımcıların cinsiyetlerine göre görüş farklılıkları saptanmıştır (X^2=11.277; sd=4; P=0.024; Fisher's Exact test P=0.042).

Bilimsel araştırmalarda hayvanların kullanılmasına karşı olanların yüzdesi, kadınlarda (%17.65) erkeklerden (%2.4) daha yüksek bulunmuştur. Gerek kısmen gerekse tamamen karara katılanların toplam oranı, erkeklerde (%50.8+%36.1=%86.9) kadınlardan (%35.3+%29.4=%64.7) %22.2 daha fazladır (Çizelge 3). Diğer yandan bu konuda kadınlarda kararsızların olmadığı gözlenmiştir. İzmirli (2009) hayvanların bilimsel araştırmalarda tamamen (%62.3) veya kısmen (%24.3) kullanılabileceğini saptarken, %3.4 oranında katılımcının bu görüşe karşı olduğunu bildirmiştir.

Çizelge 2. Hayvanların kullanılma durumuna ilişkin soru
Table 2. Question regarding the right of animals to be used

Anket sorusu	Seçenekler	Adet	%
Ancak başka bir bilimsel seçenek kalmadığı durumlarda hayvan deneylerinde acı ve stres kabul edilebilir.	Katılıyorum	69	49.6
	Kısmen katılıyorum	42	30.2
	Kararsızım	6	4.3
	Kısmen katılmıyorum	9	6.5
	Katılmıyorum	13	9.4

Çizelge 3. "Her türlü bilimsel araştırmada hayvanlar kullanılabilir" sorusuna tepkilerin cinsiyete göre durumu
Table 3. Responses to the question "Animals can be used in any kind of scientific research" by gender

	Erkek		Kadın		Toplam	
Seçenekler	Adet	%	Adet	%	Adet	%
Katılıyorum	62	50.8	6	35.3	68	48.9
Kısmen katılıyorum	44	36.1	5	29.4	49	35.3
Kararsızım	4	3.3	-	-	4	2.9
Kısmen katılmıyorum	9	7.4	3	17.65	12	8.6
Katılmıyorum	3	2.4	3	17.65	6	4.3
Toplam	122	100	17	100	139	100

Katılımcılara bir tüketici olarak da bazı sorular sorulmuş, elde edilen cevaplar Çizelge 4'te özetlenmiştir. Katılımcılara hayvansal ürünleri satın alırken verdikleri kararlarında ürününü aldıkları hayvanların yetiştirilme sırasındaki refah durumlarını düşünüp düşünmedikleri sorulmuş, %59'u çoğunlukla, %30.2'si bazen, %8.6'sı çok az düşündüğünü belirtmiş, %2.2'si ise hiç düşünmediğini ifade etmiştir. İzmirli (2009) hayvansal ürünleri satın almada refah olgusunu hiç düşünmeyen veteriner hekimlerin oranını, bu çalışmada gözlenen düzeyden daha yüksek (%16.4) saptamıştır. Alışveriş sırasında hayvan refahını düşünen vatandaşların oranı, ülkeler arasında da önemli farklılıklar göstermektedir. Nitekim Blokhuis ve ark. (2013) bu oranın Norveç ve Hollanda'da %12-13, İngiltere'de %23, İtalya'da ise %41 olduğunu bildirmiştir.

Çizelge 4. Bir tüketici olarak katılımcıların hayvan refahı konusundaki algı ve tutumları
Table 4. Perceptions and attitudes of participants as consumers on animal welfare

Anket sorusu	Seçenekler	Adet	%
Hayvansal ürünleri satın alırken hayvanların refah durumlarını düşünüyor musunuz?	Çoğunlukla	82	59.0
	Bazen	42	30.2
	Çok az	12	8.6
	Hiç düşünmem	3	2.2
Hayvansal ürünleri satın alırken hayvan refahına uygun şartlarda yetiştirilmiş hayvanlardan elde edilip edilmediğinin bildirilmesini ister misiniz?	İsterim	122	87.8
	İstemem	2	1.4
	Fark etmez	15	10.8
Hayvanların refah koşullarında yetiştirilmeleri için satın alacağınız hayvansal ürünlere ne kadar fazladan ücret ödemeyi kabul edersiniz?	%0	29	20.9
	%1-5	45	32.4
	%6-10	34	24.5
	%11-15	16	11.5
	%16-20	10	7.2
	%21-25	2	1.4
	%25'ten fazla	3	2.2

