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Quantification of Biochemical and Antioxidant Properties of Contrasting Common Purslane Populations

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ABSTRACT

Plants play a crucial role in human nutrition and health, yet many species with high nutritional and antioxidant potential remain underutilized. Common purslane (*Portulaca oleracea*), a fast-growing and easily cultivable plant, is a rich source of phytoconstituents and bioactive compounds, making it a promising candidate for functional food development. This research aimed to explore common purslane's viability as a unique Mediterranean vegetable. We collected seeds from 25 distinct populations across Turkey, addressing a significant research gap in their biochemical and antioxidant properties. Among these 25 populations, lycopene and lutein levels ranged from 52.59 to 10.52 and 37.33 to 12.63 mg g⁻¹ fresh weight (FW), respectively. Ferric Reducing Antioxidant Power (FRAP) values ranged from 8.23 to 3 mg g⁻¹ FW, Cupric Reducing Antioxidant Capacity (CUPRAC) from 123.44 to 24.17 mg g⁻¹ FW, and Trolox Equivalent Antioxidant Capacity (TEAC) from 135.33 to 66.25 mg g⁻¹ FW. In conclusion, our study not only provides an innovative approach for expanding unexploited markets but also highlights the potential for developing valuable functional foods.

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INTRODUCTION

The Mediterranean region is rich in both wild and cultivated plant species that have served as a crucial source of food, feed, and medicine for humans over hundreds of years (Ceccanti et al., 2018). Common purslane (*Portulaca oleracea* L.) is one among such wild plants and is often deemed a weed in many summer crops. This plant belongs to the Portulacaceae family, which has around 258 plant species as its members (Petropoulos et al., 2018, Ocampo et al., 2018, WFO, 2024). Common purslane has a previous history of being used as a vegetable or as a part of fresh salads. The plant exhibits glabrous, unifacial stems with sessile leaves arranged in an alternate phyllotaxis, displaying a variegation of green and yellow (Butnariu, 2018).

Scientific evidence suggests that the use of common purslane as a nutraceutical and pharmaceutical source dates back to ancient Egyptian times, with records appearing as early as the pharaonic period (Mohamed and Hussein, 1994). Common purslane shoots emerge as a nutritional powerhouse, surpassing many other plants considering their content of health-promoting compounds. They are particularly rich in omega-3 fatty acids that are essential for brain function and heart health. Additionally, they boast significant levels of α -tocopherol (vitamin E), a potent antioxidant, ascorbic acid (vitamin C) crucial for immune function, β -carotene, and a precursor to vitamin A important for vision, and glutathione, a detoxifying molecule. Beyond its impressive omega-3 content, purslane shoots boast a comprehensive vitamin profile, encompassing vitamins A, and B complex (including B1, B2, B3, B6, and B9), and vitamin E. They are further enriched with essential minerals like potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), sodium (Na), phosphorus (P), copper (Cu), and manganese (Mn) (Butnariu, 2018; Unsal et al., 2014). Pharmaceutical research on common purslane suggests potential health benefits of this plant. *In vitro* studies have demonstrated its ability to inhibit cancer cell growth, combat bacterial infections, promote skeletal muscle relaxation, and exert anti-inflammatory effects (Yan et al., 2012). Further, biologically active molecules within common purslane have demonstrated their ability to suppress the acetylcholinesterase (ACHE) enzyme, a pivotal target in Alzheimer's disease therapy (Montoya-García et al., 2018, Chen et al., 2016). Such health advantages have led the World Health Organization to recognize common purslane as one of the most widely

employed medicinal plants (Uddin et al., 2014). Common purslane extends its utility beyond a nutritional powerhouse. Extracts from its roots and leaves exhibit a robust allelopathic capacity, suggesting potential applications in organic and sustainable agriculture. Common purslane has substantial allelopathic effects, impacting the growth and development of nearby plants by releasing bioactive substances into the environment (Hamad, 2021). These allelochemicals, consisting of phenolic acids and flavonoids, are released to the environment by roots, leaf leachates, and plant decomposition (Moh et al., 2024). Additionally, depending on the species they target, they can either hinder or promote the emergence and several other physiological processes in other plants, including seed germination, root elongation, and growth (Moh et al., 2024). The allelopathic properties of common purslane are especially pertinent to agricultural settings, where they can play a role in suppressing weeds naturally or as a component of sustainable crop management techniques (Li et al., 2019). These extracts could function as natural growth regulators and organic herbicides (El-Shora et al., 2015).

Despite its long history of use and native status in the Mediterranean region, common purslane remains a relatively understudied wild species. Some limited studies include (Keser et al., 2021) examining common purslane from Sivrice/Elazığ (Turkey), Osma et al. (2014) from Istanbul (Turkey), Binici and Binici (2021) from Mersin and Antalya (Turkey), and Gul et al. (2017) from Diyarbakir (Turkey). There has been a scarcity of data on biochemical and antioxidant attributes of various common purslane populations found across diverse Turkish regions. Consequently, this is the first study to investigate the biochemical makeup of common purslane from across Turkey. This study aimed to fill this gap in knowledge by looking at the different biochemical compounds found in common purslane from various regions of Turkey. This information could be useful for developing new healthy foods and supplements. Furthermore, this research helps farmers choose the most beneficial common purslane plants. By selecting those with high levels of healthy compounds, they can create new types of leafy vegetables that are both nutritious and profitable.

MATERIALS and METHODS

Plant Material and Growth Conditions

Seeds from 25 different geographical locations in Turkey were obtained that represented the country's biodiversity. These populations, totalling 25 in number, including Agr Tohum, Agri, Aktarix, Arzuman, Assem, Asya, Balikesik, BT Altinkulak, Dogal, Duzce, ERO-I, Ero-II, Gungurler, Mercan, Noroz Nazlisi, Pasa, Rita, Sari Ferik, Simagro, Sun Agri, Tunc, Yagmur, Yerli, Yesim-I, and Yesim-II. These populations were studied under greenhouse conditions for their comparative production of biochemical compounds and antioxidants. The study was conducted according to a completely randomized design (CRD) with four replications. Pots measuring 22.5 cm in depth and 16.5 cm in diameter were filled using a mixture having a 2:1 compost-perlite combination. Each pot witnessed the immediate planting of seeds corresponding to a specific population. Four seeds were planted carefully in each pot, and the sowing depth was about 2-2.5 cm. After germination, the pots were irrigated regularly based on the plant's needs, and the pots were subjected to a rouging procedure to maintain a pair of common purslane plants per pot. The plants received a split application of nitrogen fertilizer at a total rate of 46 kg ha⁻¹. The fertilizer was applied twice in equal amounts, on days 10 and 20 after planting. Importantly, no pesticides or other chemicals (agrochemicals) were used at any point while growing the common purslane.

Experimental Site

This study was conducted at the Department of Plant Productions and Technologies, Nigde Omer Halisdemir University, Turkey, between 2021 and 2022. The plants were grown in a semi-controlled greenhouse that mimicked natural conditions, without using artificial lights. The greenhouse replicated a natural day-night cycle with temperatures set at 25°C during the day and 15°C at night. Humidity in the greenhouse was around 50%.

The experiment was conducted twice during the summer season, employing two independent repeats following identical methods for each. Harvesting was done 30 days after germination, corresponding to the onset of the flowering stage.

Lycopene contents

The method of Davis et al. (2003) was used to determine the lycopene in common purslane. A 0.5 g plant sample was used for the determination of lycopene in common purslane plants. Then, 5 ml of 80% acetone was added and the sample tubes were placed on the shaker (200 rpm) for 2 h. After this, the samples were centrifuged at 13000 rpm for 5 min and the supernatant was collected, and the volume was raised to 25 mL by adding 80% acetone. Then, 10 mL of extract was taken, and 10 mL of hexane was added. Finally, the upper phase was taken in a separate tube and measured on a spectrophotometer at 503 nm using hexane as a blank.

Lutein contents

Spectrophotometric analysis was used to assess the lutein content of the leaves of common purslane, as previously described by Bulda et al. (2008). A frozen sample of liquid nitrogen was pulverized in a mortar and pestle using an acetone-to-ethanol ratio of 1:1. By adding freshly made KOH (1 g m L⁻¹) to the extract, the saponification procedure was carried out to remove the chlorophylls and lipids from the extract. The sample was allowed to settle after the mixture was incubated for 5 min at 45°C and cooled on ice. For analysis, the uppermost colored percent was chosen. The final lutein content in common purslane leaves was calculated using the following equation after samples were read on a spectrophotometer at 480 and 495 nm.

Formula = $11.51A_{480} - 20.61A_{495}$.

Total antioxidant activity

Total antioxidant activity was calculated by using four different methods i.e., cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and TEAC (Total Equivalent Antioxidant Capacity).

Ferric Reducing Antioxidant Power (FRAP)

A leaf sample (1 g) was obtained and combined with a 10 mL solution of methanol and HCl (99:1 v/v). A 1mL of sample material was obtained and the FRAP protocol outlined by Benzie and Strain (1996) was followed. The buffers were made by combining 0.1 mol L⁻¹ acetate (pH 3.6), 10 mmol L⁻¹ 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 20 mmol L⁻¹ ferric chloride solutions (10:1:1). Before analysis, an aliquot of the material was combined with FRAP reagent. After 30 min, the absorbance of the mixture was measured using a spectrophotometer at 593 nm.

Cupric Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC technique (Apak et al. 2004) is based on antioxidants reducing copper I to copper II. A 10 M Cu (II) solution was made, and test tubes were filled with 1mL Cu (II), Nc, and NH₄Ac (pH: 7) buffer solution. After 30 minutes of incubation, the absorbance at 450 nm (A₄₅₀) was measured against a blank reagent.

Trolox Equivalent Antioxidant Capacity (TEAC)

The sample was made by adding 15 mL of a 99:1 v/v solution of methanol and HCl to 0.5 g of the sample material. Following the TEAC technique as detailed by Ozgen and Sekerci (2003), 1 mL of the sample was obtained. Potassium bisulfate was combined with 2,2-aniso-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), which was then mixed and incubated with aliquots of the sample. After 10 min, 2 mL of the prepared buffer was added to 1 mL of the sample extract, followed by measurement at a wavelength of 734 nm in the spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH scavenging assay was carried out using the Berwal et al. (2021) technique. Ethanolic extracts of common purslane leaves (100 mL) were permitted to interact with 2.9 mL of 0.006% ethanolic DPPH for 10 min in the dark. In addition, instead of extract, 100 mL of distilled water was used as a control. At 517 nm, the absorbance was measured.

Reducing power

The Oyaizu (1996) technique was used to estimate the reducing power of the samples in the study. A 25 µL of common purslane sample was taken and 25 µL ethanol was added, mixed with 2500 µL of 0.2 M phosphate buffer (pH 6.6), then 2500 µL of 1% potassium ferricyanide solution was added and incubated at 50°C for 20 min. After the reaction mixture had been incubated, 2500 µL of TCA 10% was added, and it was vortexed. Samples were centrifuged for 10 min at 4000 rpm. A 2500 µL supernatant was collected, combined with 2500 µL distilled water, and 500 µL FeCl₃ was introduced. The samples were measured at 700 nm.

Total soluble protein contents

It was carried out according to Manual (1998). A working reagent albumin (BSA) standard and bicinchoninic acid (BCA) were prepared. A volume of 1.6 mL of working reagent and 0.1 mL of each standard and leaf extract sample were mixed well in separate, labeled microcentrifuge tubes. After that, the tubes were kept for 30 minutes at 37°C. The samples were then measured at 562 nm.

Glycine betaine

A technique published by Grieve and Grattan (1983) was used to measure the glycine betaine content. H₂SO₄ (1.5 mL 2N) was mixed with 1 mg lyophilized cells, and the samples were heated in an Eppendorf for 10 min at 60°C. Samples were centrifuged at 14000 rpm at room temperature. The tubes were kept at 4°C for 16 h before being centrifuged at 14,000 rpm for 30 min. Only crystals of glycine betaine remained adhered to the walls and bottom of the tubes after the supernatant was carefully removed. A spectrophotometer of 550 nm was used to measure absorbance.

Statistical analysis

Following data collection, all measurements were subjected to rigorous analysis using Analysis of Variance (ANOVA) to identify and statistically validate any significant variations among the samples. The Least Significant Difference (LSD) test was employed to statistically differentiate between treatment groups.

Further, a statistical tool called the Pearson correlation coefficient was used to assess connections between various aspects measured in the purslane samples. In pursuit of dimensional reduction and pattern recognition, Principal Component Analysis (PCA) was harnessed. Principal Component Analysis (PCA) was used to analyze the data and uncover hidden patterns using the software Origin 2021 (Origin Pro, Version 2021. Origin Lab Corporation, Northampton, MA, USA).

RESULTS

Lycopene content exhibited significant variation ($p < 0.05$) across the different common purslane populations (Figure 1). Among the populations, "Assem" displayed the highest lycopene concentration, reaching 52.95 mg g⁻¹ of fresh weight (FW). Following closely were the "Balikesik" and "Pasa" populations. Meanwhile, the "Arzuman" and "Gungurler" populations showcased statistical similarities with the "Balikesik" and "Pasa" variants. Conversely, the "Yagmur" population displayed the lowest lycopene concentration (10.52 mg g⁻¹ FW), statistically similar to the "Sari Farik" population (Figure 1). Looking into lutein content, population "Assem" again took the lead with a substantial 37.33 mg g⁻¹ of FW, as shown in Figure 3. Similar to lycopene content, the "Agr Tohum" and "Yesim-I" populations exhibited lutein concentrations statistically close to "Assem" (Figure 2). Conversely, the "Arzuman" population displayed the lowest lutein content, measured at 12.63 mg g⁻¹ FW (Figure 2).

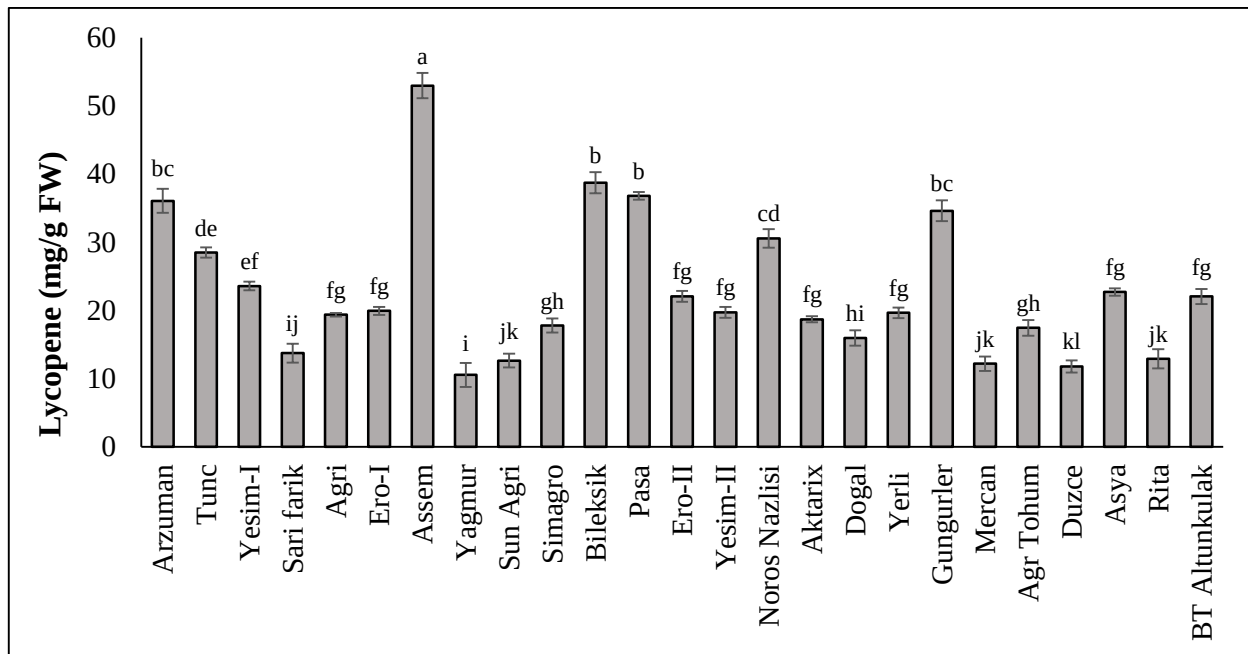


Figure 1. Quantitative assessment of lycopene concentration in common purslane populations
Şekil 1. Semizotu popülasyonlarında likopen konsantrasyonunun kantitatif değerlendirmesi

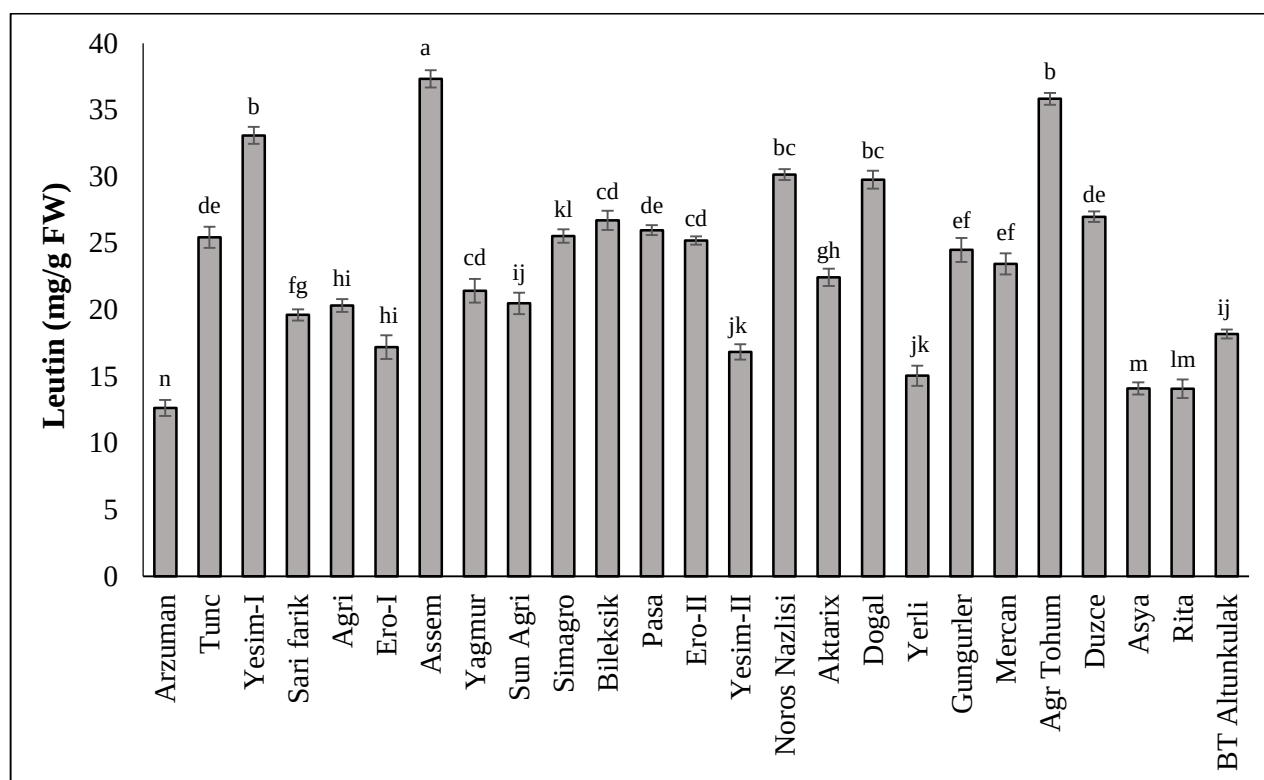


Figure 2. Assessment of Lutein in common purslane populations
Şekil 2. Semizotu popülasyonlarında Lutein konsantrasyonunun değerlendirilmesi

This exploration extended to determining the antioxidant activities exhibited by the collected common purslane populations. The Ferric Reducing Antioxidant Power (FRAP) values exhibited significant variation among the common purslane populations (Figure 3). Among the populations, "BT Altinkulak" displayed the highest FRAP value (8.23 mg g⁻¹ FW), while "Rita" exhibited the lowest (3.0 mg g⁻¹ FW) (Figure 3). Notably, the FRAP value of "BT Altinkulak" was statistically similar to those of the "Yesim-I" and "Sari Farik" populations (Figure 3). The Cupric Reducing Antioxidant Capacity (CUPRAC) assay showed similar patterns, indicating notable differences among the different common purslane populations, as depicted in Figure 4. Interestingly, "BT Altinkulak" notably achieved the highest CUPRAC value, measuring at 123.44 mg per g of FW, while "Tunc" recorded the lowest value of 24.17 mg per g of FW.

The DPPH assay results (Figure 5) revealed the "Sun Agri" population possessed the highest antioxidant activity, exhibiting 0.79% DPPH radical scavenging capacity. Conversely, the "Agri" population displayed the lowest DPPH radical scavenging capacity (0.39%) (Figure 5). Interestingly, statistical analysis exposed a high degree of similarity in antioxidant activity among most of the populations.

Furthermore, this investigation encapsulated the Total Equivalent Antioxidant Capacity (TEAC) values, with "Yagmur" boasting the highest concentration at 135.33 mg per g of FW, and "Duzce" revealing the lowest at 66.25 mg per g of FW (Fig. 6).