Hayvan refahına uygun şartlarda yetiştirilmiş hayvanlardan elde edilen ürünlerin ambalajlarında bu özelliğin belirtilmesini isteyenlerin oranı %87.8 saptanırken, geriye kalanlar bu durumu ya talep etmemiş veya fark etmez demiştir (Çizelge 4). Ankette 'hayvansal ürünlerin ambalajlarında bu özelliğin belirtilmesini isteme' olgusuna verilen cevaplar katılımcının yaş grubuna göre önemli (X^2=16.496; sd=6; P=0.047) değişkenlik göstermiştir. Fakat Fisher's Exact test sonuçlarına göre bu ilişki önemli bulunmamıştır (P=0.138). Bununla birlikte eğilim olarak düzeylere bakıldığında yaşın en yüksek olduğu grupta (51-60 yıl) bu bilgilendirmeyi isterim diyenlerin oranı %94'e kadar yükselmiştir. Etiket bilgilendirmesini istemem diyenlerin oranı, en genç grupta (21-30 yıl) %20 iken, yaşın artması ile istemem diyenlerin oranı %0'a kadar düşmüştür (Çizelgede verilmemiştir). Bu soruya 'fark etmez' diyenler ise genellikle orta yaş gruplarında (31-50 yıl) bir yığılma göstermiştir. Daha önce yapılan bir çalışmada (İzmirli, 2009) bu tip bilgilerin etikette belirtilmesini isteyen veteriner hekimlerin oranı (%93.7) bu çalışmada saptanan orandan (%87.8) daha yüksek bulunmuştur. 200 öğrenci üzerinde yapılan bir diğer ankette (Gökcam ve ark., 2012) ise öğrencilerin %70.5'inin hayvan refahı açısından etiketleri kontrol ettikleri ve etikette 'hayvan refahına uygun şartlarda yetiştirilmiş hayvanlardan üretilmiştir' ibaresini %96 oranında görmek istedikleri saptanmıştır. Çelik ve ark. (2024) Bingöl ilinde bu oranı %66.2 olarak saptamıştır.

'Refah koşullarında yetiştirilmiş hayvanlara ait ürünlere fazladan ne kadar ücret ödeyebilirsiniz?' sorusuna katılımcıların %20.9'u fazla ücret ödemek istemediğini ifade ederken geri kalan %79.1'i çeşitli düzeylerde fazla ödeme yapabileceklerini belirtmişlerdir. Fakat fazladan ödenebilecek miktar arttıkça ek ödeme yapmayı onaylayan kişi sayısında azalmalar gözlenmiştir (Çizelge 4). İzmirli (2009)'nin çalışmasında da veteriner hekimler benzer ek ödeme yapma eğilimi sergilemişlerdir. Öğrenciler üzerinde yapılan anket çalışmasında (Gökcam ve ark., 2012) bile öğrencilerin %70'i hayvan refahı standartlarına uygun üretilen ürünler için daha fazla ücret ödemeyi kabul etmişlerdir. Söz konusu çalışmada fazla ücret ödemeyi kabul etme durumunun öğrencilerin gelirinden bağımsız olduğu saptanmıştır. Çelik ve ark. (2024) hayvanların refahına dikkat edilmesi durumunda fazla ücret ödemeye razı olanların oranını Bingöl ilinde %53 olarak saptamıştır. Lagerkvist & Hess (2011) hayvan refahına yönelik daha fazla ödeme yapma olgusuna, gelir ve yaşın yanında tüketicinin refahla ilgili bilgi düzeyinin de önemli etki yaptığını saptamıştır. Turan (2018), 500 kişi ile yürüttüğü anket çalışmasında tüketicilerin %83'ünün hayvan refahı konusunda bilgi sahibi olmadıklarını ortaya koymuştur. Bununla birlikte gelişmiş ülkelerdeki tüketicilerin toplumda hayvan refahı algısının yüksekliğine rağmen alışverişlerinde hayvan refahını yeterince dikkate almadıkları da bilinmektedir (Sert & Uzmay, 2017).

Gıda ürünlerinde hayvan refahı kurallarına uyulup uyulmadığının etiket üzerinde yer alması hem kalite hem de sistemin sürdürülebilirliği açısından önemlidir. Bununla birlikte etiketleme sisteminin zorunlu hale getirilmesinin ek maliyetler getirecek olması sistemi zorlayan nedenlerden birisidir. İtalya, Hollanda ve İngiltere gibi ülkelerdeki büyük süpermarket zincirlerinin bazı hayvan refahı kalite güvence sistemleri oluşturduğu da bilinmektedir (Bozkurt & Koçak, 2017). Bu gibi durumlarda gerçekleşen özel etiketleme uygulamaları zorunlu etiketlemeye bir alternatif olabilir. Bozkurt (2016), uluslararası ticaret koşullarının, hayvan refahının tanımlanması için ortak bir kalite standardına ve etiketlemeye ihtiyaç duyduğunu bildirmiştir. Gıdaların güvenilir hale getirilmesi için birçok ülkede 'İyi Üretim Uygulamaları (GMP)', 'İyi Hijyen Uygulamaları (GHP)', 'Standart Operasyon Uygulamaları (SOP)' ve HACCP sistemi gibi çeşitli standart ve yönetim sistemleri geliştirilmiş ve uygulanmaya başlanmıştır. ISO 22000 ve HACCP gibi programlar tedarikçilere ve hayvansal kökenli hammadde üreticilerine, hayvan refahı ile ilgili şartları işletmelerinde uygulamaları mecburiyetini getirmiştir (Demirel & Çak, 2016).

Avrupa Birliği ile uyum çerçevesinde hazırlanıp yürürlüğe konulan 5996 sayılı 'Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu' ile gıda güvenliği ve denetimi konuları ortaya konmuştur. 26.01.2017 tarihinde 'Türk Gıda Kodeksi Etiketleme ve Tüketicileri Bilgilendirme Yönetmeliği' hazırlamış, Avrupa Birliği mevzuatı ile farklılıklar azaltılmıştır. Günümüz Türkiye'sinde hayvan refahını belirten özel bir etiketleme sistemi bulunmamaktadır (Demirel & Çak, 2016, Turan, 2018). Sadece serbest dolaşımlı tavuklardan elde edilen yumurtalar ile organik sertifikalı ürünleri gösteren etiketler mevcuttur. Bununla birlikte ürünün organik olması, hayvan refahına uygun üretildiğinin bir göstergesi değildir. Ayrıca hayvansal ürünlerin AB gibi ülkelere ihracında refah uygulamalarının aranan kriterler arasında olduğu da unutulmamalıdır.

Veteriner hekimlere sahadaki gözlemlerine dayanarak çiftlik hayvanlarının refahına yönelik algıları hakkında bazı sorular yöneltilmiş, elde edilen sonuçlar Çizelge 5'te sunulmuştur. Çiftlik hayvanları yetiştiriciliğinde refahla ilgili sorunlar bulunduğunu katılımcıların %74.1'i tamamen, %23'ü ise kısmen kabul ederken, %1.4'ü bu konuda kararsız kalmıştır. Sonuç olarak çiftliklerde refah sorunları olduğunu katılımcıların tamamına yakını kabul etmiştir. Benzer şekilde İzmirli (2009) de %91.1 oranında ülkemiz çiftliklerinde hayvan refahı yönünden problem olduğunu, ayrıca ankete katılan veteriner hekimlerin %56.3'ünün Türkiye'de hayvan refahın AB'den daha kötü olduğunu bildirmiştir. Blokhuis ve ark. (2013)'nın bildirdiğine göre sığırlarda refah problemi olduğunu düşünenlerin oranı İsveç ve Norveç'te %3-5 iken İtalya ve Macaristan'da %16 düzeyindedir. Bu ülkelerde

tavukların kötü refah koşullarında yetiştirildiğini düşünenlerin oranı ise çok daha yüksek olup genellikle %40-57 arasındadır.