The study also examined the reducing power of the purslane samples, and clear differences were found between the populations (Figure 7). The reducing power ranged from a high of 0.701 to a low of 0.316 Trolox mg per g of fresh weight. Among the populations, "Ero-I" exhibited the highest reducing power (Figure 7), suggesting strong antioxidant capabilities. Conversely, the "Sari Farik" population displayed the lowest reducing power (Figure 7), indicating weaker antioxidant activity.

The concentration of Total Soluble Protein (TSP) exhibited significant variation ($p < 0.05$) across the common purslane populations, ranging from 14.00 to 8.20 mg per g of fresh weight (FW) (Figure 8). The "Pasa" population exhibited the highest TSP concentration, whereas the "Agri" population displayed the lowest (Figure 8).

The Glycine Betaine (GB) concentration exhibited significant variation ($p < 0.05$) across the common purslane populations, ranging from 4.80 to 1.30 mg per g of fresh weight (FW) (Figure 9).

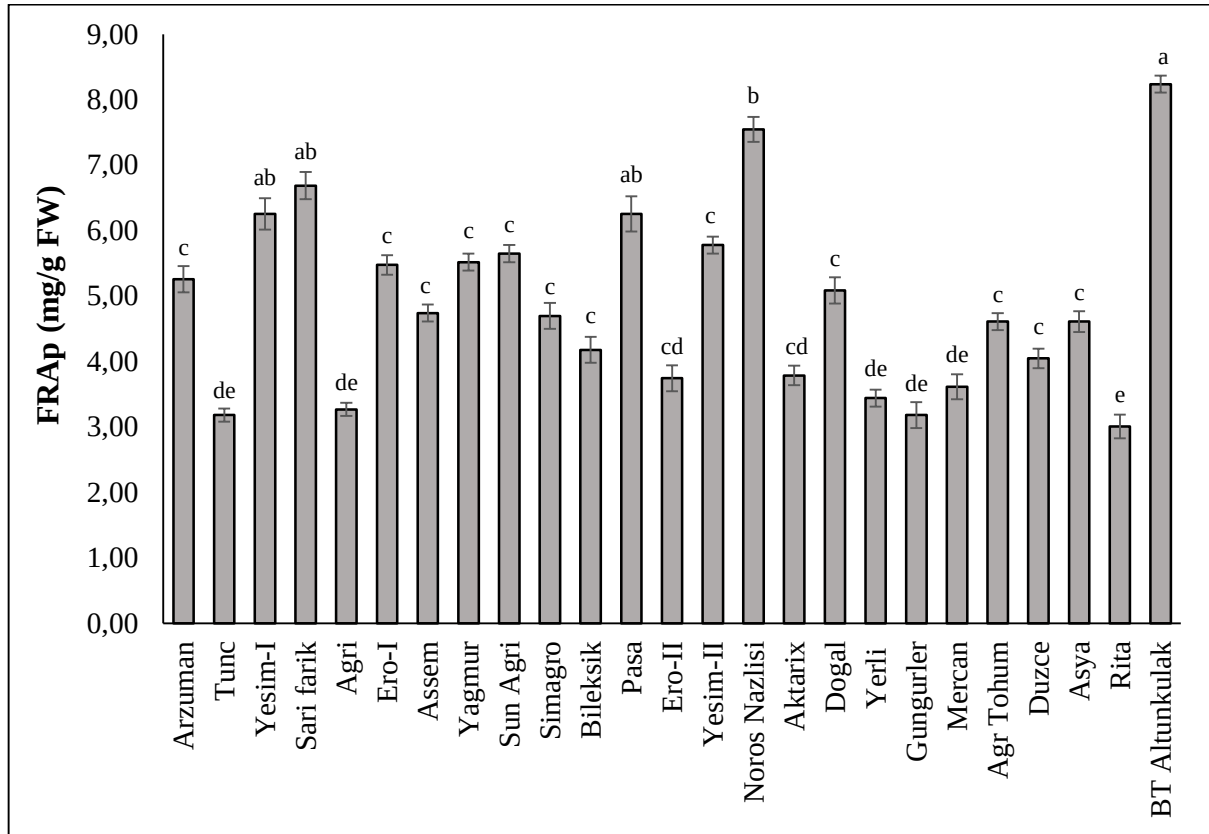


Figure 3. FRAP analysis of common purslane plant: Assessing antioxidant capacity in common purslane populations

Şekil 3. Semizotunun bitkisinin FRAP analizi: Semizot popülasyonlarında antioksidan kapasitesinin değerlendirilmesi

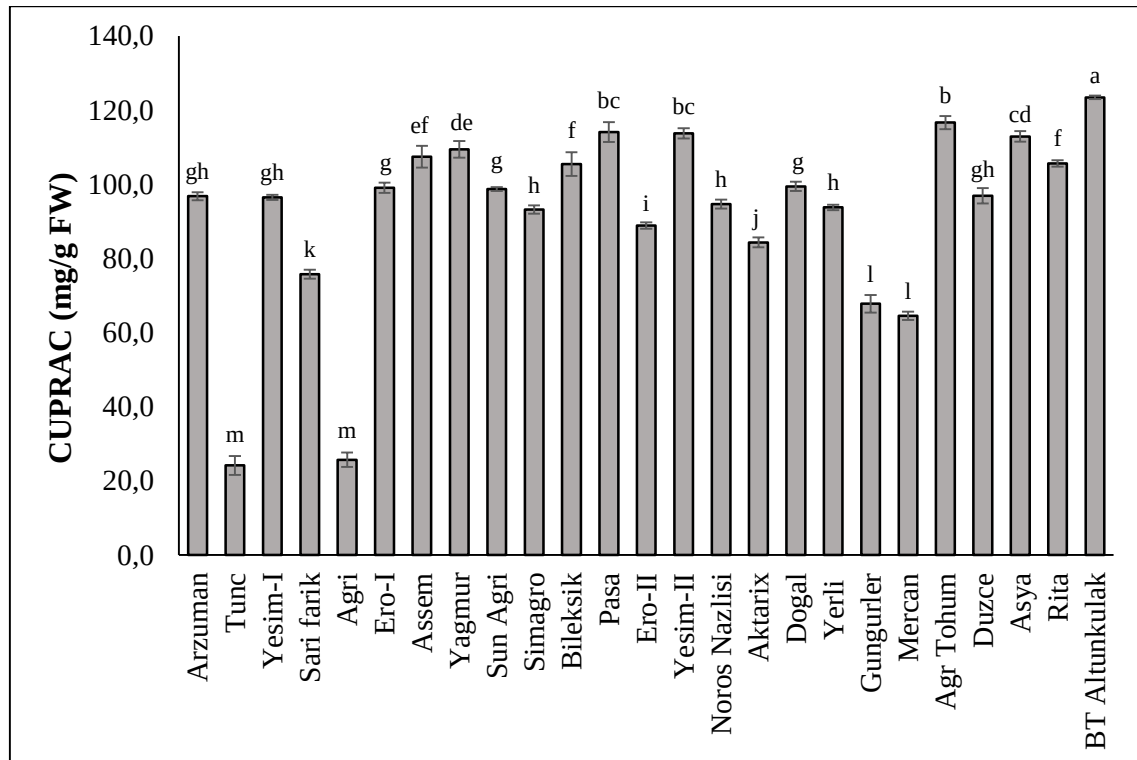


Figure 4. CUPRAC assessment of antioxidant activity in common purslane populations

Şekil 4. Semizotu popülasyonlarında antioksidan aktivitenin CUPRAC değerlendirmesi

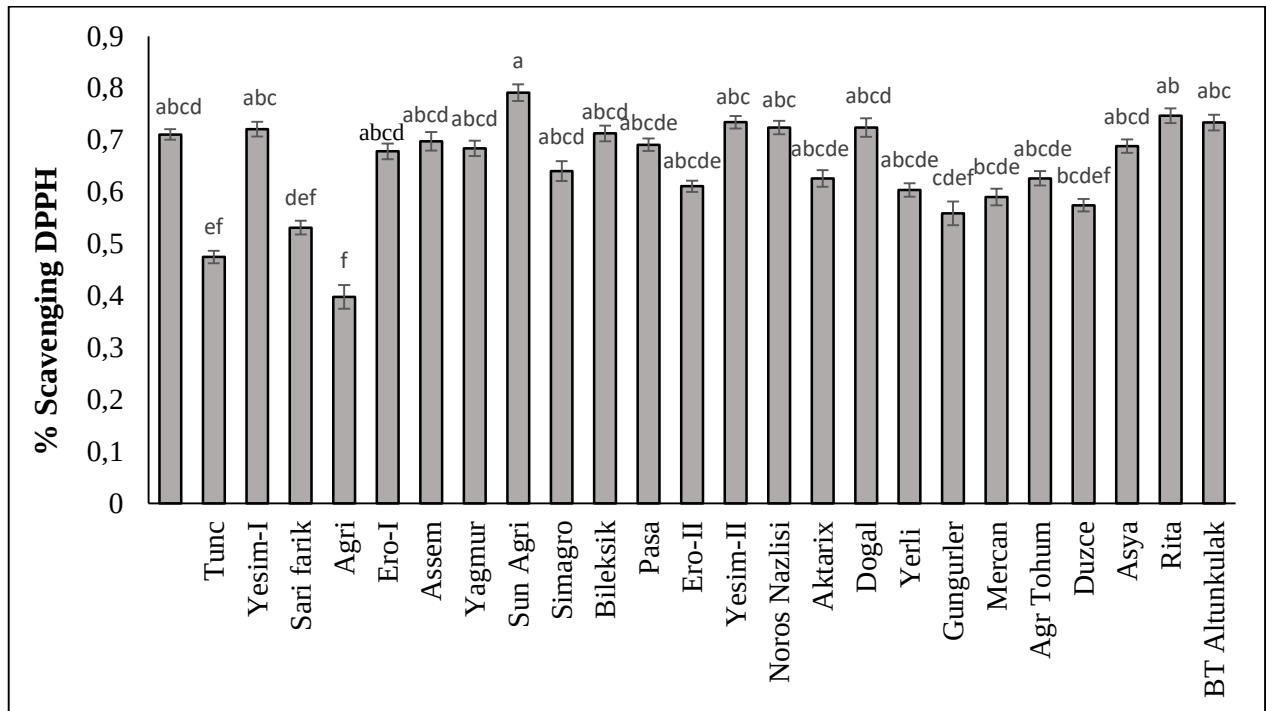


Figure 5. DPPH assay: Common purslane's antioxidant capacity
Şekil 5. DPPH testi: Semizotunun antioksidan kapasitesi

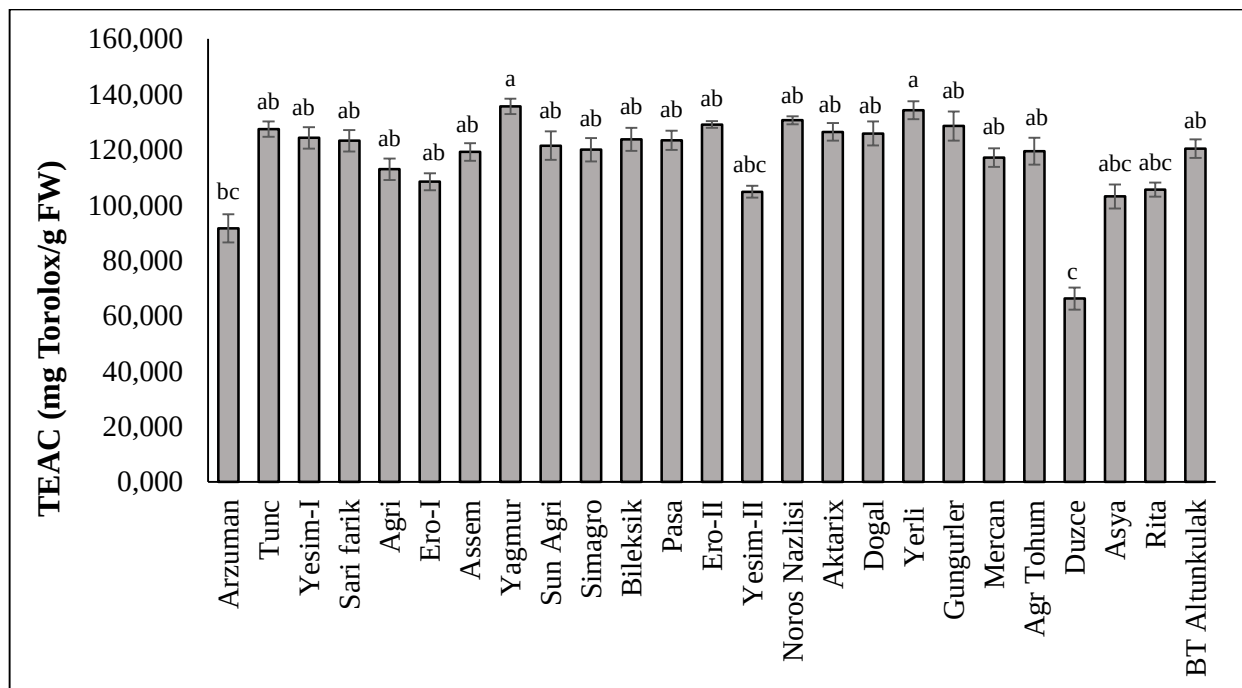


Figure 6. Quantifying antioxidant capacity in common purslane populations via TEAC analysis
Şekil 6. TEAC analizi yoluyla semizotu popülasyonlarında antioksidan kapasitesinin ölçülmesi

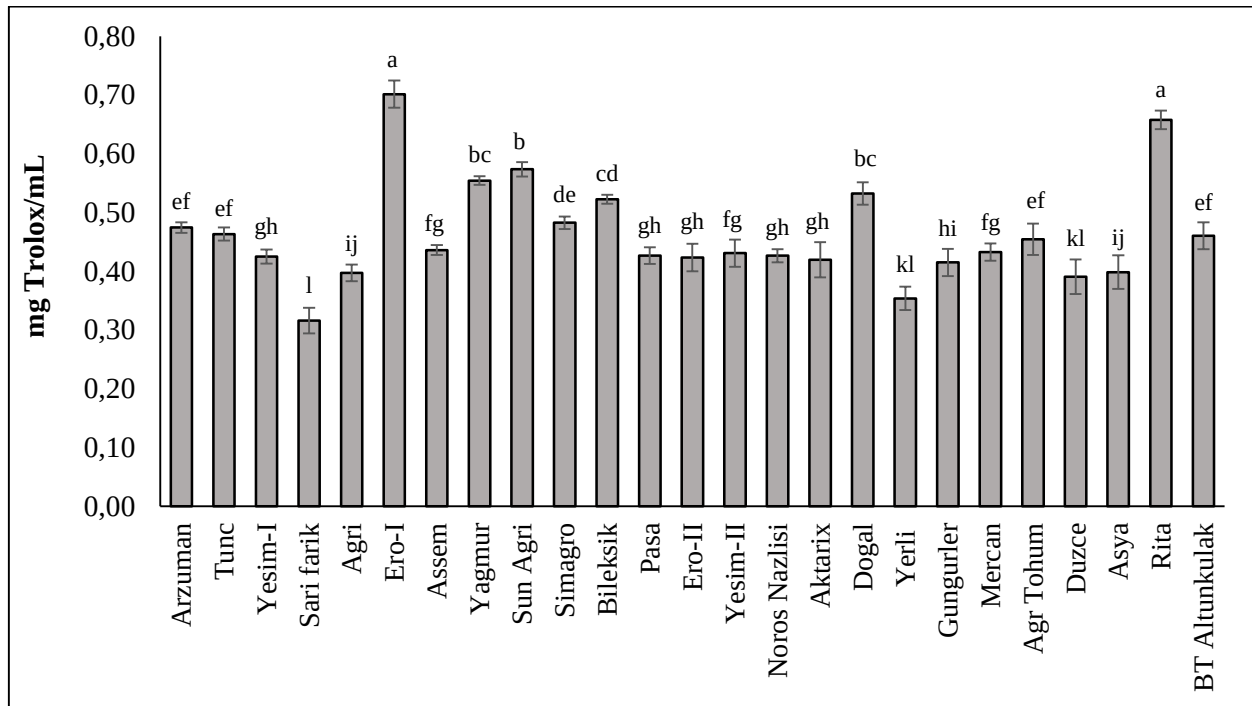


Figure 7. Quantitative analysis of reducing power in common purslane populations
Şekil 7. Semizotu popülasyonlarında indirgeyici gücün nicel analizi

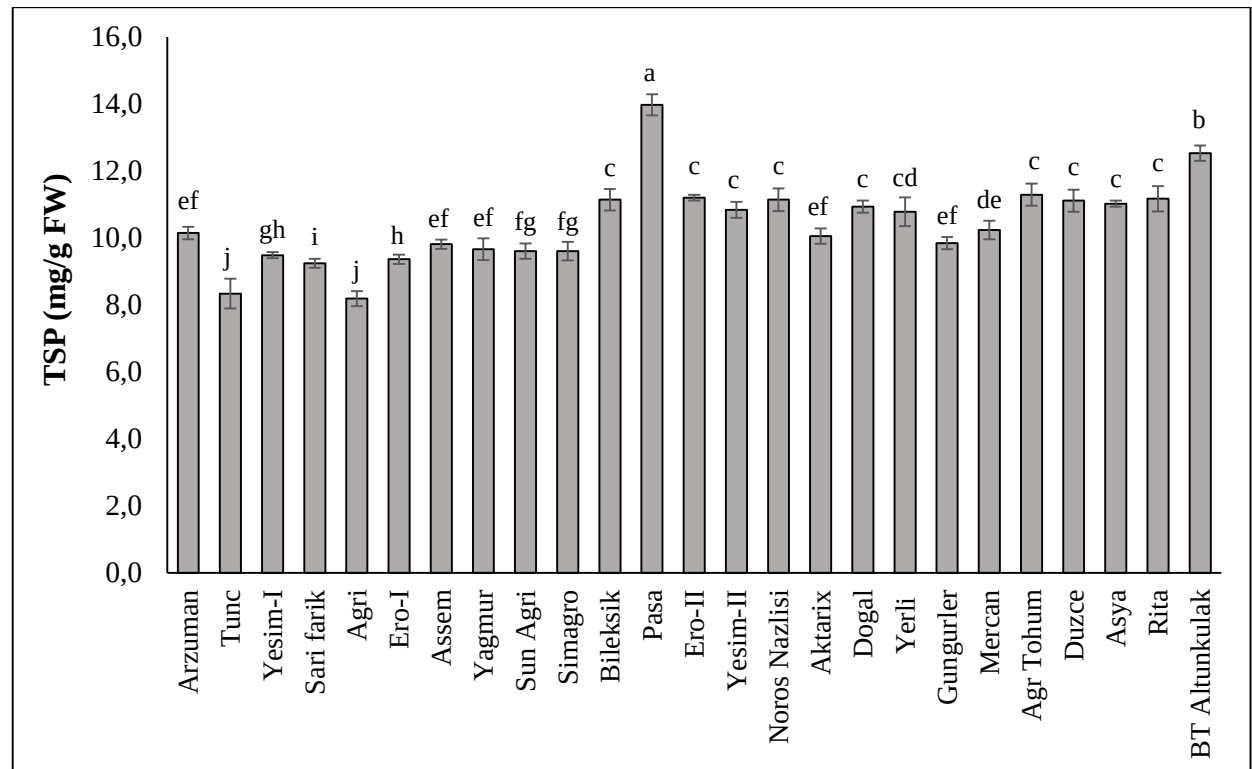


Figure 8. Analysis of total soluble protein content in common purslane populations
Şekil 8. Semizotu popülasyonlarındaki toplam çözünür protein içeriğinin analizi

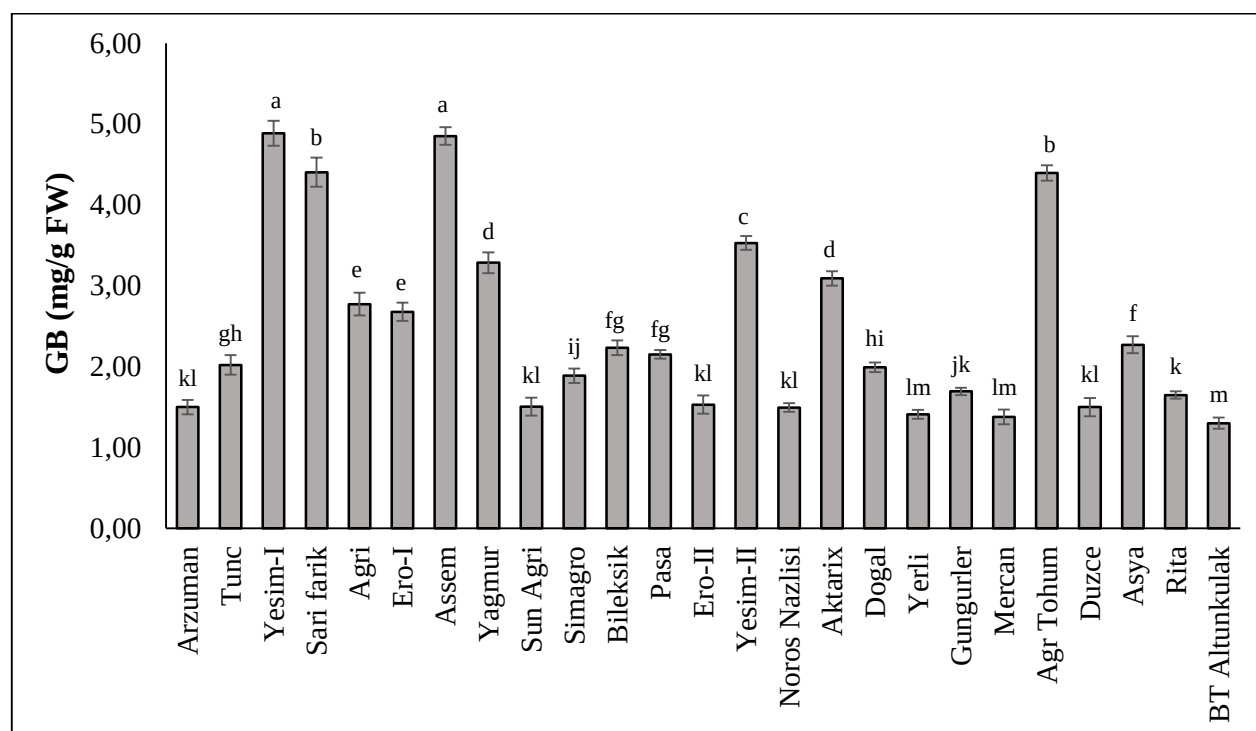


Figure 9. Exploring glycine betaine levels in common purslane populations
Şekil 9. Semizotu popülasyonlarında glisin betain düzeylerinin incelenmesi

DISCUSSION

The results showed that the plant populations studied were significantly different from each other in their antioxidant and biochemical profiles. The results of this confirmed the high antioxidant attributes and presence of other important biochemical components in common purslane plants. Common purslane, despite its often-labeled status as a weed, stands as a pivotal herb that is found ubiquitously, in gardens and lawns.