'Tüm hayvan türlerinde en önemli beş refah problem kaynağı nedir' sorusuna katılımcıların %75.5'i barınak koşulları, %55.4'ü stres, %44.6'sı bakım ve besleme koşulları, %33.1'i açlık, %31.7'si acı, %27.3'ü ise korku kaynaklı olduğu ifade etmişlerdir (Çizelge 5). İzmirli (2009) de en önemli refah problemi olarak uygun olmayan barınak koşullarını bildirmiştir. Barınak başlığı altında sıcaklık, havalandırma, aydınlatma, hayvan başına ayrılan alan, ekipmanlar, zemin ve altlık yapısı gibi birçok yetiştirme faktörünün hayvan refahını etkilediği bilinmektedir. Şahanoğlu & Koçak (2014), Afyonkarahisar ilindeki 101 süt sığırcılığı işletmesinde barınak tipi, bölme ve duraklar, altlık kullanımı ve suluk sayısı bakımından refaha uygun olmayan durumları saptadıklarını bildirmiştir.

Çizelge 5. Çiftlik hayvanlarında refaha ilişkin gözlem ve algılar
Table 5. Observations and perceptions of welfare in farm animals

Anket sorusu	Seçenekler	Adet	%
Çiftlik hayvanlarında refah problemi vardır.	Katılıyorum	103	74.1
	Kısmen katılıyorum	32	23.0
	Kararsızım	2	1.4
	Kısmen katılmıyorum	2	1.4
	Katılmıyorum	-	-
Tüm hayvan türlerinde refah konusunda önemli problem kaynakları hangileridir? (Çoklu seçim mümkün)	Barınaklar	105	75.5
	Stres	77	55.4
	Bakım ve Besleme	62	44.6
	Açlık	46	33.1
	Acı	44	31.7
	Korku	38	27.3
Hayvanlarda verim artışına yönelik çalışmalar refah sorunlarına yol açmaktadır.	Katılıyorum	44	31.7
	Kısmen katılıyorum	54	38.8
	Kararsızım	7	5.0
	Kısmen katılmıyorum	6	4.3
	Katılmıyorum	28	20.1

Altınçekiç & Koyuncu (2012b) hayvanların doğal davranışlarını gösterebilecekleri koşulların oluşturulmasının hayvan refahının dikkate alınmasıyla yakından ilişkili olduğunu bildirmiştir. Örneğin hayvan türlerine göre birim alana konulacak maksimum hayvan sayısının aşırı tutulması, birim alandan daha fazla gelir elde edilmesini sağlarken barınak içerisindeki hava kalitesinin düşmesine, nem oranının yükselmesine ve altlık kalitesinin bozulmasına neden olmakta, hayvanlarda stres, solunum sistemi ve ayak hastalıklarının artması sonucu karkas kalitesinin düşmesine ve yönetim zorluklarına da sebep olmaktadır. Bu nedenle hayvanların refahı ve sağlığı için kullanılan bütün ekipmanlar günde en az bir kez kontrol edilmeli, varsa sorun en kısa sürede giderilmelidir. Hayvanların beslenme durumu önemli bir refah problemi oluşturabileceğinden hayvanlar türüne ve yaşına uygun rasyonlarla beslenmeli, besin madde ihtiyaçları tam olarak karşılamalıdır. Ayrıca yemlik ve suluklar, kirlenme ve hayvanlar arasındaki rekabeti azaltacak şekilde tasarlanmalıdır. Aydın ili Nazilli ilçesine bağlı Hamidiye, Arpaz, Uzunçam, Beyerli ve Toygar köylerinde işletme büyüklüğü en az 10 baş süt sığırı olan 22 işletmenin incelendiği çalışmada (Acar & Konyalı, 2015) barınak içi koşulları iyileştikçe hayvan refahının da arttığı bildirilmiştir. Barınak koşullarındaki iyileştirmelere bağlı olarak bireysel süt verimlerinin arttığı ve bu artışın istatistiksel olarak önemli olduğu ifade edilmiştir.

Çalışmaya katılan veteriner hekimlerin %70.5'i (%31.7+38.8) hayvanlarda verim artışı sağlayan çalışmaların, refah sorunlarına neden olduğunu ifade etmişlerdir (Çizelge 5). Diğer yandan katılımcıların %5'i bu konuda kararsız kalmış, %24.4'ü (%4.3+%20.1) ise verim artışına yönelik yapılan çalışmaların bir refah problemi doğurmadığını bildirmişlerdir. Üreticiler geçmişte sadece üretimin artırılmasına odaklanırken, son yıllarda hayvan refahıyla birlikte ürün kalitesinin de artırılması gerektiğini düşünmeye başlamışlardır.

İzmirli (2009)'nin bildirdiği %90 oranına karşın bu çalışmada katılımcıların %98.6'sı (%92.1+%6.5) çiftlik hayvanlarının refah koşullarında yetiştirilmeleri durumunda elde edilecek hayvansal ürünlerin, refah koşullarında yetiştirilmeyenlere göre daha kaliteli olabileceğini ifade etmiştir (Çizelge 6). Turan (2018) ise bu şekilde düşünenlerin oranını %56 olarak saptamıştır.

Ankette katılımcıların çoğunluğu (%33.1+36.7=69.8) ürün kalitesindeki artış işlemlerinin hayvan refahı ile birlikte yürütülmesi gerektiğini ifade etmiştir. Diğer yandan hayvan refahına göre yetiştirilmiş hayvansal ürünlerin tercih edilmesinin, hayvan refahına bakışı olumlu yönde etkileyeceği düşünülürken (%79.9+%13.7=%93.6), katılımcıların sadece %4.3'ü (%2.9+%1.4) bu görüşe katılmadıklarını ifade etmişlerdir

(Çizelge 6). İzmirli (2009), söz konusu oranları sırasıyla %87.1 ve %1.7 olarak saptamıştır. Turan (2018) ise refahın sağlanmasında tüketicilerin rolünün ankette son sırada çıktığını, katılımcıların esas sorumlu olarak üretici, devlet ve sivil toplum kuruluşlarını gördüklerini bildirmiştir.

Katılımcıların %95'i (%74.8+%20.2) hayvan refahına uygun hayvan yetiştiriciliği yapılmasını doğru bulduğunu, %1.4'ü ise bu görüşe hiç, %2.9'u ise kısmen katılmadıklarını ifade etmişledir (Çizelge 6). Bu sorudaki görüş farklılıkları cinsiyete göre önemli (X^2=10.165; sd=4; P=0.038) değişim gösterse de ankete katılan kadın sayısının azlığı bu ilişkiye ait sonucun tutarlı olmadığını ortaya koymaktadır (Fisher's Exact Test P=0.081). (Çizelgede verilmemiştir).

Çizelge 6. Çiftlik hayvanlarında refah, ürün kalitesi ve halk sağlığı ilişkisi
Table 6. Relationship between welfare, product quality and public health in farm animals

Anket sorusu	Seçenekler	Adet	%
Çiftlik hayvanlarının refah koşullarında yetiştirilmeleri, bu hayvanlardan sağlanacak ürünlerin daha kaliteli olmasını sağlar.	Katılıyorum	128	92.1
	Kısmen katılıyorum	9	6.5
	Kararsızım	-	-
	Kısmen katılmıyorum	1	0.7
	Katılmıyorum	1	0.7
Geçmişte üreticiler sadece üretimin artırılması çabası içerisindeyken, günümüzde ürün kalitesinin artırılması ve bununda refah standartları doğrultusunda gerçekleştirilmesi gerektiğini düşünmektedir.	Katılıyorum	46	33.1
	Kısmen katılıyorum	51	36.7
	Kararsızım	9	6.5
	Kısmen katılmıyorum	15	10.8
	Katılmıyorum	18	12.9
Hayvan refahına göre yetiştirilmiş ürünlerinin satın alınması hayvan refahı olgusuna olumlu etki yapar.	Katılıyorum	111	79.9
	Kısmen katılıyorum	19	13.7
	Kararsızım	3	2.2
	Kısmen katılmıyorum	2	1.4
	Katılmıyorum	4	2.9
Hayvan refahına uygun yetiştiriciliğini onaylıyorum.	Katılıyorum	104	74.8
	Kısmen katılıyorum	28	20.2
	Kararsızım	1	0.7
	Kısmen katılmıyorum	4	2.9
	Katılmıyorum	2	1.4
Halk sağlığı, hayvan sağlığı ve hayvan refahı arasında yakın ilişkiler vardır.	Katılıyorum	121	87.1
	Kısmen katılıyorum	16	11.5
	Kararsızım	-	-
	Kısmen katılmıyorum	1	0.7
	Katılmıyorum	1	0.7