Plant antioxidants are complex mixtures, and a single test may not be enough to capture all their antioxidant abilities (Chu et al., 2000, Yurt et al., 2024). Because of this, scientists often use several in vitro tests to assess the overall antioxidant power of plants, especially for leafy vegetables. Remarkable diversity in antioxidant activity was found among the collected common purslane populations during this analysis. Many plants contain natural chemicals that can fight oxidative stress in the body. These chemicals might therefore help treat various diseases (Ketnawa et al., 2022; Kario et al., 2022; Çelik et al., 2024). Increasing fruit and vegetable consumption is a widely recognized approach to preventing or treating chronic diseases (İzol and Turhan, 2024).

Lycopene and lutein are carotenoids, natural pigments found in many plants and foods. These pigments have received a lot of interest because of their potential health benefits (Mapelli-Brahm et al., 2020). Lycopene, a key carotenoid pigment found in many plants, has gained attention for its potential health benefits, particularly in reducing the risk of various diseases (Khan et al., 2021; İzol et al., 2024). Studies suggest that taking lycopene supplements in reasonable amounts appears safe (Hedayati et al., 2021). Furthermore, lycopene is being explored as a potential nutraceutical due to its promise in improving blood vessel function and lowering blood pressure. Notably, the study shows the presence of substantial lycopene content in the collected common purslane populations, consistent with prior findings such as Sakil et al. (2018).

Common purslane, being a green leafy vegetable, is unsurprisingly rich in lutein, a type of carotenoid pigment also found in vegetables like kale, spinach, and broccoli (Mitra et al., 2021). Lutein offers several potential health benefits, including protecting against brain and eye problems, as well as skin irritation (Vijay et al., 2018; Yılmaz et al., 2023). A study by Ejoh et al. (2021) found similar levels of lutein in *Basella alba*. Another study by Mrowicka et al. (2022) compared lutein content in various green leafy vegetables, and common purslane stood out for its significantly higher nutritional value compared to other plants.

Measuring antioxidant activity involves using various techniques, each revealing different aspects of how effective the plant is at fighting off harmful molecules. In this study, we employed four methods—FRAP, CUPRAC, TEAC, and DPPH radical scavenging—to ascertain the antioxidant prowess of collected common purslane. "BT Altunkulak" emerged with the highest FRAP and CUPRAC values, while "Sun Agri" and "Yagmur" showed superior

TEAC and DPPH radical scavenging activities, respectively. Notably, a positive correlation was evident among FRAP, CUPRAC, TEAC, and DPPH radical scavenging activity, as depicted in Figure 10.

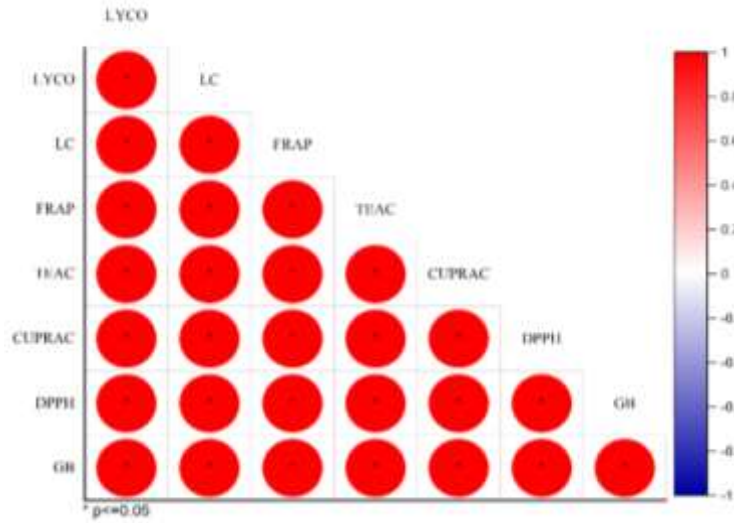


Figure 10. Correlation analysis of biochemical traits in common purslane: unveiling interconnections and associations

Şekil 10. Semizotunda biyokimyasal özelliklerin korelasyon analizi: bağlantıların ve ilişkiler

Note. *Significant ($p < 0.05$). LYCO, lycopene; LC, Lutein; FRAP; TEAC; CUPRAC; GB, glycine betaine; TSP; total soluble protein

The FRAP test measures hydrophilic antioxidants, which explains why it shows lower values for common purslane since its main antioxidants are likely fat-soluble, and this aligns with previous findings (Apak et al., 2004; Uddin et al., 2014; Alam et al., 2014; İzol et al., 2024). Similarly, the CUPRAC assay is a common method used to assess the effectiveness of bioactive compounds against oxidative damage and overall antioxidant activity in biological samples or food (Uzunboy et al., 2017; Bayarsaikhan et al., 2019; Inci et al., 2023). These findings are supported by the results presented by Banerjee et al. (2013). The DPPH scavenging activity outcomes partially align with Uddin et al. (2014) and Alam et al. (2014) also established similar TEAC values. The resemblance between the carried results and those of Gunathilake and Ranaweera (2016) demonstrates consistency. Since plant extracts contain many different antioxidant compounds, scientists need to use several methods to measure their overall antioxidant capacity (Ketnawa et al., 2022; İzol et al., 2021).

Plant proteins are becoming increasingly popular as alternatives to animal proteins in human diets. This study supports previous research by Sarkar et al. (2020) on protein content in *Marsilea quadrifolia* L. The leaves of common purslane were found to be high in protein, making them a promising source of protein for our diet. Additionally, the presence of glycine betaine in the plants aligns with findings by Xu et al. (2018) and Li et al. (2019) who stated that the GB helps plants maintain photosynthesis during stressful conditions.

CONCLUSIONS

In this study, the common purslane seeds were collected from various regions of Turkey, and were grown under greenhouse conditions to determine their biochemical contents. Significant variations were observed among the collected populations, highlighting the wide range of antioxidant and biochemical properties inherent to common purslane. These differences may be attributed to the genetic variability of the seeds rather than external factors such as climate, soil composition, and sunlight exposure.

Turkey Interestingly, some populations exhibited higher antioxidant potential, suggesting they could be valuable resources for medicine. Future research can explore how to cultivate these high-antioxidant purslane varieties and determine their effectiveness in treating specific health conditions.

Understanding these differences can unlock the full potential of common purslane as a medicinal plant. Common purslane is easy to grow and thrives in many environments, making it a potentially sustainable source of bioactive compounds. Additionally, its historical use in traditional medicine suggests promise for further investigation. By adding to this research, we can explore the potential of common purslane as a safe and natural alternative to conventional medications.

Conflict of Interest

The authors declare no conflict of interest.

Contribution of authors

KJ and MYN conceived the idea. MYN conducted the study and prepared the initial draft. KJ approved the final draft.

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Ethics approval and consent to participate

This article lacks any study related to human or animal participants performed by any of the authors.

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REFERENCES

- Alam, M., Juraimi, A. S., Rafii, M. Y., Abdul Hamid, A., Aslani, F., Hasan, M. M., & Uddin, M. (2014). Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) populations. *BioMed Research International*, 296063, 1-10.
- Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, 52(26), 7970-7981.
- Banerjee, D., Hazra, A. K., Chakraborti, S., Ray, J., Mukherjee, A., & Mukherjee, B. (2013). Variation of total phenolic content, flavonoid and radical scavenging activity of *Ipomoea pes-caprae* with respect to harvesting time and location. *Indian Journal of Geo-Marine Sciences*, 106-109.
- Bayarsaikhan, G., Avan, A. N., Çekiç, S. D., & Apak, R. (2019). Use of modified CUPRAC and dinitrophenylhydrazine colorimetric methods for simultaneous measurement of oxidative protein damage and antioxidant defense against oxidation. *Talanta*, 204, 613-625.
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76.
- Berwal, M. K., Haldhar, S. M., Ram, C., & Saroj, P. L. (2021). Determination of total phenolic & flavonoids and antioxidant activity in *Calligonum polygonoides* L. from Thar Desert. *Journal of Environmental Biology*, 42(5), 1347-1354.
- Binici, H. I., Şat, İ. G., & Aoudeh, E. (2021). The effect of different drying methods on nutritional composition and antioxidant activity of purslane (*Portulaca oleracea* L.). *Turkish Journal of Agriculture and Forestry*, 45(5), 680-689.
- Bulda, O. V., Rassadina, V. V., Alekseichuk, H. N., & Laman, N. A. (2008). Spectrophotometric measurement of carotenes, xanthophylls, and chlorophylls in extracts from plant seeds. *Russian Journal of Plant Physiology*, 55(4), 544-551.
- Butnariu, M. (2018). *Portulaca Oleracea* Phytochemistry and pharmacological considerations. *Annal Pharmacology and Pharmacy*, 3(3), 1149.
- Ceccanti, C., Landi, M., Benvenuti, S., Pardossi, A., & Guidi, L. (2018). Mediterranean wild edible plants: Weeds or "new functional crops"? *Molecules*, 23(9), 2299.
- Çelik, Ş., Dervişoğlu, G., İzol, E., Seçzyk, Ł., Özdemir, F. A., Yilmaz, M. E., Yilmaz, M. A., Gülçin, İ., Al-Anazi, K. M., Farah, M. A., Zafar, M., Makhkamov, T., & Khan, M. A. (2024). Comprehensive phytochemical analysis of *Salvia hispanica* L. callus extracts using LC-MS/MS. *Biomedical Chromatography*, e5975. <https://doi.org/10.1002/bmc.5975>
- Chen, Y.-X., Li, G.-Z., Zhang, B., Xia, Z. Y., & Zhang, M. (2016). Molecular evaluation of herbal compounds as potent inhibitors of acetylcholinesterase for the treatment of Alzheimer's disease. *Molecular Medicine Reports*, 14(1), 446-452.
- Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture*, 80(5), 561-566.
- Davis, A. R., Fish, W. W., & Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene

- content in tomato and tomato products. *Postharvest Biology and Technology*, 28(3), 425-430.
- Ejoh, S. I., Wireko-Manu, F. D., Page, D., & Renard, C. M. (2021). Traditional green leafy vegetables as underutilised sources of micronutrients in a rural farming community in south-west Nigeria I: estimation of vitamin C, carotenoids and mineral contents. *South African Journal of Clinical Nutrition*, 34(2), 40-45.
- El-Shora, H. M., & Abd El-Gawad, A. M. (2015). Evaluation of allelopathic effect of white lupin (*Lupinus termis* L.) leaf extract on the biochemical dynamics of common purslane (*Portulaca oleracea* L.). *Egyptian Journal of Botany*, 54(2), 317-332.
- Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for determination of water-soluble quaternary ammonium compounds. *Plant and Soil*, 70(2), 303-307.
- Gul Guven, R., Guven, K., Matpan Bekler, F., Acer, O., Alkan, H., & Dogru, M. (2017). Purification and characterization of polyphenol oxidase from purslane. *Food Science and Technology*, 37, 356-362.
- Gunathilake, K. P. P., & Ranaweera, K. K. D. S. (2016). Antioxidative properties of 34 green leafy vegetables. *Journal of Functional Foods*, 26, 176-186.
- Hamad, S. W. (2021). Bioherbicidal actions of common purslane on seed germination and growth of some crop and weed species. In *IOP Conference Series: Earth and Environmental Science* 910(1), p. 012107. IOP Publishing.
- Hedayati, N., Oskouei, Z., Tabeshpour, J., & Naeini, M. B. (2021). Berberine and lycopene as alternative or add-on therapy to metformin and statins, a review. *European Journal of Pharmacology*, 913, 174590.
- İnci, H., İzol, E., Yılmaz, M. A., İlkaya, M., Bingöl, Z., & Gülçin, İ. (2023). Comprehensive phytochemical content by LC/MS/MS and anticholinergic, antiglaucoma, antiepilepsy, and antioxidant activity of Apilarnil (drone larvae). *Chemistry & Biodiversity*, 20(10), e202300654. <https://doi.org/10.1002/cbdv.202300654>
- İzol, E., Bursal, E., Yapıcı, İ., Yılmaz, M. A., Yılmaz, İ., & Gülçin, İ. (2024). Chemical content by LC-MS/MS, antiglaucoma, and antioxidant activity of propolis samples from different regions of Türkiye. *Journal of Food Biochemistry*, 2024(1), 7488590. <https://doi.org/10.1155/2024/7488590>
- İzol, E., Temel, H., Yılmaz, M. A., Yener, I., Ölmez, O. T., Kaplaner, E., & Ertaş, A. (2021). A detailed chemical and biological investigation of twelve *Allium* species from Eastern Anatolia with chemometric studies. *Chemistry & Biodiversity*, 18(1), e2000560. <https://doi.org/10.1002/cbdv.202000560>
- İzol, E., & Turhan, M. (2024). In-depth phytochemical profile by LC-MS/MS, mineral content by ICP-MS, and in-vitro antioxidant, antidiabetic, antiepilepsy, anticholinergic, and antiglaucoma properties of *Bitlis* propolis. *Life*, 14, 1389. <https://doi.org/10.3390/life14111389>
- İzol, E., Turhan, M., Yılmaz, M. A., Çağlayan, C., & Gülçin, İ. (2024). Determination of antioxidant, antidiabetic, anticholinergic, antiglaucoma properties and comprehensive phytochemical content by LC-MS/MS of Bingöl honeybee pollen. *Food Science & Nutrition*. <https://doi.org/10.1002/fsn3.4531>
- Kario, K., Kagitani, H., Hayashi, S., Hanamura, S., Ozawa, K., & Kanegae, H. (2022). A Japan nationwide web-based survey of patient preference for renal denervation for hypertension treatment. *Hypertension Research*, 45(2), 232-240.
- Keser, F., Karatepe, M., Keser, S., Tekin, S., Türkoğlu, İ., Kaygılı, O., & Kirbag, S. (2021). In vitro biological activities and phytochemical contents of *Portulaca oleracea* L. (purslane). *Journal of Physical Chemistry and Functional Materials*, 4(1), 1-7.
- Ketnawa, S., Reginio Jr, F. C., Thuengtung, S., & Ogawa, Y. (2022). Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition*, 62(17), 4684-4705.
- Khan, U. M., Sevindik, M., Zarrabi, A., Nami, M., Ozdemir, B., Kaplan, D. N., & Sharifi-Rad, J. (2021). Lycopene: Food sources, biological activities, and human health benefits. *Oxidative Medicine and Cellular Longevity*, 2713511, 1-10.
- Li, D., Zhang, T., Wang, M., Liu, Y., Brestic, M., Chen, T. H., & Yang, X. (2019). Genetic engineering of the biosynthesis of glycine betaine modulates phosphate homeostasis by regulating phosphate acquisition in tomato. *Frontiers in Plant Science*, 9, 401291, 1-13.
- Li, Z. R., Amist, N., & Bai, L. Y. (2019). Allelopathy in sustainable weed management. *Allelopathy Journal*, 48(2), 109-138.
- Mapelli-Brahm, P., Barba, F. J., Remize, F., Garcia, C., Fessard, A., Khaneghah, A. M., & Meléndez-Martínez, A. J. (2020). The impact of fermentation processes on the production, retention, and bioavailability of carotenoids: An overview. *Trends in Food Science & Technology*, 99, 389-401.
- Mrowicka, M., Mrowicki, J., Kucharska, E., & Majsterek, I. (2022). Lutein and zeaxanthin and their roles in age-related macular degeneration—Neurodegenerative disease. *Nutrients*, 14(4), 827.
- Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44(6), 307-315.
- Uddin, M., Juraimi, A. S., Hossain, M. S., Nahar, M., Un, A., Ali, M., & Rahman, M. M. (2014). Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes.

The Scientific World Journal, 951019, 1-6.

- Uzunboy, S., Çekiç, S. D., Eksin, E., Erdem, A., & Apak, R. (2017). CUPRAC colorimetric and electroanalytical methods determining antioxidant activity based on prevention of oxidative DNA damage. *Analytical Biochemistry*, 518, 69-77.
- Yılmaz, M. A., Çakır, O., İzol, E., Tarhan, A., Behçet, L., & Zengin, G. (2023). Detailed phytochemical evaluation of a locally endemic species (*Campanula baskilensis*) by LC-MS/MS and its in-depth antioxidant and enzyme inhibitory activities. *Chemistry & Biodiversity*, 20(12), e202301182. <https://doi.org/10.1002/cbdv.202301182>
- Yurt, B., Sağlamtaş, R., Demir, Y., İzol, E., Diril, H., & Çağlayan, C. (2024). Determination of in vitro antioxidant, anticholinergic and antiepileptic activities of some medicinal and aromatic plant extracts. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 27(Suppl. 1), 1-15. <https://doi.org/10.18016/ksutarimdogavi.1472403>



Purification and Characterization of Lactoperoxidase from Goat Milk, Investigation of Inhibition Effects of Cefotaxime Sodium

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ABSTRACT

It has been shown in the literature that LPO is important for natural immunity. The purpose of this study is to purify the lactoperoxidase (LPO) enzyme from goat milk and to investigate the inhibition property of cefotaxime sodium on the enzyme. In this study, the LPO enzyme was purified from goat milk and the inhibition effect of cefotaxime sodium was examined. Enzyme purification processes were carried out by various chromatographic methods and the enzyme inhibitory effect was analyzed with Lineweaver-Burk graphs. Inhibition of cefotaxime sodium on LPO was determined as competitive inhibition. The enzyme was purified by first partially purifying milk using Amberlite CG-50 H+ resins, CM-Sephadex C-50 ion-exchange chromatography, and Sephadex G-100 gel filtration chromatography. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate was used to determine the enzyme's Km and Vmax values. Enzyme purity was determined using SDS-PAGE electrophoresis. A specific activity of 7.21 EU mg⁻¹ of protein per liter was detected. The LPO was purified 13.35 times from 1 liter of defatted milk, and 1.9 mg of enzyme was obtained. ABTS substrate ($\epsilon_{412nm}=32400M^{-1}cm^{-1}$) was used for the enzyme-activity assays.

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Laktoperoksidaz Enziminin Keçi Sütünden Saflaştırılması ve Karakterizasyonu, Cefotaxime Sodyumun İnhibisyon Özelliklerinin Araştırılması

ÖZET

LPO' nun doğal bağışıklık için önem arzettiği literatürlerde gösterilmiştir. Bu çalışmanın amacı, keçi sütünden laktoperoksidaz (LPO) enzimini saflaştırmak ve sefotaksim sodyumun enzim üzerindeki inhibisyon özelliğini araştırmaktır. Bu çalışmada, keçi sütünden LPO enzimi saflaştırılmış ve sefotaksim sodyumun inhibisyon etkisi incelenmiştir. Enzim saflaştırma süreçleri çeşitli kromatografik yöntemlerle gerçekleştirilmiş ve enzim inhibitör etkisi Lineweaver-Burk grafikleri ile analiz edilmiştir. Sefotaksim sodyumun enzim üzerindeki inhibisyonu yarışmalı inhibisyon olarak belirlenmiştir. Enzimin keçi sütünden saflaştırılması için, Amberlite CG-50 H+ reçinesiyle kısmi saflaştırma yapılarak, CM-Sephadex C-50 iyon değişim kromatografisi, Sephadex G-100 jel filtrasyon kromatografisi, sırasıyla çalışıldı. ABTS (3-ethylbenzothiazoline-6-sulphonicacid) substratı için Km ve Vmax değerleri ile optimum pH, optimum sıcaklık belirlendi. Enzim saflığı SDS-PAGE elektroforezi ile tespit edildi. Enzimin bir litre keçi sütünden spesifik aktivitesi 7.21 EÜ mg⁻¹ protein miktarı belirlendi. LPO saflaştırma işlemleri sonucunda 1 litre yağı alınmış taze süttten 13.35 kat saflaştırıldı ve 1.9 mg ($R_z=0.7$) elde edildi. Enzim saflaştırılması sırasında aktivite tayinleri için ABTS substratı ($\epsilon_{412nm}=32400M^{-1}cm^{-1}$) kullanıldı.