Katılımcılara göre hayvan refahı ve sağlığının, ürün kalitesi ve insan sağlığı açısından çok önemli faktörlerdir. Nitekim halk sağlığı, hayvan sağlığı ve hayvan refahı arasında yakın bir bağlantı olduğunu sadece bir katılımcı kabul etmemiş, diğer katılımcılar çeşitli derecelerde bu görüşü onaylamıştır (Çizelge 6). Yıbar & Çetin (2013) hayvan refahının et kalitesine etkisini ele almış, özellikle kasaplık hayvanların taşınmasında yaşanan problemlere ışık tutmuştur. Kesim öncesi hayvan refahı uygulamalarına dikkat edilmediğinde yüksek et Ph'sı, daha fazla su tutma kapasitesi, koyu et rengi ve sert et oluşumu gibi karkas ve et kalite özelliklerinin etkilendiği ve bu yüzden önemli ekonomik kayıplar oluştuğu bildirilmiştir. İşletmelerin maliyetleri düşürme ekseninde hayvan refahına yatırım yapmaktan kaçınmaları durumunda, refahtan uzaklaşma düzeyi ile verimlilikteki ekonomik kayıpların doğru orantılı olarak artacağı bildirilmiştir (Canan, 2023).

Hayvansal üretim yapılan işletmelerde, hayvan refahının sağlanmasında sorumlu tarafların belirlenmesi amacıyla yöneltilen soruda en büyük sorumluluğun hayvan sahiplerine (%67.5) ve politikacılara (%15.8) daha sonra sırası ile bakıcılara, veteriner hekimlere ve yerel yönetimlere ait olduğu belirtilmiştir (Çizelge 7). İzmirli (2009) ise hayvan refahından sorumlu olanları önem sırasına göre hayvan sahipleri, veteriner hekimler ve bakıcılar olarak sıralamıştır. Bu nedenle tüm tarafların, özellikle de hayvan sahibi ve bakıcıların davranışlarının hayvanlarda stres yaratabileceği dikkate alınarak, bu kesimlerin hayvan refahı konusundaki bilgi eksikliklerinin mutlaka giderilmesi gereklidir. Nitekim Şahanoğlu & Koçak (2014) 101 adet süt sığırcılığı işletmesinde çalışanların gerek sığır yetiştiriciliği gerekse hayvan refahı konusunda eğitim alanların oranının oldukça düşük olduğunu bildirmiştir (sırasıyla %32.70 ve %3). Bozkurt ve ark. (2018) koyunculukta da durumun farklı olmadığını, çiftçilerin refah gereksinimlerini ve özellikle refahla ilgili standart ve mevzuatı bilmediklerini

saptamıştır. Afyonkarahisar'da 103 koyunculuk işletmesinde çalışan 177 kişi üzerinde yapılan anket çalışmasında yetiştiricilerinin hayvan refahı konusunda bilgi sahibi olmadıkları bildirilmiştir (Kılıç ve ark., 2013). Halbuki çiftlik hayvanlarının refahında, mesleki kabiliyet ve bilgiye sahip yeterli sayıda personelin bulunması son derece önemlidir. Kılıç ve ark. (2013), çiftlik personelinin mutluluğu ve işini severek yapması için işletme sahiplerinin önlemler almasının, hayvan refahı ve verimliliği de artıracağı önerisinde bulunmuştur. Altınçekiç & Koyuncu (2012b) yetiştirici ekseninde insan-hayvan etkileşimini ve bunun hayvan refahına etkisini ele almış, hayvan refahı ve verimlilik adına yapılabilecekleri sıralamışlardır.

Çizelge 7. Hayvan refahına etki eden taraflar veya olgular
Table 7. Parties or facts affecting animal welfare

Anket sorusu	Seçenekler	Adet	%
Hayvan refahının gerçekleştirilmesinde sorumluluk kimlerdedir?	Hayvan sahiplerine	94	67.6
	Politikacılara	22	15.8
	Hayvan bakıcılarına	10	7.2
	Veteriner hekimlere	9	6.5
	Yerel yönetimlere	2	1.4
	Diğer	2	1.4
Refah eksikliğinin sebebi hangileridir? (çoklu seçim mümkün)	Hayvan sahiplerinin bilgisizliği	125	89.9
	Ekonomik sebepler	124	89.2
	Mevzuatın yetersizliği	71	51.1
	Kamuoyu duyarsızlığı	64	46.0
	Ziraat Mühendislerinin ilgisizliği	8	5.8
	Veteriner hekimlerin ilgisizliği	19	13.7
Hayvan refahın gerçekleşmesinde en büyük engel nedir?	Ekonomik sorunlar	77	55.4
	Üreticilerin tutumu	41	29.5
	Tarım politikaları	15	10.8
	Tüketicilerin tutumu	2	1.4
	Hepsi	4	2.9