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INTRODUCTION

The enzyme lactoperoxidase (LPO) (E.C.1.11.1.7) is available found in liquid form in milk, saliva, tears, and gastric juices (Prince & Ratner, 2000; Kumar & Bhatla, 1995; Amornkul & Henning, 2007; Boots & Floris, 2006). Raw milk is a thick white nutritious substance produced by the secretory glands of mammals. It is at the primary level in the food chain of young individuals. Milk contains large amounts of compounds that protect against infections. One of the important proteins is the LPO enzyme (Wolf et al., 2000). Studies have shown that the LPO enzyme is important for immune system activities. It creates a barrier that protects babies against infections after birth. In studies, it has been stated that the LPO system has been tested at different concentrations and has antibacterial and antifungal activity. (Uğuz & Özdemir, 2005). They form a defensive barrier that protects the young from microorganisms in the postnatal period. The structure of milk contains many compounds that have the ability to protect itself against infections. One of these structures is the LPO enzyme (Wolf et al., 2000). The use of the LPO system is important against the risk of encountering reasons that cannot prevent milk spoilage, such as transportation difficulties in the cold chain, power outages, and device maintenance problems, especially in countries with weak economies (Seifu et al., 2005). Milk and its products used as a food source can quickly deteriorate due to microbial contamination. Some preservatives are added to milk to prevent this negative process. The LPO system is one of them. LPO is a natural preservative system found in fresh raw milk. Approximately 30 mg-1 of LPO is found in bovine milk, and its concentration is stable during lactation. In the goat milk LPO system, oxidation of thiocyanate to antibacterial hypothiocyanite may help protect the mammary gland from involution (Zou et al., 2021). LPO does not have an antimicrobial effect on its own; it requires the presence of hydrogen peroxide. Thus, it turns into hypothiocyanate and has an antibacterial effect. Lactoperoxidase is responsible for the oxidation reaction of many organic molecules, as well as catalyzes its antimicrobial activity with compounds with phenol and thiol aromatic rings. Not only do secondary products such as halide and thiocyanate formed in the metabolic reactions of lactoperoxidase change the compounds, but also chemical change can occur with different compounds. Lactoperoxidase can show its catalytic activity in the lactating mammary glands of mothers as well as in the gastrointestinal tract of babies. Researchers have shown that the LPO system has antibacterial and antifungal properties at different concentrations. (Uğuz & Özdemir, 2005). Although it has a bacteriostatic effect on Lactobacilli settling in the intestinal flora, it also has a bactericidal effect on some pathogenic bacteria such as Esherichia coli and Pseudomonas. (Roger et al., 1994). Recently, the desire to understand the role of milk chemicals in biochemical mechanisms has been the focus of the attention of researchers (Y.S. Qin et al., 2021). LPO, thiocyanate ion (SCN⁻) into the antibacterial hypothiocyanate ion (OSCN⁻) (Figure 1), and this is important for the natural immune system. (Wolfson & Summer, 1993; Kumar & Bhatla, 1995; Shin et al, 2001; Reiter & Harnulv, 1984). The source of LPO activity is the secondary products formed as a result of the reaction it catalyzes. The antibacterial effect is due to the reaction of the hypothiocyanate formed with the protein thiol groups. The effect of LPO on bacteria is that the sulfhydryl -SH groups undergo oxidation, and thus, the ions cannot be transported through the bacterial membrane structure (Sisecioglu et al., 2010a). The LPO system acts as a strong and effective inhibitor together with hydrogen peroxide and thiocyanate ions (Reiter & Harnulv, 1984). LPO thus suppresses enzymes used in bacterial defense (Hoogendorn et al., 1977; Pruitt et al., 1982). LPO enzyme is a single polypeptide chain protein with a molecular weight of 80–85 kDa and 612 amino acids, isoelectric pH of 9.2, carrying a heme group. (Thomas & Bozeman, 1991; Paul & Ohlsson., 1985; Elegamy et al., 2010; Hashimoto et all, 2005). The best-known substrates of enzymes used in metabolic reactions are Guaiacol, (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and catechol (Shindler and Bardsley, 1975; Daniel et al., 1997; Brück et al., 2001). Despite this, there are few studies in the literature on the purification of LPO from goat milk and the inhibition of the enzyme by cefotaxime sodium. In addition, the fact that this study was conducted for the first time in a geographical region, that antibiotics have many side effects, and that the LPO enzyme inhibition activity of cefotaxime sodium is known shows the importance of the study. Research has shown that immunity weakens as a result of increased bacterial resistance (Sisecioglu et al., 2010a). At the end of the study conducted to check whether cefotaxime sodium has an inhibitory effect on LPO, it was determined that cefotaxime sodium inhibited the enzyme . The aim of the study is to purify the LPO enzyme from goat milk with a good yield and to investigate the inhibition effect of cefotaxime. It will contribute to the literature since there is no study in the literature on goat milk LPO. It is reported that LPO will not cause any adverse effects on consumer health (Sarkar and Misra, 1994).

LPO

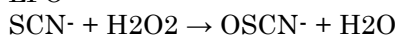


Figure 1 Enzymatic reaction for Lactoperoxidase

Şekil 1. Laktoperoksidaz'ın enzimatik reaksiyonu

MATERIAL and METHOD

Purifying the LPO Enzyme from Goat's Milk

First, 1 L of fresh raw goat's milk was centrifuged 3 times at 2700 rpm for 15 min each time, until the oil was completely removed (Kumar & Bhatla, 1995). Then the weakly acidic cation exchanger Amberlite CG 50 H⁺ resin (equilibrated with 5 mm of sodium acetate, at pH =6.8) was added at a ratio of 22 g L⁻¹ of milk (Doumonted & Rousset, 1983; Kumar & Bhatla, 1995). The milk mixture was mixed with a magnetic stirrer in the refrigerator for 1 hour. It was allowed to rest for 30 minutes. Then, the eluate was washed with distilled water at pH 6.8 and 20-mM sodium acetate until the absorbance decreased below 0.01 at 280 nm. It was eluted using 500 mM sodium acetate at pH 6.8 with the aid of a Buchner Funnel. Protein and enzyme activities of the extracts were measured, and the extracts were stored in the refrigerator at 4°C. (NH₄)₂SO₄ solution was slowly added to the eluate to precipitate. The residue formed by centrifugation at 12,000 rpm at 5°C for 1 hour was dissolved in 25 mL of 5 mM sodium phosphate buffer at pH 6.8. Dialysis was performed overnight in the refrigerator in a 5 mM sodium phosphate buffer of pH 6.8. The supernatant was equilibrated using 10 mM sodium phosphate buffer pH 6.8 and placed in a CM-Sephadex C-50 column. For the washing process, 100 mL of 10 mM pH 6.8 sodium phosphate buffer containing 100 mM NaCl was used. The enzyme was eluted with a linear gradient of 100-200 mM NaCl in 10 mM sodium phosphate buffer at pH 6.8 followed by a 90% saturated ammonium sulfate precipitate II treatment. The enzyme solution was then dialyzed overnight in 5mM Sodium phosphate buffer at pH 6.8. The eluate was collected in 10 mL fractions. RZ value by looking at absorbance at 280 nm and 412 nm against the blank (RZ = A412/A280). Eluates with RZ values greater than 0.75 were combined. The LPO sample was dialyzed. Then it is placed on the Sephadex G-100 column. The enzyme bound to the column was eluted with 0.1 M sodium phosphate buffer at pH 6.8 and removed with 90% saturated ammonium sulfate solution III. The enzyme solution was dialyzed overnight with 0.5 M sodium phosphate buffer at pH 6.0. (Sisecioglu et al., 2010a; Koksall et al., 2017).

Determining Protein Concentration

Protein concentration, enzyme activity, specific activity, and purification rates were determined according to the Lowry method using bovine serum albumin as the standard (Lowry et al., 1951, Boroujeni et al., 2024, Kandasamy et al., 2014).

Determining LPO Activity

LPO activity was measured according to the Shindler procedure. Enzyme activity can be measured by monitoring the absorbance of the resulting-colored compound at 412 nm. (Shindler & Bardsley, 1975, Koksall & Alim, 2020). Working procedure: 2.8 mL of 1 mM ABTS in 0.1 M sodium phosphate buffer at pH 6.0, pH 6.0, and 0.1 mL of LPO enzyme in 1 mM sodium phosphate buffer were mixed. Then 0.1mL of 3.2 mM H₂O₂ solution was added. The absorbance at 412 nm was then measured every 15 seconds for 3 minutes.

Kinetic Studies

To calculate K_m and V_{max} criteria, 5 different (0.2 mL, 0.5 mL, 0.8 mL, 1.1 mL, and 1.5 mL) ABTS substrate concentrations were measured for enzyme activity at pH 6.0 at room temperature. For each concentration, the total volume was adjusted to 2.8 mL. 0.1 mL enzyme solution and 0.1 mL H₂O₂ were added to each tube and absorbance values were measured with a spectrophotometer at 412 nm. Each measurement was repeated twice. Values of 1/V-1/[S] were determined and a Lineweaver-Burk plot was plotted using least squares regression line parity. K_m and V_{max} values were calculated from this graph and presented in Table 1. LPO enzyme activity was calculated similarly. To determine the optimum temperature of the enzyme, 5 substrate concentrations were measured at temperatures ranging from 5-80°C, and these temperatures were achieved using a water bath. To determine the K_m and V_{max} values, first, the optimal pH and temperature values were measured using ABTS activity in 5 substrate concentrations: 0.2 mL, 0.5 mL, 0.8 mL, 1.1 mL, and 1.5 mL. For each concentration, the total volume used, including the buffer solution, was 2.8 mL. Then, 0.1 mL of enzyme solution and 0.1 mL of H₂O₂ were added to each tube, and absorbance values were measured at 412 nm using a spectrophotometer. Each measurement was repeated twice. The 1/V-1/[S] values were calculated, and a Lineweaver-Burk graph was plotted. (Fatemeh et al., 2016).

Table 1. K_i, K_m ve V_{max} values of three different drug concentrations obtained from Lineweaver-Burk graphs

Çizelge 1. Lineweaver-Burk grafiklerinden elde edilen üç farklı ilaç konsantrasyonunun K_i, K_m ve V_{max} değerleri

Drug	[Drug] M	K _i values(M)	Mean K _i (M)	Inhibition form	K _m	V _{max}
Cefotaxime	0.1	0.012	0.015	Competitive	1.4	5.89
	0.2	0.014			2.0	5.89
	0.3	0.02			2.13	5.89

Investigating The Effect of Cefotaxime Sodium on Enzyme Activity

A water-soluble drug was used to investigate the inhibition effect of the purified LPO enzyme. To measure the inhibitor concentration (I_{50}) value of the drug due to the ABTS substrate At room temperature and optimal pH, saturated ABTS (26.67×10^{-2} mM) and drug without H_2O_2 were added at different concentrations. Activity percentage-concentration graphs were drawn, assuming the enzyme activity was 100% without drug addiction. For this purpose, activity percentage was calculated by adding six different volumes of the drug to the fixed substrate concentration of ABTS, and I_{50} values causing 50% inhibition were determined for each drug used (Figure 3). Then, activity values were studied at five different ABTS substrate concentrations and three different drug concentrations. Inhibition types and K_i values were found by drawing Lineweaver-Burk graphs. (Sarıkaya et al., 2015).

RESULTS

Partial purification of the enzyme was done by adding Amberlite CG-50 H^+ , a weakly acidic cation exchanger, to goat milk. (Köksal et al., 2017). The purification steps of the LPO enzyme are given in Table 2, respectively.

Table 2. Purification steps of lactoperoxidase from goat milk

Çizelge 2. Keçi sütünden laktoperoksidazın saflaştırma basamakları

Step	Activity, (EÜmL ⁻¹)	Total volume, (mL)	Protein (mg)	Total Protein, (mg)	Total Activity, (EÜmL ⁻¹)	Specific Activity, (EÜmg ⁻¹)	Recovery (%)	Purification (fold)
Crude homogenate	0.76	120	1.40	168	91.2	0.54	100	1.00
Ammonium sulfate	3.81	20	1.58	28.6	76.2	2.66	83.5	4.92
CM Sephadex C-50 column	0.25	240	0.05	13.2	60.0	4.54	65.8	8.40
Ammonium sulfate	1.98	20	0.35	7.0	39.6	5.65	43.4	10.46
Sephadex G-100 column	0.25	90	0.04	3.6	22.5	6.25	24.6	11.57
Ammonium sulfate and dialyse	0.98	14	0.14	1.9	13.7	7.21	15.0	13.35

LPO enzyme activity was measured by a modification of the Shindler and Bardsley method based on measuring at 412 nm the increased absorbance of the colored compound formed by oxidation of the ABTS chromogenic substrate by H_2O_2 (Shindler & Bardsley, 1975). Enzyme activity measurement results in 1 enzyme unit; It was defined as “the amount of enzyme that catalyzes the oxidation of 1 μ mol ABTS in 1 minute at 20°C.” Six purification steps were performed, at the end of which the measured activity increased from 0.54 EU m g⁻¹ to 7.21 EU mg⁻¹. The enzyme was purified 13.35-fold. After purification, the yield value was 15%. Using the obtained $1/V-1/[S]$ values, the Lineweaver-Burk graph was drawn (Figure 2), and K_m and V_{max} values were determined.

According to ABTS usage, the V_{max} value was found to be 5.89 EU mL⁻¹ and the K_m value was 2. For kinetic studies of the LPO enzyme, Lineweaver-Burk graphs were drawn by measuring spectrophotometric at six different ABTS substrate concentrations and three different drug concentrations. Inhibition types and K_i values were determined. Lineweaver-Burk graphs were created with the help of the obtained $1/V-1/[S]$ values (Figure 2). For these values, it was found that 1g of cefotaxime sodium had a significant inhibitory effect, the K_i value of 1g of cefotaxime sodium for ABTS was 0.015 M. The results showed that 1 g of cefotaxime sodium significantly inhibited the LPO enzyme. According to these values, cefotaxime (1g Cefotaxime Sodium) shows a competitive inhibition effect. The activity-concentration graphs for the ABTS substrate were plotted against the values obtained in the kinetic study using 1 g of cefotaxime sodium, and the I_{50} values were determined. For this purpose, activity values were calculated by adding 1g of cefotaxime sodium at various concentrations of ABTS (26.67×10^{-2} mM) and H_2O_2 (10.67×10^{-2} mM) at constant substrate concentrations. Thus, the reaction rate was found to be dependent on ABTS. Figure 3 shows this graph.

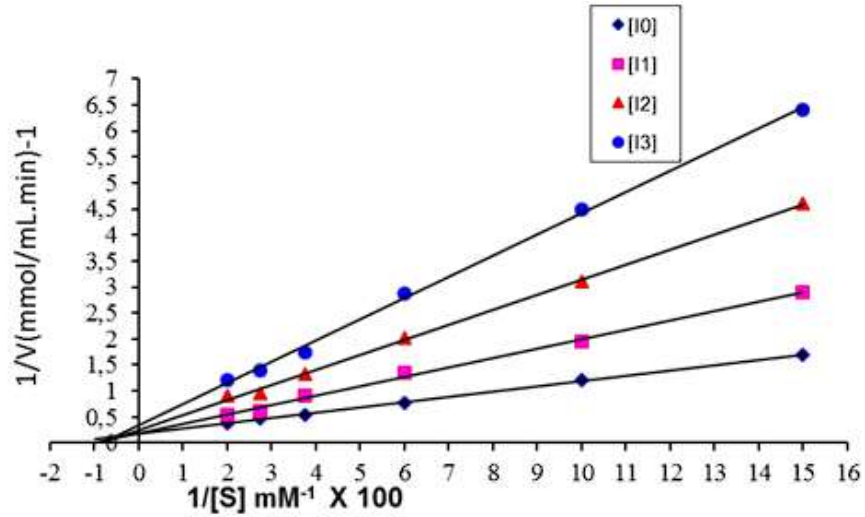


Figure2. Lineweaver-Burk graphics for three different Cefotaxime Sodium (1g Cefotaxime sodium) concentrations and different ABTS concentrations

Şekil 2. Üç farklı Sefotaksim Sodyum (1g Sefotaksim sodyum) konsantrasyonu ve farklı ABTS konsantrasyonları için Lineweaver-Burk grafikleri

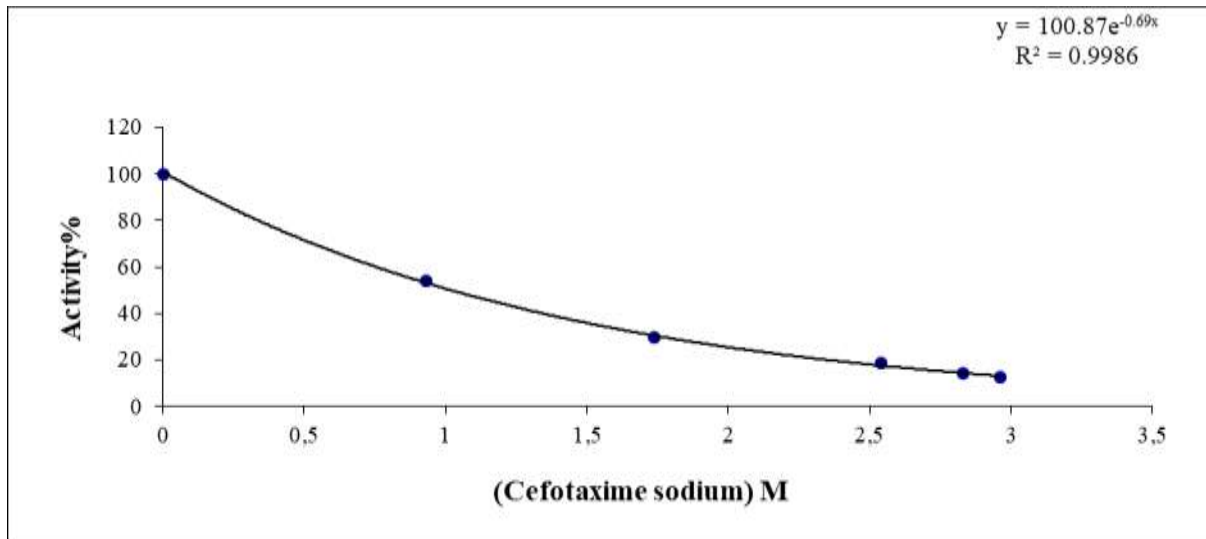


Figure3. Activity %Concentration analysis graphs for Goat Milk LPO of Cefotaxime Sodium

Şekil 3. Sefotaksim Sodyumun Keçi Sütü LPO'su için Aktivite %Konsantrasyon analiz grafikleri

According to this result, the I_{50} value for cefotaxime sodium is 0.93 M. After the chromatographic steps, SDS-polyacrylamide gel electrophoresis was performed to determine the purity of the purified lactoperoxidase enzyme. Presented in Figure 4. Thus, LPO was compared with markers with different molecular weights

DISCUSSION

Purification of the investigated LPO enzyme from goat milk is not frequently seen in the literature. In previous studies, LPO has been purified from goat milk by different methods. A single-step purification process was performed using affinity chromatography. (Koksal et al., 2017). Antimicrobial inhibition studies are being investigated due to the high side effects of antibiotics, which are generally used in some bacterial diseases, and their antibacterial, antifungal, and antiviral effects. (Gobbetti et al., 2004). The Schindler and Bardsley method are based on the oxidation of the ABTS substrate by H_2O_2 and the increase in the absorbance of the resulting colored compound. Many researchers said that they used this method themselves. (Sisecioglu et al., 2011; Ozdemir & Uguz, 2005). The enzyme was first purified to a total of 5mg from 1 liter of bovine milk using Amberlite CG-50 H^+ resins, CM-Sephadex C-50 ion exchange chromatography, and Sephadex G-100 gel filtration chromatography. While the average lactoperoxidase concentration determined by purification processes in the goat milk sample was

1.9 mgL⁻¹ LPO (Rz = 0.75), it was determined that the total protein in the human milk sample was 0.77 ± 0.38 mgL⁻¹ (Kouichirou Shin et al., 2001). The difference in the amount of pure lactoperoxidase we obtained may be due to regional and racial characteristics. (Kumar & Bhatla, 1995).



Figure 4. SDS- PAGE for the purity of the enzyme lactoperoxidase from the purified goat milk

1. Bovine Carbonic Anhydrase (M.W.29 kdal), 2. Chicken Egg Albumin (M.W. 45 kdal), 3. Bovine Serum Albumin (M.W. 66 kdal), 4. Goat milk LPO (M.W. 80-85 kdal).

Şekil 4. Safleştirilmiş keçi sütündeki laktoperoksidaz enziminin saflığı için SDS-PAGE

1. Sığır Karbonik Anhidraz (M.W.29 kdal), 2. Tavuk Yumurta Albümini (M.W. 45 kdal), 3. Sığır Serum Albümini (M.W. 66 kdal), 4. Keçi sütü LPO (M.W. 80-85 kdal).

The specific activity of LPO obtained as a result of purification steps and dialysis was found to be between 0.54 EU mg⁻¹ and 7.21 EU mg⁻¹. Koksall and her colleagues used the affinity chromatography method to purify LPO from different milk sources and purified 53-fold LPO with a yield of 49.5%. (Koksall et al., 2017) In our study, LPO was obtained in pure form with 13.35 times 15% yield. The increase in the number of purification steps reduces the efficiency. Thus, LPO was obtained with lower efficiency.

Inhibition types and K_i values were found by drawing Lineweaver-Burk graphs (Koksall et al., 2017; Sisecioglu et al., 2010). It was found that the Lactoperoxidase enzyme showed maximum activity at pH: 6 and 50 °C (Sisecioglu et al., 2010b). According to ABTS usage, the V_{max} value was found to be 5.89 EU mL⁻¹ and the K_m value was 2. K_m value was found as 0.358 µmol, also Vmax values were calculated as 18.86 EU mL⁻¹. (Sisecioglu et al., 2009). K_i constant values for melatonin and serotonin were calculated as 0.82 ± 0.28 and 0.26 ± 0.04 µM, respectively. Inhibition of melatonin and serotonin has been demonstrated. For this purpose, activity values were calculated by adding 1 g cefotaxime sodium at different concentrations of ABTS and H₂O₂. For this, the H₂O₂ concentration was taken above saturation, while the ABTS substrate concentration was taken below saturation. (Sisecioglu et al., 2011; Ozdemir & Uguz, 2005). In their study, Kalın and his colleagues investigated the enzyme inhibition of cefazolin on LPO, which they purified from bovine milk.

In previous studies, LPO enzyme from large animal production was purified by ion exchange chromatography and gel filtration chromatography. Specific activity was determined in its purification step. LPO has many applications. The shelf life of pasteurized milk in transportation and storage may be increased, especially if it is added to milk in milk processing units. (Barrett et al., 1999). Purification of the LPO enzyme from goat's milk has seldom been discussed in the literature. This study was conducted because LPO enzyme activity varies in various bacterial strains. It has been reported that thiocyanate, the precursor of hypothiocyanate found in animal cell secretion, is known as the substrate with antimicrobial activity for lactoperoxidase. (Thomas et al., 1991; Reiter & Perraudin 1991). Similarly, the inhibitory effect of LPO activity on melatonin and serotonin was determined (Sisecioglu et al., 2010; Barrett et al, 1999). Clinical research has demonstrated that numerous side effects arise from the unnecessary use of antibiotics. The most significant of these is that overuse diminishes the natural immunity of the immune system.

CONCLUSION

Since the purification of lactoperoxidase (LPO) enzyme from goat milk and the study on the inhibitory activity of cefotaxime sodium on the enzyme have not been widely reported in the literature, this study gains importance. Studies on cefotaxime sodium, which inhibits the enzyme, are also rare, but it has been shown to inhibit the lactoperoxidase enzyme. In addition, since some species are routinely treated with antibiotics, the antimicrobial effects of the tested drug have also been expressed. Due to the antimicrobial activity of LPO, ongoing human

studies will provide more information on the physiological roles of LPO. Less use of antibiotics will protect the immune system's health. Although the study on goat milk lactoperoxidase was conducted regionally, the results were found to be consistent with the studies of other researchers. The findings of this study showed the kinetic parameters and competitive inhibition of cefotaxime on LPO. However, more research should be done on lactoperoxidase to know its physiological effects in human milk in detail. Since the current study showed that cefotaxime sodium inhibits the LPO enzyme, a more comprehensive study of the LPO enzyme is recommended.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

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

REFERENCES

- Amornkul, Y., Henning, D.R. (2007). Utilization of microfiltration or Lactoperoxidase system or both for manufacture of Cheddar Cheese from raw milk. *Journal Dairy Science*, 90, 4988-5000.
- Barrett, N.E., Grendison, A.S., Lewis, M.J. (1999). Contribution of the lactoperoxidase system to the keeping quality of pasteurized milk. *Journal Dairy*, 66 (1), 73-75.
- Boots, J.W., Floris, R. (2006). Lactoperoxidase: From catalytic mechanism to practical applications. A review. *International Dairy Journal*, 16, 1271-1276.
- Boroujeni, M.B., Aghamaali, M.R., Nayeri, H., Maal, K.B. (2024). Stabilization of whey lactoperoxidase using betaine. *Spectroscopic and computational approaches*, 1301, 137329.
- Brück T.B., Fielding R.J., Symons M.C.R., Harvey P.J. (2001). Mechanism of nitrite-stimulated catalysis by lactoperoxidase. *Eur. J. Biochem*, 268, 3214-3222.
- Daniel, R., Rao, L.D., Mona, IC. (1997). Identification of colored guaiacol oxidation product produced by peroxidases. *Analytical Biochem*, 250 (1), 10-17.
- Doumonted, C., Rousset, B. (1983). Identification, purification and characterization of a non-heme Lactoperoxidase in bovine milk. *Journal Biological Chemistry*, 258, 14166-14172.
- Elegamy, E., Ruppanner, R., İsmail, A., Champagne, C.P., Assal, R. (1996). Purification and characterization of lactoferrin, lactoperoxidase, lysozyme and immunoglobulins from camel's milk. *Journal Dairy Research*, 6 (2), 129-145.
- Fatemeh, B., Mohammad, M., Jaleh, V., Shariat, S., Seyed, S.S. (2016). Purification of lactoperoxidase from bovine whey and investigation of kinetic parameters. *Advanced Biomedical Research*, Mumbai. 5 (1), 1-6.
- Gobbetti, M., Minervini, F., Rizzello, C.G. (2004). Angiotensin I-converting-enzyme inhibitory and antimicrobial bioactive peptides. *International Journal of Dairy Technology*, 57, 173-188.
- Hashimoto, K., Sato, K., Nakamura, Y., Ohtsuki, K. (2005). Development of a large-scale (50L) apparatus for ampholyte-free isoelectric focusing (autofocusing) of peptides in enzymatic hydrolysates of food proteins. *J Agric Food Chem*, 53, 3801-3806.
- Hoogendorn, J.P., Piessens, W., Scholtes, A.S. (1977). Hypothiocyanite ion: The inhibitor formed by the system Lactoperoxidase-thiocyanate-hydrogen peroxide. *Caries Research*, 11, 77-84.
- Jacob, B.M., Monoj, N.K., Haridas, M. (1998). Antibacterial property of goat milk Lactoperoxidase. *Indian Journal of Experimental Biology*, 31, 808.
- Kandasamy, V.V., Shivanu, S., Rathnasamy, S. (2014). Lactoperoxidase Extraction from Caprine Milk using Conventional and Ionic Liquid Based ATP System. *International Journal of ChemTech Research*, 6 (5), 2887-2893.
- Koksal, Z., Alim, Z. (2020). Lactoperoxidase, an antimicrobial enzyme, is inhibited by some indazoles. *Drug and chemical toxicology*, 43 (1), 22-26.
- Koksal, Z., Kalin, R., Gerni, S., Gulcin, İ., Ozdemir, H. (2017). The inhibition effects of some natural products on lactoperoxidase purified from bovine milk. *J. Biochem. Mol. Toxicol*, 31 (9), 1-6.
- Koksal, Z., Usanmaz, H., Bayrak, S., Ozdemir, H. (2017). Improved chromatographic method for purification of lactoperoxidase from different milk sources. *Preparative biochemistry and biotechnology*, 47 (2), 129-136.
- Kouichirou, S., Hirotooshi, H., Bo, L. (2001). Purification and quantification of lactoperoxidase in human milk with use of immunoadsorbents with antibodies against recombinant human lactoperoxidase. *The American Journal of Clinical Nutrition*, 73 (5), 984-989.

- Kumar, R., Bhatla, K.L. (1995). Purification, crystallization and preliminary x-ray crystallographic analysis of Lactoperoxidase from buffalo milk. *Acta Crystallographica Section D*, 51, 1094-1096.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin Phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.
- Metodiewa, D., Reszka, K., Dunfurd, H.B. (1989). Oxidation of the substituted catechols dihydroxyphenylalanine methyl ester and trihydroxyphenylalanine by Lactoperoxidase and Its compounds. *Archives of Biochemistry and Biophysics*, 274 (2), 601-608.
- Ozdemir, H., Aygul, I., Kufrevioglu, O.İ. (2001). Purification of Lactoperoxidase from Bovine Milk and Investigation of the Kinetic Properties. *Prep. Biochem. Biotechnol*, 31, 125-134.
- Ozdemir, H., Uguz, M. (2005). In vitro effects of some anaesthetic drugs on Lactoperoxidase enzyme activity. *Journal Of Enzyme Inhibition And Medicinal Chemistry*, 20, 491-495.
- Paul, K.G., Ohlsson, P.I. (1985). In The Lactoperoxidase System: Chemistry and biological significance. Oten. ovue. *Journal of Educational Sciences*, 15-29.
- Prince, A., Ratner, A.J (2000). Lactoperoxidase. *American Journal of Respiratory Cell and Molecular Biology*, 22, 642-644.
- Pruitt, K.M., Tenovuo, J.O., Andrews, R.W., Mckane, T. (1982). Lactoperoxidase catalysed oxidation of thiocyanate: Polarographic study of the oxidation products. *Biochemistry*, 21, 562-567.
- Qin, Y. S., Jiang, H., Wang, C. F., Cheng, M., Wang, L. L., Huang, M. Y., Zhao, Q.X. (2021). Physicochemical and functional properties of goat milk whey protein and casein obtained during different lactation stages. *J. Dairy Sci.*, 104 (4), 3936-3946.
- Reiter, B., Harnulv, G. (1984). Lactoperoxidase antibacterial system: Natural occurrence, biological functions and practical applications. *Journal of Food Protection*, 47, 724-732.
- Reiter B, Perraudin J-P. (1991). Lactoperoxidase: biological functions. Everse J, Everse KE, Grisham MB (Eds.), *Peroxidases in chemistry and biology*, 1., CRC Press, Boca Raton, 143-180
- Roger, V., Tenovuo, J., Lenander-Lumikari, M., Söderling, E., Vilja, P. (1994). Lysozyme and lactoperoxidase inhibit the adherence of Streptococcus mutans NCTC 10449 (serotype c) to salivatreated hydroxyapatite in vitro. *Caries Res*, 28 (6), 421-428.
- Sarkaya, B.O., Sisecioglu, M., Cankaya, M., Gulcin, İ., Ozdemir, H. (2015). Inhibition profile of a series of phenolic acids on bovine lactoperoxidase enzyme. *J Enzyme Inhib Med Chem*, 30 (3), 479-483.
- Sarkar S, Misra AK. 1994. Implication of LP-system on manufacture of fermented milk products. *Indian Journal Dairy Science*, 47 (2), 133-139.
- Shin, K., Hayasawa, H., Lonnerdal, B. (2001). Inhibition of Escherichia coli respiratory enzymes by the Lactoperoxidase hydrogen peroxide-thiocyanate antimicrobial system. *Journal of Applied Microbiology*, 90, 489-493.
- Shindler, J., Bardsley, W.G. (1975). Steady-state kinetics of Lactoperoxidase with ABTS as chromogen. *Biochemical and Biophysical Research Communications*, 67, 1307-1312.
- Sisecioglu, M., Cankaya, M., Gulcin, I., Ozdemir, H. (2009). The Inhibitory Effect of Propofol on Bovine Lactoperoxidase. *Protein and Peptide Letters*, 16 (1), 46-49.
- Sisecioglu, M., Cankaya, M., Gulcin, I., Ozdemir, H. (2010). Interactions of melatonin and serotonin to Lactoperoxidase enzyme. *Journal Of Enzyme Inhibition And Medicinal Chemistry*, 25, 779-783.
- Sisecioglu M, Gulcin I, Cankaya M, Atasever A, Ozdemir H. (2010b). The effects of norepinephrine on lactoperoxidase enzyme. *Scientific Research and Essays*, 5, 1351-1356.
- Sisecioglu, M., Uguz, M.T., Cankaya, M., Ozdemir, H., Gulcin, I. (2011). Effects of ceftazidime pentahydrate, prednisolone, amikacin sulfate, ceftriaxone sodium and teicoplanin on bovine milk lactoperoxidase activity. *International Journal of Pharmacology*, 7, 79-83.
- Thomas, E.L., Bozeman, D.B. (1991). Lactoperoxidase: Structure and catalytic properties. In peroxidases in chemistry and biology. *Journal Everse K.E., Everse M.B. Grisham Editors. 1*, CRC Press. Boca Raton. FL. 123-142.
- Uguz, M.T., Ozdemir, H. (2005). Purification of bovine milk Lactoperoxidase and investigation of antibacterial properties at different thiocyanate mediated. *Applied Biochemistry and Microbiology*, 41, 349-353.
- Wolf S.M, Ferrari RP, Traversa S, Biemann K (2000). Determination of the carbohydrate composition and the disulfide bond linkages of bovine lactoperoxidase by mass spectrometry. *J. Mass. Spectrom*, 35, 210-217.
- Wolfson, L.M., Summer, S.A. (1993). Antibacterial activity of the Lactoperoxidase system. *A Review Journal of Food Protection*, 56, 887-892.
- Zou, Z., Bauland, J., Hewavitharana, A. K., Al-Shehri, S. S., Duley, J. A., Cowley, D. M., ... & Bansal, N. (2021) A sensitive, high-throughput fluorescent method for the determination of lactoperoxidase activities in milk and comparison in human, bovine, goat and camel milk. *Food Chem*, 339 Article 128090



Agroinfection-Mediated Virus Induced Gene Silencing of Fungal Pathogen *Fusarium verticillioides* CYP51 Gene to Reduce its Pathogenicity during Maize (*Zea mays*) Germination

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ABSTRACT

Fusarium verticillioides is a fungal pathogen, resulting in devastating diseases in cereals, especially in maize (*Zea mays*) and causing massive economic losses in agriculture. *F. verticillioides* infections in the field are managed by chemical fungicides, mainly azoles which target cytochrome P450 lanosterol C-14 α -demethylase (CYP51). Alternative to chemical fungicide, this study evaluated the potential of agroinfection-mediated Virus Induced Gene Silencing (VIGS) approach in controlling *F. verticillioides* pathogenicity, which is based on naturally occurring RNA interference (RNAi) mechanism. For this purpose, *F. verticillioides* was co-cultivated with *Agrobacterium tumefaciens* transformed with a Brome Mosaic Virus (BMV3) derived vector carrying a 313 nucleotide length fragment common to the three existing *F. verticillioides* CYP51 genes and comparatively evaluated with the mock group treated similarly but with empty vector at morphologically, biochemically and transcriptionally. It was detected that agroinfection-mediated VIGS treatment of *F. verticillioides* reduced expression levels of CYP51A by 49%, CYP51B by 65% and CYP51C by 51% and the growth rate by 13% while the germination rate was 20% and ROS amount 56% higher compared to mock-treated *F. verticillioides*. These findings pointed out that silencing of CYP51 caused ROS accumulation in cells leading to inhibition of the pathogenicity of *F. verticillioides*. This study represented the potential of targeting CYP51 gene by agroinfection-mediated VIGS treatment as an agriculturally sustainable and environmentally friendly alternative method to control *F. verticillioides*-caused plant diseases.

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Mısır (*Zea mays*) Bitkisinin Çimlenmesi Sırasında Fungal Patojen *Fusarium verticillioides*'in Patojenitesini Azaltmak için Fungal CYP51 Geninin Agroenfeksiyon Aracılığı ile Virüs Kaynaklı Gen Susturulması

ÖZET

Fusarium verticillioides, tahıllarda özellikle mısır (*Zea mays*) bitkisinde yıkıcı hastalıklara neden olan ve tarımda büyük ekonomik kayıplara yol açan bir fungal patojendir. Günümüzde, *F. verticillioides* enfeksiyonları ile mücadelede başlıca, sitokrom P450 lanosterol C-14 α -demetilazı (CYP51) hedef alan azol içerikli kimyasal fungisitler kullanılmaktadır. Buna alternatif olarak, bu çalışmada, patojenin kontrolü için endojen RNA interferans (RNAi) mekanizmasına dayanan ve agroenfeksiyon aracılığıyla Virüs Kaynaklı Gen Susturma (VIGS) yaklaşımının potansiyeli değerlendirilmiştir. Bu amaçla, *Agrobacterium tumefaciens*, *F. verticillioides*'in üç CYP51 geninde ortak olan 313 nükleotidlik fragmenti taşıyan Brome Mozaik Virüs (BMV3) türevi bir vektörle transforme edildikten sonra fungal patojen ile birlikte kültüre alınmıştır. Sonuçlar, benzer şekilde fakat boş vektörle muamele edilmiş kontrol grubuyla morfolojik, biyokimyasal ve transkripsiyonel olarak karşılaştırmalı olarak analiz edilmiştir. Buna göre, CYP51A, CYP51B ve CYP51C genlerinin ifade

Mikrobiyoloji

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RNA interferans

Brome mozaik virüsü

Agrobacterium tumefaciens

Sitokrom P450 lanosterol C-14 α -demetilaz

VIGS

seviyelerinde, sırasıyla, %49, %65 ve %51 oranında azalış saptanmıştır. Fungal büyüme oranının %13 oranında azaldığı; çimlenme oranının %20 ve ROS miktarının %56 oranında kontrole göre daha yüksek olduğu tespit edilmiştir. Bu bulgular, CYP51'in susturulmasının hücrelerde ROS birikimine neden olarak *F. verticillioides*'in patojenitesinin inhibisyonuna yol açtığını göstermektedir. Bu çalışma, *F. verticillioides* kaynaklı bitki hastalıklarını kontrol etmek için tarımsal olarak sürdürülebilir ve çevre dostu bir alternatif yöntem olarak agroenfeksiyon aracılı VIGS uygulamasıyla CYP51 geninin hedeflenmesinin potansiyelini ortaya koymuştur.

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INTRODUCTION

Fusarium verticillioides, an endophytic fungal plant pathogen, belongs to the Ascomycota phylum of fungi kingdom (Omotayo *et al.*, 2023). *F. verticillioides* is phylogenetically diverged from the African clade of the monophyletic *Fusarium fujikuroi* species complex (FFSC) group (Blacutt *et al.*, 2018). Seedling blight, Fusarium ear rot (FER), crown root rot and stalk rot may occur due to *F. verticillioides* infection in maize (*Zea mays*), which is one of the most valuable crops worldwide (Lanubile *et al.*, 2017; Kılınç *et al.*, 2018; Zhang *et al.*, 2023). *F. verticillioides* infection significantly reduces maize yield in the field. Its pathogenicity arises from a mycotoxin, fumonisin B1 (FB1) production, which blocks the ceramide synthase and leads to changes in sphinganine and sphingosine levels (Chen *et al.*, 2023). FB1-contaminated maize consumption may have hepatotoxic, neurotoxic, and possibly carcinogenic effects in animals and humans (Voss *et al.*, 2001; Stockmann-Juvala & Savolainen, 2008).

Control of infections caused by plant fungal pathogens is strongly based on commercial chemical fungicides which are harmful to the environment and humans. Azoles comprise the largest fungicide class and they are sterol 14 α -demethylase inhibitors (DMI) (Fan *et al.*, 2013; Jørgensen & Heick, 2021). DMIs catalytically restrict the activity of both 14 α -demethylase and cytochrome P450 lanosterol C-14 α -demethylase (*CYP51*) (Doughty *et al.*, 2021). *CYP51* belongs to the cytochrome P450 monooxygenase (CYP) superfamily, and is involved in the fungal kingdom-specific ergosterol biosynthesis. Catalytic restriction of CYP51 inhibits eburicol and lanosterol demethylation and ergosterol biosynthesis, deficiency of which leads to disruption of cell membrane fluidity and active transport system in fungi (Price *et al.*, 2015). In the fungi kingdom, five CYP51 gene families have been known up to now, which are *CYP51*, *CYP51A*, *CYP51B*, *CYP51C* and *CYP51D* (Pintye *et al.*, 2024). However, *Fusarium spp.* contain only *CYP51A*, *CYP51B* and *CYP51C* paralogs, sequences of which have nearly 60% identity (van Rhijn *et al.*, 2021). Virus-induced gene silencing (VIGS) was revealed as an RNA-mediated defense mechanism against viruses (Voinnet, 2001). It has been developed as a method to downregulate gene expression by a modified viral vector for insertion of the gene of interest. Viral vector infection triggers RNA interference (RNAi) mechanism due to double-stranded RNA (dsRNA) that is released from the vector (Pooggin, 2017). RNA interference (RNAi), which is a naturally occurring post-transcriptional gene silencing mechanism, is highly conserved among eukaryotes (Koepe *et al.*, 2023; Padilla-Roji *et al.*, 2023). RNAi not only regulates gene expression levels in a sequence-specific way with the help of small RNAs, which are microRNAs or small interfering RNAs (siRNAs), but also defend cells against transposons, retroelements and viruses (Buchon & Vaury, 2005).