Katılımcılar, hayvanlardaki refah problemlerinin altında yatan sebeplerin, hayvan sahiplerinin bilgisizliği (%89.9), ekonomik sebepler (%89.2), yasal mevzuatın durumu (%51.1) ve kamuoyu duyarsızlığı (%46) şeklinde ifade etmişlerdir (Çizelge 7). Diğer yandan ekonomik sorunlar (%55.4), üreticilerin tutumu (%29.5) ve tarım politikalarının (%10.8) çiftlik hayvanlarındaki refah problemlerinin aşılmasında baskın rol oynadığını göstermektedir. İzmirli (2009) ise refah eksikliğinin en önemli iki sebebinin ekonomik sebepler ve hayvan sahiplerinin bilgisizliği olduğunu, refah problemlerinin aşılmasında benzer engellerin bulunduğunu saptamıştır.

SONUÇ ve ÖNERİLER

Tarım ve Orman Bakanlığı Aydın İl/İlçe Müdürlüklerinde görevli veteriner hekimlerle gerçekleştirilen anketlere verilen cevaplara göre; katılımcıların genellikle hayvan refahı konusunda duyarlı olduğunu, hayvan refahına uygun yetiştirilmiş hayvansal ürünleri tercih etmenin hayvan refahı olgusuna katkı sağlayacağını, hayvansal ürün alırken etiketlerinde hayvan refahına uygun şartlarda yetiştirilmiş olup olmadıklarını bilmek istediklerini ve eğer refaha uygun şartlarda yetiştirilmişlerse ürüne daha fazla ücret ödemeyi kabul edeceklerini ifade etmişlerdir. Genel olarak bakıldığında üreticiler, üretimin refah standartları doğrultusunda artırılmasını, toplum ise kendi ihtiyaçlarının artan kalite ve hayvan refahı olgusunun dikkate alınarak karşılanmasını istemeye başlamıştır. Bu talepleri iyi bir başlangıç noktası kabul edip hayvan refahını da önceleyen üretim sistemlerinin kurulması gereklidir. Çünkü hayvansal üretimde hayvan refahı konusunun ihmal edilmesi mümkün değildir. Ek alt yapı gereksinimi ve bakım-besleme pratikleri nedeniyle hayvan refahı uygulamalarının ürün maliyetlerini bir miktar artırması normal karşılanmalıdır. Dolayısıyla hayvan refahı uygulamaları kademeli bir şekilde zamana yayılarak yaygınlaştırılmalı, yetiştiricilere bu konuda destekler verilmeli, yetiştiriciler ve toplum hayvan refahı konusunda bilinçlendirilmeli, hayvan refahının etkin uygulandığı işletme ürünlerinin teşviki için ürün reklamı ve uygun fiyat koşulları geliştirilmelidir. Bu aşamada hayvansal üretimde görev alan paydaşların ve genel olarak da toplumun verilecek eğitimlerle hayvan refahı konusunda bilgilendirilmesi son derece önemlidir. Bu yüzden hayvanlara yapılan her işlemin hayvan refahı açısından durumu değerlendirilmeli, etik olup olmadığı sorgulanmalı ve bu konudaki mevzuata uygun tutum alınmalıdır. Başlangıçta yasal düzenlemelerin uygulanmasında bazı sıkıntıların yaşanması normal kabul edilmelidir. Refah şartlarına sahip çiftliklerin ödüllendirilmesi ve belki de hayvan refahına uymayan işletme sahiplerinin cezalandırılması yönlendirici uygulamalar olabilir. Bununla birlikte mevzuattaki bazı uygulamaların yürürlük tarihlerinin devamlı

ertelenmesi, refah uygulamalarına yönelik alt yapı yatırımlarını ve refah koşullarının tam olarak gerçekleştirilmesini önemli ölçüde geciktirmektedir.

TEŞEKKÜR

Aydın ilinin farklı ilçelerinde kamu personeli olarak görev yapan ve anketimize katılan 139 veteriner hekime katkılarından dolayı teşekkür ederiz.

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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