Many plant viruses have been developed as vectors for transient gene expression or silencing in plants (Yang *et al.*, 2018). Brome mosaic virus (BMV) belonging to the Bromoviridae family has been modified for genetic modification of monocot species, which is difficult to stably transform (Ding *et al.*, 2017). RNAi also occurs via the bidirectional exchange of RNA between host and pathogen, which is called "Cross-kingdom RNAi" (Huang *et al.*, 2019). RNAi mechanism initiates with dsRNA cleavage by Dicer, thereby producing small-interfering RNAs (siRNAs) (Halder *et al.*, 2022). The guide strand of siRNA separates from the passenger strand and is integrated into an RNA-induced silencing complex (RISC) together with Argonaute (AGO) proteins to bind complementary mRNA, which is degraded by endonuclease activity within the RISC causing gene silencing (Waheed *et al.*, 2021).

In this study, an agroinfection-mediated VIGS approach based on RNAi was used to silence the *Fusarium verticillioides* *CYP51* (*Fv-CYP51*) gene to inhibit fungal growth and pathogenicity on maize seeds (Mascia *et al.*, 2014; Zulfiqar *et al.*, 2023). To this end, a fragment conserved in three existing *Fv-CYP51* gene paralogs was cloned into a derived vector and transformed into *Agrobacterium tumefaciens*. Transformed-*A. tumefaciens* and *F. verticillioides* co-cultivated and the transcript level of *CYP51* paralogs, endogenous ROS production and fungal growth, and pathogenicity inhibition were analyzed at morphological, biochemical, and transcriptional levels. This study aims to contribute to the development of a VIGS-based fungicide that can avoid the use of chemical azoles for the control of *F. verticillioides* infection.

MATERIAL and METHOD

Culture Conditions of Bacterial and Fungal Strains

Bacterial *E. coli* DH5α and *A. tumefaciens* EHA105 strains were used and cultured in Luria Bertani (LB) broth (Caisson Labs, England) at 37°C and 28°C, respectively. *Fusarium verticillioides* isolates were cultured on potato dextrose agar (PDA, Biolife, Italy) for 1-2 weeks or in potato dextrose broth (PDB) for 1-3 days at 28 °C. PDB was prepared by boiling 200 g L⁻¹ potato in distilled water, supplementing 200 g L⁻¹ D-glucose and autoclaving at 121°C for 20 min. Bacterial and fungal strains are stored at -80°C in 15% glycerol.

Cloning *CYP51* into BMV3 Vector

The VIGS method was followed according to Ding *et al.* (2017) to clone *Fv-CYP51* (NCBI Sequence ID: XM_018901738.1) fragment into the BMV3 vector. For this end, total *F. verticillioides* RNA was isolated according to TRIzol (Invitrogen, Thermo Fischer Scientific, USA) method and cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher) using 2 µg of the extracted total RNA as a template (Rymen *et al.* 2007). A 313 bp fragment of conserved in *Fv-CYP51* paralogs was amplified by forward (5'-CCTCCTAGGCCCTTGACGTGGGAGAATTTA-3') containing *AvrII* restriction site and reverse (5'-CTTCCATGGTGACGGACTTGCTCATCATAC-3') containing *NcoI* restriction endonuclease site by PCR using Phusion High-Fidelity DNA Polymerase (Thermo Fisher). PCR condition was followed by an initial denaturation step at 97°C and 30 s, then 30 cycles of denaturation at 98°C for 10 s, annealing at 57°C for 20 s, extension at 72°C for 20 s, and final extension at 72°C for 10 min.

The amplified insert fragment was ligated into pC13/F3CP5 (abbreviated as BMV3 in this article) vector linearized by *AvrII* and *NcoI* restriction enzymes (New England Biolabs). The vector and PCR product were subjected to overnight ligation with the T4 DNA ligase (Invitrogen) at +4°C. To confirm the positive clone, the BMV3: *CYP51* vector was subjected to restriction digestion with *AvrII* and *HindIII* (New England Biolabs) and 2% agarose gel electrophoresis. The unraveled insert fragment was extracted with the PCR Clean Up & Gel Extraction kit (Thermo Fisher) and sequenced. The obtained sequence aligned to the database with BLASTn algorithm (Altschul *et al.* 1990). The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11) based on comparing nucleotide sequences (Tamura *et al.*, 2021).

BMV3 vector contains RNA3 of BMV encoding 3a protein and a capsid protein (CP) required for movement of virus, while pC13/F1+2 (abbreviated as BMV1 in this article) plasmid contains genomic RNA 1 and 2 of BMV, encoding proteins 1a and 2a, respectively (Ding *et al.*, 2017; Wang *et al.*, 2021). These proteins are required for virus replication processes. BMV1 and BMV3 vectors containing the kanamycin resistance gene were used to transform *A. tumefaciens*. Firstly, *E. coli* and *A. tumefaciens* EHA105 competent cells were prepared as described in (Sambrook and Russell, 2006). *Escherichia coli* (*E. coli*) and *A. tumefaciens* competent cells were transformed separately with BMV1, BMV3 and BMV3:CYP51 vectors by heat shock method.

Sensitivity of *F. verticillioides* to Kanamycin

To test the resistance of *F. verticillioides* to bactericide kanamycin; 10³, 10⁴, and 10⁵ spores mL⁻¹ *F. verticillioides* were cultivated in PDB for 1 d and on PDA for 3 d supplemented with 5, 10, 50, and 100 µg mL⁻¹ kanamycin. The resistance of *F. verticillioides* to kanamycin was confirmed (Poudyal *et al.*, 2024); therefore, this antibiotic could be supplemented in a fungal selective PDA medium.

Agroinfection-Based VIGS Treatment of *Fusarium verticillioides* to Silence *Fv-CYP51*

Agroinfection applied based on previous studies of Bundock *et al.* (1995), Sayari *et al.* (2019), Dong *et al.* (2020) and Yoon *et al.* (2023). *A. tumefaciens* EHA105 strain was transformed with BMV1, BMV3 or BMV3:CYP51 and grown in 5 mL LB medium containing 10 µg mL⁻¹ gentamicin and 100 µg mL⁻¹ kanamycin antibiotics overnight at 28°C with shaking at 150 rpm. *Agrobacterium* transformant cultures were diluted to 20 mL with LB medium and

grown to an optical density (OD) of 0.6 at 600 nm measured with a UV-1800 Spectrophotometer (Shimadzu, Japan). 500 µL aliquots of each transformant culture were centrifuged for 5 min at 3000 rpm and resuspended in 25 µL of induction buffer (containing 10 mM MES (2-(N-morpholino) ethanesulfonic acid); pH:5,6), 10 mM MgCl₂, 0.1 mM Acetosyringone). 25 µL of BMV3:CYP51 agrotransformant culture was mixed with 25 µL of BMV1 agrotransformant culture (1:1, v:v). Subsequently, 50 µL of resuspended agrotransformant mixture and 25 µL of 10x10⁴ spore mL⁻¹ *F. verticillioides* cultures were mixed and spread on sterile parchment paper placed on minimal medium (MM) (containing 2.05 K₂HPO₄ g L⁻¹, 1.45 KH₂PO₄ g L⁻¹, 0.15 NaCl g L⁻¹, 0.50 MgSO₄·7H₂O g L⁻¹, 0.1 CaCl₂·6H₂O g L⁻¹ and 0.5 g L⁻¹ (NH₄)₂SO₄, 40mM MES (ph:5.3), 200 µM acetosyringone, 10 mM glucose, 0,5 % glycerol and 1.5% agar) (Bundock *et al.* 1995). For mock treatment, *F. verticillioides* was co-cultured similarly but with BMV1 and empty BMV3 transformed *Agrobacterium*.

After 2 d co-cultivation at 25°C, the parchment papers were transferred to a fungal selective PDA medium containing 100 µg mL⁻¹ kanamycin and 200 µg mL⁻¹ timentin to kill bacterial cells and incubated for 7 d at 25°C in the dark (Sayari *et al.* 2019). Lastly, the parchment papers were suspended in 20 mL PDB and grown overnight. These fungal cultures were used in ROS production assay, fungal growth zone measurement, transcriptional analysis of *CYP51* paralogous genes and seed germination assay.

Transcriptional Analysis of *Fv-CYP51* Paralogous Genes

Primers specific to *CYP51* paralogs which are *CYP51A* (XM_018899353.1), *CYP51B* (XM_018887915.1), and *CYP51C* (XM_018901738.1) and *β-tubulin* (FVEG 05512) (*F. verticillioides* 7600; assembly ASM14955v1; <https://www.ncbi.nlm.nih.gov/genome>) were designed with IDT OligoAnalyzer 3.1 (<https://eu.idtdna.com>) by taking into account specific parameters (Melting temperature (T_m) between 58-61°C; hairpin and self- or hetero- dimer formation at 50°C with G> -3 kcal mol⁻¹ and -6 kcal mol⁻¹, respectively). The specificity of primers was evaluated with the BLASTn tool (www.blast.ncbi.nlm.nih.gov).

Triplicate 1 mL aliquots were taken from the agroinfected *F. verticillioides* cultures and centrifuged at 13,000 rpm for 15 min. The supernatant was discarded. Total RNA and cDNA synthesis was conducted similarly to those represented above. RT-qPCR reactions were prepared in triplicate biological and duplicate technical replicates with SYBR Green qPCR Mix (AidLab, China); 10 µM forward and reverse primers (Table 1) and 3 µd cDNA as template. The conditions for RT-qPCR run on StepOne Real-Time PCR (ABI, Foster City, CA, USA) machine were as follows: 95°C for 3 min (initial denaturation); 40 cycles of 10 s at 95°C (denaturation), 20 s at 60°C (hybridization) and 15 s at 72°C (elongation) and for melting curve analysis 15 s at 95°C, 1 min at 58°C and 15 s at 95°C. The results were normalized with endogenous control *β-tubulin*. 2^{-ΔΔCT} values were evaluated to assess relative gene expression levels (Pfaffl, 2001).

Table 1. The list of forward and reverse primers used in transcriptional analysis

Çizelge 1. Transkripsiyonel analizde kullanılan ileri ve geri primerlerin listesi

Gene (Gen)		Primer sequence (5'→3') (Primer sekansı (5'→3'))
<i>CYP51A</i> (XM_018899353.1)	Forward primer <i>İleri primer</i>	TCTATTCTGCGAAAGGTCAAGAG
	Reverse primer <i>Geri primer</i>	CAGTTGGTGAAGCGAGGATAA
<i>CYP51B</i> (XM_018887915.1)	Forward primer <i>İleri primer</i>	GTGGCGATGACGAAGAGAAG
	Reverse primer <i>Geri primer</i>	AGCACCAAAGGGCAGATAAG
<i>CYP51C</i> (XM_018901738.1)	Forward primer <i>İleri primer</i>	CCCTTGACGTGGGAGAAATTTG
	Reverse primer <i>Geri primer</i>	ACTTGACCTGTCTGGAGAATAG
<i>β-tubulin</i> (FVEG 05512)	Forward primer <i>İleri primer</i>	GCAGGGCTTCCAACATCTTA
	Reverse primer <i>Geri primer</i>	TATCGACCGTTGCGGAAATC

ROS Production Assay

Fluorometric 2',7'-dichlorofluorescein diacetate (DCFH-DA) staining assay was conducted to measure the amount of intracellular reactive oxygen species (ROS) in agroinfected *F. verticillioides* cultures produced in "Agroinfection-

Based VIGS Treatment of *Fusarium verticillioides* to Silence *Fv-CYP51*. 10^6 spores mL^{-1} of cultures were incubated in 10 μM DCFH-DA (Sigma Aldrich) at 30°C for 30 min in the darkness with shaking at 150 rpm (Chen *et al.*, 2010). Fluorescence levels of cultures were measured every 10 min for 4 h with excitation wavelength at 488 nm and emission wavelength at 525 nm with Varioskan Flash Microplate Reader (Thermo Scientific) (Wang & Joseph, 1999). Intracellular ROS production level was calculated by subtracting the fluorescence levels of DCFH-DA non-treated experimental groups from fluorescence levels of DCFH-DA treated ones (Kobayashi *et al.*, 2002). This experiment was done in five replicates.

Fungal Growth Zone Measurement

Fungal growth zone assay was conducted according to Aydinoglu *et al.* (2024). PDA plates were divided into 6 parts and round wells of equal size were created in the middle of divisions. 10 μL from *F. verticillioides* cultures agroinfected with *BMV3:CYP51* or *BMV3* agrotransformants was loaded into one well in six replicates. After 48 h incubation at 28°C, the fungal growth zones were measured from the longest diameter with ImageJ&Fiji software (<https://imagej.net/software/fiji/downloads>). Experimental data was shown as average $\pm$ standard deviation ($\bar{X} \pm \text{SS}$) and Student t-test analysis was applied in Microsoft Excel 365 for significance level between variances of groups ($P\text{-value} < .05$ was considered as a significant difference.). This experiment was done in six replicates for each experimental group.

Seed Germination Assay

Maize (*Zea mays*) hybrid line PEHLIVAN developed by Maize Research Institute (Sakarya, Türkiye) was used for seed germination assay. Maize seeds were sterilized in 5% sodium hypochlorite (NaOCl) solution for 5 min and then in 70% ethanol solution for 1 min and 3 times in an Erlenmeyer flask. They were washed five times with autoclaved distilled water. For inoculation, ten seeds per experimental group were shaken for 1 hour with *F. verticillioides* spore solutions (5×10^3 spores mL^{-1}) which were not agroinfected, agroinfected with *BMV3* or *BMV3:CYP51 Agrobacterium* transformants. Non-inoculated seeds were used as the control group. For seed germination assay, seeds were placed between sterile filter papers moistened with autoclaved dH_2O in glass plates for 7 d at 25°C/ 20 °C (day/ night), 15.000 lux light intensity and 70% humidity (Khaeim *et al.*, 2022; Akgul & Aydinoglu, 2025). After 7 d, the germinated seeds were counted and compared. This experiment was done in ten replicates for each experimental group.

Statistical Analysis

Two-tailed *t*-test analysis was performed in Microsoft Excel 365 by assuming unequal or equal variances depending on the result of *F*-test analysis for variances of two samples. Data represent average $\pm$ standard deviation ($\bar{X} \pm \text{SS}$). $P\text{-value} < .05$ was accepted as a statistically significant difference.

RESULTS and DISCUSSION

Cloning *Fv-CYP51* Gene Fragment into *BMV3* Vector

BMV3:CYP51 clone was confirmed by restriction digestion followed by electrophoresis and sequencing analysis. Restriction digestion revealed the expected 313-bp-long inserted fragment and 3 vector fragments bigger than 1000 bp (Figure 1B). After being extracted from the gel and sequenced, the BLASTn results confirmed the sequence displayed 91% similarity to *F. verticillioides* sterol 14-demethylase (XM_018901738.1) with 439 coverage out of 486 (Figure 1A) ($P < .01$). This gene is highly conserved between *Fusarium* species. The sequence fragment showed 99% similarity to *Cytochrome P450 lanosterol C-14 α -demethylase* (GU785046.1) gene of *F. proliferatum*, while it showed 95%, 91%, 91%, 88%, 88% similarity with *F. proliferatum* (GU785047.1), *F. mangiferae* (XM_041826468.1), *F. musae* (XM_044825370.1), *F. oxysporum* (XM_018393093.1), *F. subglutinans* (GU785054.1), respectively (Figure 1C).

The Transcript Level of *Fv-CYP51* Paralogs Decreased in Response to Agroinfection- Mediated VIGS Treatment of *Fusarium verticillioides*

The relative transcript levels of *Fv-CYP51* paralogous genes, *CYP51A*, *CYP51B*, and *CYP51C*, were determined to determine whether cross-kingdom gene silencing between *Agrobacterium* and *F. verticillioides* via RNAi induction was achieved or not in response to agroinfection mediated VIGS treatment. The results showed that, the transcript amounts of *CYP51A*, *CYP51B* and *CYP51C* were decreased by 49% ($t(4) = -2.87$, $p = .022$), 65% ($t(4) = -2.24$, $p = .044$), 51% ($t(4) = -3.37$, $p = .014$) in *BMV3:CYP51* agroinfected-*F. verticillioides* compared to mock-treated *F. verticillioides*, respectively (Figure 2). This finding represented the successful targeting of *Fv-CYP51* paralogs by selecting the conserved region of three paralogs to clone into the VIGS vector. Mao *et al.* (2023) showed that

deletion of *CYP51A*, *CYP51B*, and *CYP51C* in *F. fujikuroi* had no obvious effect on morphology, conidia germination, or pathogenicity but displayed differential sensitivity to DMI fungicide. These findings revealed the silencing of *CYP51* genes as an efficient management strategy for pathogenic *Fusarium spp.* The transcriptional analysis of *Fv-CYP51* paralogs proved that the VIGS mechanism served the *CYP51* gene silencing, which is the purpose of the present study. Agroinfection-mediated VIGS treatment of *F. verticillioides* with *BMV3:CYP51* resulted in the silencing of *CYP51A*, *B* and *C* genes, as determined by a statistically significant reduction of the expression profiles of these genes by nearly 50% compared to mock.

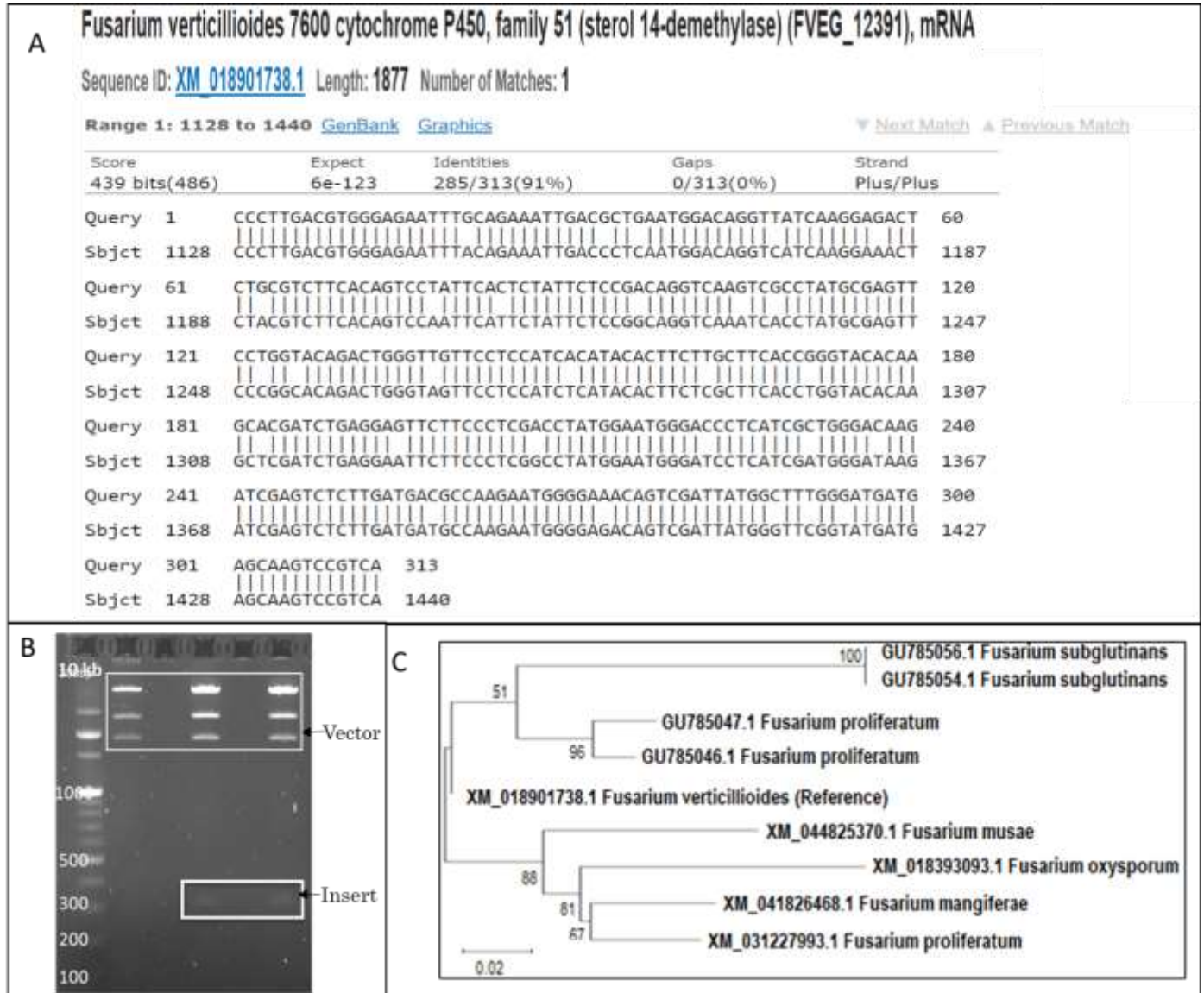


Figure 1. Confirmation of *BMV3:CYP51* clone. A) BLASTn alignment result of the sequenced fragment (Query) that was extracted from the gel. B) Restriction endonuclease (RE) digestion followed by gel electrophoresis of *BMV3:CYP51* clone treated to *AvrII* and *HindIII*. RE confirmed the insert fragment that was 313 bp long. Vector revealed 3 bands longer than 1000 bp. C) Phylogenetic tree constructed with the sequenced insert fragment using the Neighbor Joining method showing evolutionary relation within *Fusarium* species. The percentage of replicate trees where related taxa clustered together by bootstrapping (500 replicates). The branch lengths proportional to evolutionary distances were calculated using the MCL (Maximum Composite Likelihood) method and shown as a percentage.

Şekil 1. *BMV3:CYP51* klonunun doğrulanması. A) Jelden izole edilen dizilenmiş parçanın BLASTn hizalama sonucu. B) *AvrII* ve *HindIII*'e tabi tutulan *BMV3:CYP51* klonunun jel elektroforezini restriksiyon endonükleaz (RE) kesimi izledi. RE, ek parçanın 313 bp uzunluğunda olduğunu doğruladı. Vektör, 1000 bp'den daha uzun 3 bant ortaya çıkardı. C) Neighbor Joining yöntemi kullanılarak dizilenmiş ek parça ile oluşturulan filogenetik ağaç, *Fusarium* türleri içindeki evrimsel ilişkiyi gösteriyor. İlgili taksonların önyükleme yoluyla (500 tekrar) birlikte kümelendiği tekrar eden ağaçların yüzdesi. Evrimsel mesafelere orantılı dal uzunlukları, MCL (Maximum Composite Likelihood) yöntemi kullanılarak hesaplandı ve yüzde olarak gösterildi.

The result pointed out the possibility of dsRNA of *Fv-CYP51* transferred from *A. tumefaciens* to *F. verticillioides* in a cross-kingdom way and processed by host fungal RNAi machinery to release viral small-interfering RNAs (siRNAs) targeting endogenous fungal *CYP51* paralogs (Qin *et al.*, 2017). *Agrobacterium tumefaciens* is well known as a bacterial plant pathogen resulting in tumorigenic crown gall disease and has been long since utilized for *Agrobacterium*-mediated transformation of plants (Hooykaas, 2023). However, Bundock *et al.* (1995) reported that *Saccharomyces cerevisiae* transformed by T-DNA of *A. tumefaciens* in acetosyringone containing co-cultivation medium. de Groot *et al.* (1998) performed *Agrobacterium*-mediated transformation in a wide range of filamentous fungal species such as *Fusarium venenatum* and *Neurospora crassa*, thereby approving that this transformation technique application is not unique to the plant kingdom.

In this study, *F. verticillioides* and *A. tumefaciens* were co-cultivated for 48 hours and at 25°C on a specific co-cultivation medium containing 200 µM acetosyringone. These co-cultivation conditions were determined based on common usage in previous studies (Sayari *et al.*, 2019; He *et al.*, 2021). In the study of Visentin *et al.* (2012), which focuses on the *Agrobacterium*-mediated transformation of *F. verticillioides*, the co-cultivation conditions were also 48 hours and at 25°C. Nevertheless, the acetosyringone concentration in the medium was 100 µM in this study. In the present study, 200 µM acetosyringone was preferred in the co-cultivation medium, as it has been the general usage of concentration since the study of Bundock *et al.* (1995).

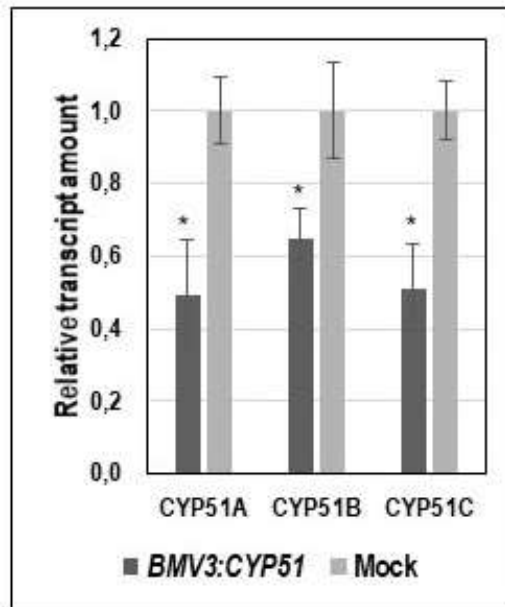


Figure 2. The transcript levels of *CYP51A*, *CYP51B*, and *CYP51C* in *BMV3:CYP51* and mock-treated *Fusarium verticillioides*. *CYP51*= cytochrome P450 lanosterol C-14 α -demethylase, *BMV*: Brome mosaic virus, n=3, average $\pm$ standard deviation ($X \pm SS$), *= $P < .05$ according to Student T-test.

Şekil 2. *BMV3:CYP51* ve boş vektör ile agrotransformasyona uğratılmış *A. tumefaciens* ile birlikte inkübe edilmiş *Fusarium verticillioides*'teki *CYP51A*, *CYP51B* ve *CYP51C* transkript düzeyleri. *CYP51*= sitokrom P450 lanosterol C-14 α -demetilaz, *BMV*: Brom mozaik virüsü, n=3, ortalama $\pm$ standart sapma ($X \pm SS$), *=*Student T*-testine göre $P < .05$.

Endogenous ROS Level of *F. verticillioides* Cell Increased in Response to *Fv-CYP51* Silencing

To observe the oxidative status of the *Fv-CYP51* silenced *F. verticillioides* cells, total ROS production in agroinfected and VIGS-treated-*F. verticillioides* was determined by DCFH-DA staining assay. The results showed that the endogenous ROS level increased linearly over time of DCFH-DA incubation in both agroinfected *F. verticillioides* with *BMV3* (mock) or *BMV3:CYP51* (Figure 3). Moreover, the latter has statistically significantly higher ROS level than the former in each measurement ($F(1, 8) = 9.73$, $p = .02$). At the end of 4 h incubation with DCFH-DA, it was measured as 70.9 OD in *BMV3:CYP51* agroinfected-*F. verticillioides* and 39.8 OD in mock-treated-*F. verticillioides*. This data represented that *CYP51* gene silencing increased total ROS production by 78% in cells ($t(8) = 18.78$, $p < .001$).

ROS (Reactive oxygen species) such as superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) are a common part of oxygen metabolism at low levels; however, at excessive levels, it disrupts cellular signaling

mechanisms and leads to oxidative stress (Collin, 2019). For total cellular ROS measurement, DCFH-DA staining has been a commonly used method due to its capability of detecting a wide range of ROS including nitrogen dioxide ($\cdot\text{NO}_2$) (Kim & Xue 2020). Non-fluorescent DCFH-DA can freely diffuse through cell membranes and intracellular esterases hydrolyze DCFH-DA to dichlorodihydrofluorescein (DCFH) which is also non-fluorescent (de Haan *et al.*, 2022).

ROS lead to the oxidation of DCFH to green fluorescent 2',7'-dichlorofluorescein (DCF), the fluorescence intensity of which provides quantification of cellular ROS production (Yu *et al.*, 2021). The increased level of ROS production in *F. verticillioides* infected with *BMV3:CYP51* agrotransformant compared to mock indicated the inhibition of *F. verticillioides* growth and the elevated intracellular oxidative stress conditions, which is an indicator of disrupted redox homeostasis and increased apoptosis rate (Kannan & Jain, 2000). It can be explained by the formation of *CYP51* dsRNA from viral vector (Padmanabhan & Dinesh-Kumar, 2009) in *A. tumefaciens* and its transfer to *F. verticillioides* due to agroinfection of *F. verticillioides* with the help of acetosyringone in the co-cultivation medium, inducing the expression of vir (virulence) genes in Ti-plasmid of *A. tumefaciens* (Sharma & Kuhad, 2010).

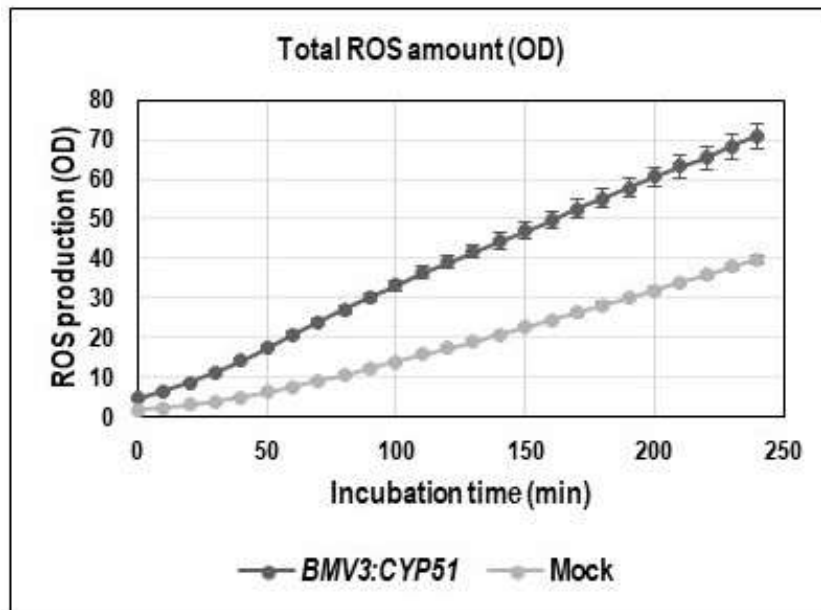


Figure 3. Total ROS production level of *F. verticillioides* infected with *BMV3:CYP51* and *BMV3* Agrotransformants. The total ROS production amount of cultures was measured with fluorometric 2',7'-dichlorofluorescein diacetate (DCFH-DA) staining assay every 10 minutes for 4 hours with 488 nm excitation wavelength and 525 nm emission wavelength with a microplate reader. Error bars represent standard deviation. ROS production is expressed as optic density (OD). n=5, average $\pm$ standard deviation ($\bar{X} \pm SS$).

Şekil 3. *BMV3:CYP51* ve *BMV3* Agrotransformatları ile enfekte edilmiş *F. verticillioides*'in toplam ROS üretim seviyesi. Kültürlerin toplam ROS üretim miktarı, 488 nm uyarım dalga boyu ve 525 nm emisyon dalga boyu ile 4 saat boyunca her 10 dakikada bir florometrik 2',7'-diklorofluorescein diasetat (DCFH-DA) boyama testi ile mikro plaka okuyucu ile ölçüldü. Hata çubukları standart sapmayı temsil eder. ROS üretimi optik yoğunluk (OD) olarak ifade edilir. n=5, ortalama $\pm$ standart sapma ($\bar{X} \pm SS$).

Agroinfection with *BMV3:CYP51* Reduces The Growth Rate of *Fusarium verticillioides*

To evaluate the fungal growth rate of agroinfected *F. verticillioides*, the fungal growth zone diameters on PDA were measured comparatively (Figure 3A, B). The growth zone of *BMV3:CYP51* agroinfected *F. verticillioides* was 1.4 ± 0.07 cm, while it was 1.6 ± 0.07 cm in mock. Therefore, *CYP51* silencing resulted in 13% decrease in the fungal growth rate which was a statistically significant result according to applied Student's t-test between VIGS untreated and treated *F. verticillioides* fungal growth zone measurements ($t(10) = -2.14$, $p = .029$) (Figure 3C).

F. verticillioides can generate a high number of spores that can easily spread through the air. *F. verticillioides* spores create a hazardous risk for not only cereals but also animals and humans. In the case of maize production, which is annually 1127 million tons on average in the world (Hoppe *et al.*, 2024), *F. verticillioides* spores infect pre-harvest nearly half of the crops in maize fields (Akanmu *et al.*, 2020). Because azole fungicides pose dangerous risks to humans such as disruption of steroid and androgen hormone balance and signaling (Draskau & Svingen,

2022), *F. verticillioides* infection in crops should be controlled more safely for the well-being of the whole environment. In this study, the procedure for growth inhibition of *F. verticillioides* pathogenesis was achieved by transient cross-kingdom gene silencing of *Fv-CYP51* with Agroinfection based on VIGS treatment. Apart from VIGS, other approaches used for gene silencing with the help of dsRNA are Host-induced gene silencing (HIGS) and Spray-induced gene silencing (SIGS). In several previous studies, *CYP51* gene silencing was achieved with both HIGS and SIGS in *Fusarium graminearum* (Koch *et al.*, 2018; Koch *et al.*, 2019; He *et al.*, 2019) and these studies have been successful in reducing virulence and infection in *Brachypodium distachyon* (He *et al.*, 2019), *Arabidopsis thaliana* and *Hordeum vulgare* plants (Koch *et al.*, 2019).

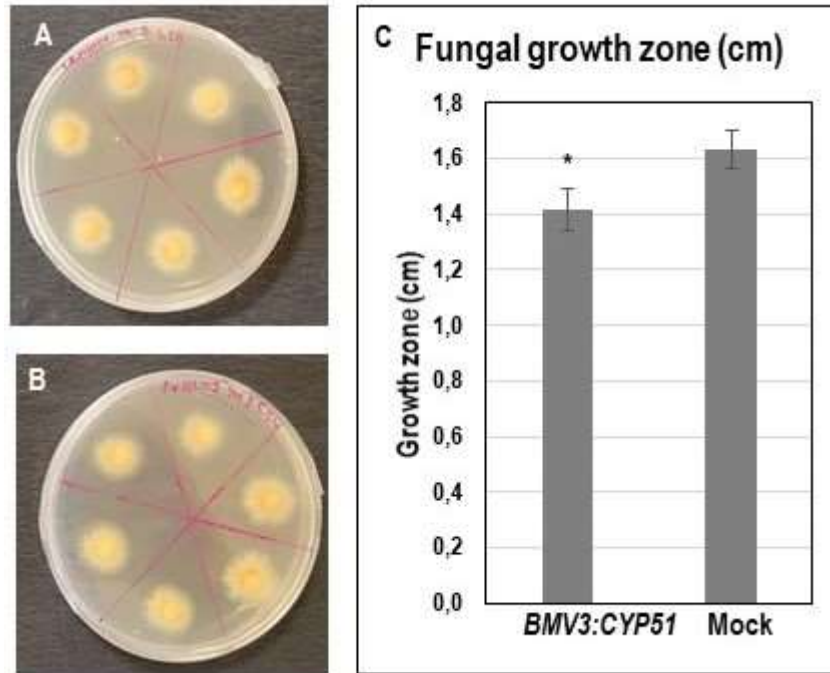


Figure 4. A) The fungal growth zone assay of *BMV3:CYP51* and *BMV3* Agroinfected *Fusarium verticillioides* cultures. B) The graph representing the mean of growth zones (cm) measured with Image J. *BMV3:CYP51* is attributed to *F. verticillioides* Agroinfected with *BMV3:CYP51* transformant and *BMV3* is attributed to *F. verticillioides* Agroinfected with *BMV3* transformant. Error bars show standard deviation. n=6, average $\pm$ standard deviation ($X \pm SS$), *p=.029 according to Student t-test.

Şekil 4. A) *BMV3:CYP51* ve *BMV3* Agroinfected *Fusarium verticillioides* kültürlerinin fungal büyüme çapı analizi. B) Image J ile ölçülen büyüme çaplarının (cm) ortalamasını gösteren grafik. *BMV3:CYP51*, *BMV3:CYP51* transformant *F. verticillioides* Agroinfected'e atfedilir ve *BMV3*, *BMV3* transformantlı *F. verticillioides* Agroinfected'e atfedilir. Hata çubukları standart sapmayı gösterir. n=6, ortalama $\pm$ standart sapma ($X \pm SS$), *Student t-testine göre p=.029.

Silencing *CYP51* Gene in *Fusarium verticillioides* Increased Seed Germination Rate of *Zea mays*

The seed germination assay was conducted to observe whether the agroinfection of *Fusarium verticillioides* with *BMV3:CYP51* transformant decreases the pathogenicity of the fungus. To test this, seeds of PEHLIVAN maize hybrid were inoculated with 5×10^3 spore mL^{-1} *F. verticillioides* alone and agroinfected with *BMV3* or *BMV3:CYP51*-agrotransformants. In addition, non-inoculated maize seeds were included as the control group. Finally, it was detected that the non-inoculated seeds germinated 100%, while seeds inoculated with *F. verticillioides* alone germinated 40% (Figure 5). *BMV3*-agroinfected-*F. verticillioides* inoculation of maize seeds increased the germination rate by 10% compared with only *F. verticillioides* inoculation. Furthermore, it rose to 70% in seeds inoculated with *BMV3:CYP51* Agroinfected-*F. verticillioides*. These findings revealed that both the agroinfection approach and VIGS treatment against *Fv-CYP51* gene alleviated fungal pathogenicity and rotting in maize, thereby raising the seed germination rate compared to non-agroinfected or non-VIGS-treated *F. verticillioides*. *CYP51* gene silencing is more effective than Agroinfection in the decrement of fungal pathogenesis. In a previous study, *CYP51* gene silencing with the HIGS method in *Fusarium graminearum* resulted in diminished fungal pathogenesis on *Arabidopsis thaliana* and barley plant leaves (Höfle *et al.*, 2020). van der Linde *et al.* also applied BMV vector-based VIGS method by silencing *terpene synthase 6/11* (*tps6/11*) gene for the

identification of *Zea mays* genes in the interaction between maize and fungus *Ustilago maydis*, forming tumors on corn smut, leaves and stem (van der Linde *et al.*, 2010).

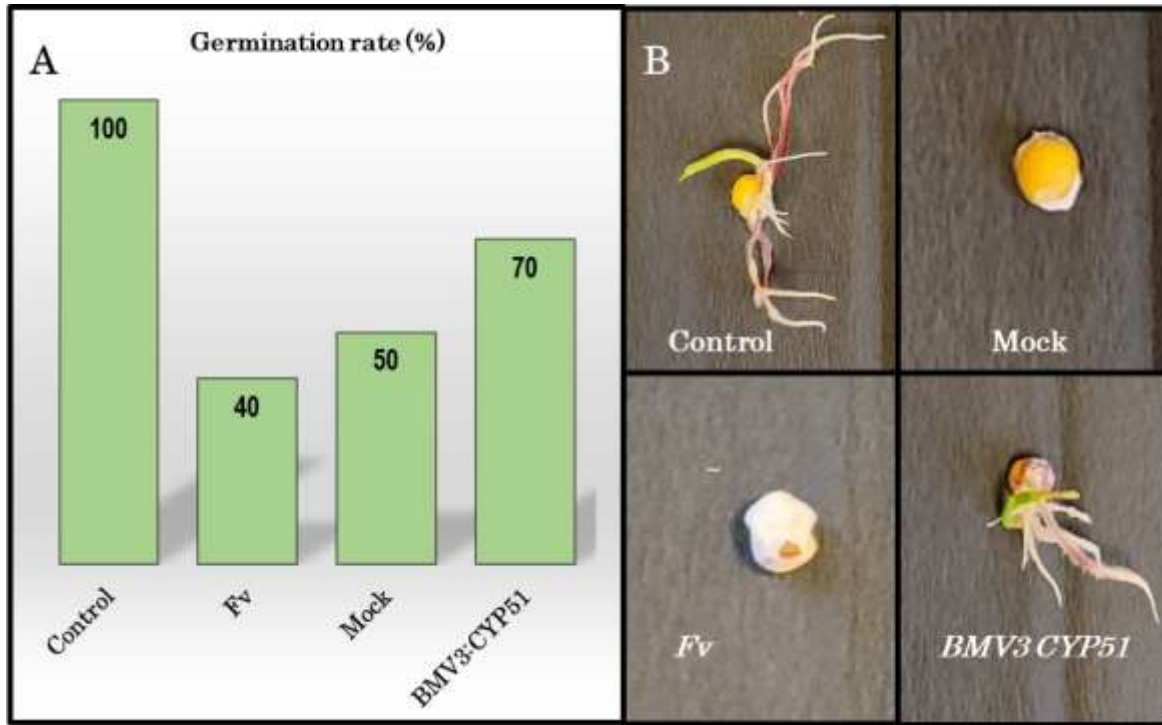


Figure 5. A) The germination rates of *Zea mays* hybrid line PEHLIVAN seeds that were infected with 5×10^3 spore mL^{-1} of non-treated *F. verticillioides* (Fv) and agroinfected with 5×10^3 spore mL^{-1} *F. verticillioides* treated to BMV3:CYP51 or BMV3 (Mock) agrotransformants. Control groups were not inoculated with *F. verticillioides*. Germination rate of the seeds in each experimental group as a percentage. B) Representative photographs of seeds after germination in the experimental groups. n=10.

Şekil 5. Mısır (*Zea mays*) hibridi PEHLIVAN tohumlarının, boş BMV3 (Mock) vektörüyle veya BMV3:CYP51 vektörü ile agrotransförmasyona uğratılmış veya uğratılmamış 5×10^3 spor mL^{-1} 'lik *F. verticillioides* ile enfeksiyonu sonucunda çimlenme oranları. Kontrol grubu *F. verticillioides* ile enfekte edilmemiştir. A) Her deney grubundaki tohumların çimlenme oranı yüzde olarak gösterilmiştir. B) Deney gruplarındaki tohumların çimlendikten sonraki temsili fotoğrafları. n=10.

CONCLUSION

This study has shown that cross-kingdom RNA exchange occurs between *Agrobacterium tumefaciens* and *Fusarium verticillioides* and that the transferred dsRNA produced in *A. tumefaciens* suppresses *CYP51* gene expression by stimulating RNAi mechanism in *F. verticillioides*. Our results showed that agroinfection-mediated VIGS based on the RNAi approach successfully decreased the transcript level of *CYP51A*, *CYP51B* and *CYP51C*. The silencing of *CYP51* genes significantly increased cellular ROS levels in *F. verticillioides*. In addition, the fungal growth zone assay represented a significant reduction in *F. verticillioides* growth by agroinfection-based VIGS approach. It was also observed that the silencing of *CYP51* genes decreased the pathogenicity of *F. verticillioides* on maize seeds. Our results suggested that *CYP51* gene silencing in *F. verticillioides* thanks to VIGS treatment altered the cell membrane lipid content and disrupted its integrity, resulting in suppression of fungal growth and pathogenicity in germination of maize seeds. In this context, agroinfection-mediated VIGS based on the RNAi approach is an applicable and profound procedure in silencing fungal genes. Hence, this research forms the basis of future studies to develop antifungal VIGS-based systems for *Fusarium verticillioides* in the field.

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Contribution Rate Statement Summary of Researchers

YN and BDP conducted the experimental works. YN wrote the manuscript. FA was responsible for designing and performing experiments and writing and reviewing the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

- Akanmu, A.O., Sobowale, A.A., Abiala, M.A., Olawuyi, O.J. & Odebode, A.C. (2020). Efficacy of biochar in the management of *Fusarium verticillioides* SACC. causing ear rot in *Zea mays* L. *Biotechnology Reports*, 26, e00474. <https://doi.org/10.1016/j.btre.2020.e00474>
- Akgul, B., & Aydinoglu, F. (2025). Evaluation of ZMA-MIR408 and its target genes function on maize (*Zea mays*) leaf growth response to cold stress by VIGS-based STTM approach. *Gene*, 938, 149-161. <https://doi.org/10.1016/j.gene.2024.149161>
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Aydinoglu, F., Gultekin, Y., Gül, E. Y., & Ecik, E. T. (2024). Bodipy-mediated photosensitization approach to control maize (*Zea mays*) pathogenic fungus *Fusarium verticillioides*. *Journal of Plant Pathology*, 107(1), 511–523. <https://doi.org/10.1007/s42161-024-01792-z>
- Blacutt, A.A., Gold, S.E., Voss, K.A., Gao, M. & Glenn, A.E. (2018). *Fusarium verticillioides*: Advancements in understanding the toxicity, virulence, and niche adaptations of a model mycotoxigenic pathogen of maize. *Phytopathology*, 108(3), 312–326. <https://doi.org/10.1094/phyto-06-17-0203-rvw>
- Buchon, N., & Vauray, C. (2005). RNAi: A defensive RNA-silencing against viruses and transposable elements. *Heredity*, 96(2), 195–202. <https://doi.org/10.1038/sj.hdy.6800789>
- Bundock, P., Dulk-Ras, A., Beijersbergen, A. & Hooykaas, P.J. (1995). Trans-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae*. *EMBO Journal*, 14, 3206–3214. <https://doi.org/10.1002/j.1460-2075.1995.tb07323.x>
- Chen, X., Abdallah, M.F., Landschoot, S., Audenaert, K., De Saeger, S., Chen, X. & Rajkovic, A. (2023). *Aspergillus flavus* and *Fusarium verticillioides* and their main mycotoxins: Global Distribution and scenarios of interactions in maize. *Toxins*, 15(9), 577. <https://doi.org/10.3390/toxins15090577>
- Chen, X., Zhong, Z., Xu, Z., Chen, L. & Wang, Y. (2010). 2', 7'-Dichlorodihydrofluorescein as a fluorescent probe for reactive oxygen species measurement: forty years of application and controversy. *Free Radical Research*, 44(6), 587-604. <https://doi.org/10.3109/10715761003709802>
- Collin, F. (2019). Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. *International Journal of Molecular Sciences*, 20(10), 2407. <https://doi.org/10.3390/ijms20102407>
- de Groot, M.J., Bundock, P., Hooykaas, P.J., & Beijersbergen, A.G. (1998). *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. *Nature Biotechnology*, 16, 839–842. <https://doi.org/10.1038/nbt0998-839>
- de Haan, L.R., Reiniers, M.J., Reeskamp, L.F., Belkous, A., Ao, L., Cheng, S., Ding, B., van Golen, R.F., & Heger, M. (2022). Experimental conditions that influence the utility of 2'7'-dichlorodihydrofluorescein diacetate (DCFH2-DA) as a fluorogenic biosensor for mitochondrial redox status. *Antioxidants*, 11(8), 1424. <https://doi.org/10.3390/antiox11081424>
- Ding, X.S., Mannas, S.W., Bishop, B.A., Rao, X., Lecoultre, M., Kwon, S., & Nelson, R.S. (2017). An improved brome mosaic virus silencing vector: Greater insert stability and more extensive VIGS. *Plant Physiology*, 176(1), 496–510. <https://doi.org/10.1104/pp.17.00905>
- Dong, Y.H., Meng, X.L., Wang, Y.N., Hu, T.L., Wang, S.T. & Cao, K.Q. (2020). Screening of pathogenicity-deficient *Fusarium oxysporum* mutants established by *Agrobacterium tumefaciens*-mediated transformation. *Canadian Journal of Plant Pathology*, 43(1), 140–154. <https://doi.org/10.1080/07060661.2020.1746402>
- Doughty, K.J., Sierotzki, H., Semar, M. & Goertz, A. (2021). Selection and amplification of fungicide resistance in *Aspergillus fumigatus* in relation to DMI fungicide use in agronomic settings: Hotspots versus coldspots. *Microorganisms*, 9(12), 2439. <https://doi.org/10.3390/microorganisms9122439>
- Draskau, M.K. & Svingen, T. (2022). Azole fungicides and their endocrine disrupting properties: Perspectives on sex hormone-dependent reproductive development. *Frontiers in Toxicology*, 4. <https://doi.org/10.3389/ftox.2022.883254>
- Fan, J., Urban, M., Parker, J.E., Brewer, H.C., Kelly, S.L., Hammond-Kosack, K.E., Fraaije, B.A., Liu, X. & Cools, H.J. (2013). Characterization of the sterol 14 α -demethylases of *Fusarium graminearum* identifies a novel genus-specific CYP51 function. *New Phytologist*, 198(3), 821–835. <https://doi.org/10.1111/nph.12193>

- Halder, K., Chaudhuri, A., Abdin, M.Z., Majee, M. & Datta, A. (2022). RNA interference for improving disease resistance in plants and its relevance in this clustered regularly interspaced short palindromic repeats-dominated era in terms of dsrna-based biopesticides. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.885128>
- He, D., Feng, Z., Gao, S., Wei, Y., Han, S. & Wang, L. (2021). Contribution of NADPH-cytochrome P450 reductase to azole resistance in *Fusarium oxysporum*. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.709942>
- He, F., Zhang, R., Zhao, J., Qi, T., Kang, Z., & Guo, J. (2019). Host-induced silencing of *Fusarium graminearum* genes enhances the resistance of *Brachypodium distachyon* to fusarium head blight. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.01362>
- Hooykaas, P.J. (2023). The ti plasmid, driver of Agrobacterium pathogenesis. *Phytopathology*, 113(4), 594–604. <https://doi.org/10.1094/phyto-11-22-0432-ia>
- Hoppe, K., Chelkowski, J., Błaszczyk, L. & Bocianowski, J. (2024). Observation of changes in fusarium mycotoxin profiles in maize grain over the last decade in Poland. *Food Control*, 158, 110248. <https://doi.org/10.1016/j.foodcont.2023.110248>
- Höfle, L., Biedenkopf, D., Werner, B. T., Shrestha, A., Jelonek, L., & Koch, A. (2020). Study on the efficiency of dsrnas with increasing length in RNA-based silencing of the *Fusarium cyp51* genes. *RNA Biology*, 17(4), 463–473. <https://doi.org/10.1080/15476286.2019.1700033>
- Huang, C.Y., Wang, H., Hu, P., Hamby, R. & Jin, H. (2019). Small RNAs—big players in plant-microbe interactions. *Cell Host & Microbe*, 26(2), 173–182. <https://doi.org/10.1016/j.chom.2019.07.021>
- Jørgensen, L.N. & Heick, T.M. (2021). Azole use in agriculture, horticulture, and Wood Preservation – is it indispensable? *Frontiers in Cellular and Infection Microbiology*, 11. <https://doi.org/10.3389/fcimb.2021.730297>
- Kannan, K. & Jain, S.K. (2000). Oxidative stress and apoptosis. *Pathophysiology*, 7(3), 153–163. [https://doi.org/10.1016/s0928-4680\(00\)00053-5](https://doi.org/10.1016/s0928-4680(00)00053-5)
- Kılınç, S., Karademir, Ç., & Ekin, Z. (2018). Bazı Mısır (*Zea mays* L.) çeşitlerinde Verim ve kalite özelliklerinin Belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 21(6), 809–816. doi:10.18016/ksutarimdoga.vi.463813
- Kim, H. & Xue, X. (2020). Detection of total reactive oxygen species in adherent cells by 2',7'-Dichlorodihydrofluorescein diacetate staining. *Journal of Visualized Experiments*, 160, e60682. <https://doi.org/10.3791/60682>
- Khaeim, H., Kende, Z., Jolánkai, M., Kovács, G. P., Gyuricza, C., & Tarnawa, Á. (2022). Impact of temperature and water on seed germination and seedling growth of maize (*Zea mays* L.). *Agronomy*, 12(2), 397. <https://doi.org/10.3390/agronomy12020397>
- Kobayashi, D., Kondo, K., Uehara, N., Otokozawa, S., Tsuji, N., Yagihashi, A. & Watanabe, N. (2002). Endogenous reactive oxygen species is an important mediator of miconazole antifungal effect. *Antimicrobial Agents and Chemotherapy*, 46(10), 3113–3117. <https://doi.org/10.1128/aac.46.10.3113-3117.2002>
- Koch, A., Stein, E., & Kogel, K.H. (2018). RNA-based disease control as a complementary measure to fight fusarium fungi through silencing of the azole target cytochrome P450 lanosterol C-14 α -demethylase. *European Journal of Plant Pathology*, 152(4), 1003–1010. <https://doi.org/10.1007/s10658-018-1518-4>
- Koch, A., Höfle, L., Werner, B. T., Imani, J., Schmidt, A., Jelonek, L., & Kogel, K. (2019). SIGS vs HIGS: A study on the efficacy of two dsRNA delivery strategies to silence *Fusarium fgcyp51* genes in infected host and non-host plants. *Molecular Plant Pathology*, 20(12), 1636–1644. <https://doi.org/10.1111/mpp.12866>
- Koepe, S., Kawchuk, L., & Kalischuk, M. (2023). RNA interference past and future applications in plants. *International Journal of Molecular Sciences*, 24(11), 9755. <https://doi.org/10.3390/ijms24119755>
- Lanubile, A., Maschietto, V., Borrelli, V.M., Stagnati, L., Logrieco, A.F., & Marocco, A. (2017). Molecular Basis of Resistance to Fusarium Ear Rot in Maize. *Frontiers in Plant Science*, 8, 1774. doi: 10.3389/fpls.2017.01774.
- Mao, C.X., Luo, J., Zhang, Y., & Zhang, C.Q. (2023). Targeted deletion of three CYP51s in *Fusarium fujikuroi* and their different roles in determining sensitivity to 14 α -demethylase inhibitor fungicides. *Pest Management Science*, 79(4), 1324–1330. <https://doi.org/10.1002/ps.7304>
- Mascia, T., Gallitelli, D. & Palukaitis, P. (2014). Something new to explore: Plant viruses infecting and inducing gene silencing in filamentous fungi. *Mobile Genetic Elements*, 4(4). <https://doi.org/10.4161/mge.29782>
- Omotayo, O.P., Omotayo, A.O., Mwanza, M. & Babalola, O.O. (2019). Prevalence of mycotoxins and their consequences on human health. *Toxicological Research*, 35(1), 1–7. <https://doi.org/10.5487/tr.2019.35.1.001>
- Padilla-Roji, I., Ruiz-Jiménez, L., Bakhat, N., Vielba-Fernández, A., Pérez-García, A., & Fernández-Ortuño, D. (2023). RNAi technology: A new path for the research and management of obligate biotrophic phytopathogenic fungi. *International Journal of Molecular Sciences*, 24(10), 9082. <https://doi.org/10.3390/ijms24109082>
- Padmanabhan, M. & Dinesh-Kumar, S.P. (2009). Virus-induced gene silencing as a tool for delivery of dsRNA into plants. *Cold Spring Harbor Protocols*, 2009(2). <https://doi.org/10.1101/pdb.prot5139>

- Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, 29(9), e45. <https://doi.org/10.1093/nar/29.9.e45>
- Pintye, A., Bacsó, R. & Kovács, G.M. (2024). Trans-kingdom fungal pathogens infecting both plants and humans, and the problem of azole fungicide resistance. *Frontiers in Microbiology*, 15. <https://doi.org/10.3389/fmicb.2024.1354757>
- Pooggin, M.M. (2017). RNAi-mediated resistance to viruses: A critical assessment of methodologies. *Current Opinion in Virology*, 26, 28–35. <https://doi.org/10.1016/j.coviro.2017.07.010>
- Poudyal, N., Subedi, Y. P., Shakespear, M., Grilley, M., Takemoto, J. Y., & Chang, C. W. T. (2024). Synthesis of kanamycin-azole hybrids and investigation of their antifungal activities. *Bioorganic & Medicinal Chemistry*, 114, 117947. <https://doi.org/10.1016/j.bmc.2024.117947>
- Price, C.L., Parker, J.E., Warrilow, A.G., Kelly, D.E. & Kelly, S.L. (2015). Azole fungicides-understanding resistance mechanisms in agricultural fungal pathogens. *Pest Management Science*, 71(8), 1054–1058. <https://doi.org/10.1002/ps.4029>
- Qin, C., Li, B., Fan, Y., Zhang, X., Yu, Z., Ryabov, E., Zhao, M., Wang, H., Shi, N., Zhang, P., Jackson, S., Tör, M., Cheng, Q., Liu, Y., Gallusci, P. & Hong, Y. (2017). Roles of dicer-like proteins 2 and 4 in intra- and intercellular antiviral silencing. *Plant Physiology*, 174(2), 1067–1081. <https://doi.org/10.1104/pp.17.00475>
- Rymen, B., Fiorani, F., Kartal, F., Vandepoele, K., Inze, D. & Beemster, G.T.S. (2007) Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. *Plant Physiology*, 143(3), 1429–1438. <https://doi.org/10.1104/pp.106.093948>
- Sambrook, J. & Russell, D.W. (2006). Preparation and transformation of competent *E. coli* using calcium chloride. *Cold Spring Harbor Protocols*, 2006(1). <https://doi.org/10.1101/pdb.prot3932>
- Sayari, M., van der Nest, M.A., Steenkamp, E.T., Adegeye, O.O., Marincowitz, S. & Wingfield, B.D. (2019). Agrobacterium-mediated transformation of *Ceratocystis albifundus*. *Microbiological Research*, 226, 55–64. <https://doi.org/10.1016/j.micres.2019.05.004>
- Sharma, K.K. & Kuhad, R.C. (2010). Genetic transformation of lignin degrading fungi facilitated by *Agrobacterium tumefaciens*. *BMC Biotechnology*, 10(1), 67. <https://doi.org/10.1186/1472-6750-10-67>
- Stockmann-Juvala, H. & Savolainen, K. (2008). A review of the toxic effects and mechanisms of action of fumonisin B1. *Human & Experimental Toxicology*, 27, 799–809. doi:10.1177/0960327108099525.
- Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- van der Linde, K., Kastner, C., Kumlehn, J., Kahmann, R., & Doeblemann, G. (2010). Systemic virus-induced gene silencing allows functional characterization of maize genes during biotrophic interaction with *Ustilago maydis*. *New Phytologist*, 189(2), 471–483. <https://doi.org/10.1111/j.1469-8137.2010.03474.x>
- van Rhijn, N., Bromley, M., Richardson, M. & Bowyer, P. (2021). CYP51 paralogue structure is associated with intrinsic azole resistance in fungi. *mBio*, 12(5). <https://doi.org/10.1128/mbio.01945-21>
- Visentin, I., Montis, V., Döll, K., Alabouvette, C., Tamiotti, G., Karlovsky, P. & Cardinale, F. (2012). Transcription of genes in the biosynthetic pathway for fumonisin mycotoxins is epigenetically and differentially regulated in the fungal maize pathogen *Fusarium verticillioides*. *Eukaryotic Cell*, 11(3), 252–259. <https://doi.org/10.1128/ec.05159-11>
- Voinnet, O. (2001) RNA silencing as a plant immune system against viruses. *Trend in Genetics*, 17(8), 449–59
- Voss, K.A., Riley, R.T., Norred, W.P., Bacon, C.W., Meredith, F.I., Howard, P.C., Plattner, R.D., Collins, T.F., Hansen, D.K. & Porter, J.K. (2001). An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium moniliforme*. *Environmental Health Perspectives*, 109(2), 259–266. doi: 10.1289/ehp.01109s2259
- Waheed, S., Anwar, M., Saleem, M.A., Wu, J., Tayyab, M. & Hu, Z. (2021). The critical role of small RNAs in regulating plant innate immunity. *Biomolecules*, 11(2), 184. <https://doi.org/10.3390/biom11020184>
- Wang, H. & Joseph, J.A. (1999). Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biology and Medicine*, 27(5-6), 612–616. [https://doi.org/10.1016/s0891-5849\(99\)00107-0](https://doi.org/10.1016/s0891-5849(99)00107-0)
- Wang, Y., Cha, C., Khatabi, B., Scheible, W.R., Udvardi, M.K., Saha, M.C., Kang, Y. & Nelson, R.S. (2021). An efficient Brome mosaic virus-based gene silencing protocol for hexaploid wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.685187>
- Yang, J., Zhang, T.Y., Liao, Q.S., He, L., Li, J., Zhang, H.M., Chen, X., Li, J., Yang, J., Li, J.B. & Chen, J.P. (2018). Chinese wheat mosaic virus-induced gene silencing in monocots and dicots at low temperature. *Frontiers in Plant Science*, 9, 1627. <https://doi.org/10.3389/fpls.2018.01627>
- Yoon, J., Kim, Y., Kim, S., Jeong, H., Park, J., Jeong, M.H., Park, S., Jo, M., An, S., Park, J., Jang, S.H., Goh, J. & Park, S.Y. (2023). *Agrobacterium tumefaciens*-mediated transformation of the aquatic fungus *Phialemonium inflatum* FBCC-f1546. *Journal of Fungi*, 9(12), 1158. <https://doi.org/10.3390/jof9121158>

- Yu, D., Zha, Y., Zhong, Z., Ruan, Y., Li, Z., Sun, L. & Hou, S. (2021). Improved detection of reactive oxygen species by DCFH-da: New insight into self-amplification of fluorescence signal by light irradiation. *Sensors and Actuators B: Chemical*, 339, 129878. <https://doi.org/10.1016/j.snb.2021.129878>
- Zhang, L., Hou, M., Zhang, X., Cao, Y., Sun, S., Zhu, Z., Han, S., Chen, Y., Ku, L. & Duan, C. (2023). Integrative transcriptome and Proteome Analysis reveals maize responses to *Fusarium verticillioides* infection inside the stalks. *Molecular Plant Pathology*, 24(7), 693–710. <https://doi.org/10.1111/mpp.13317>
- Zulfiqar, S., Farooq, M.A., Zhao, T., Wang, P., Tabusam, J., Wang, Y., Xuan, S., Zhao, J., Chen, X., Shen, S. & Gu, A. (2023). Virus-induced gene silencing (VIGS): A powerful tool for crop improvement and its advancement towards epigenetics. *International Journal of Molecular Sciences*, 24(6), 5608. <https://doi.org/10.3390/ijms24065608>



Sağlık Hizmetleri Meslek Yüksekokulu Öğrencileri ve Ailelerinin Solunum Yolu Enfeksiyonlarında ve Çeşitli Yaralarda Bitkisel Ürün Kullanım Durumlarının Araştırılması

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ÖZET

Tıbbi bitkisel ürünler antik çağlardan beri çeşitli hastalıkların profilaksisi ve tedavisinde kullanılmıştır. Bu çalışmada öğrenci grubumuzun ve ailelerinin solunum yolu enfeksiyonları ve çeşitli yaralarda bitkisel ürün kullanma durumlarının araştırılması amaçlanmıştır. Mayıs-Temmuz 2023 tarihleri arasında yapılan bu kesitsel araştırmanın örneklemini Türkiye'deki bir kamu üniversitesinde öğrenim gören öğrenci ve aileleri oluşturmaktadır (n=164). Araştırmada sosyodemografik verilerin yanı sıra araştırmacılar tarafından literatür taranarak hazırlanan anket formu ve 11 adet sorunun yer aldığı Tamamlayıcı ve Alternatif Tıbbı Karşı Tutum Ölçeği kullanılmıştır. Sorular online anket yöntemi ile katılımcıların erişimine sunulmuştur. Verilerin incelenmesinde IBM SPSS versiyon 22 programından yararlanılmıştır. Katılımcıların yarısından fazlası kadın, 18-30 yaş aralığında ve %38.4'ü bitkisel ürünleri sık sık kullanmaktaydı. Bitkisel ürünler ile ilgili en sık kullanılan bilgi alma yolu internetti (%30.1). Katılımcıların %34.1'i bitkisel ürünleri diğer ilaçlardan daha etkili bulurken, %63.4'ü ürünleri başkalarına önermişlerdi. Katılımcıların sadece %18.9'u tıbbi bitkilerin yan etkileri ve bitki ilaç etkileşimleri hakkında yeterli bilgiye sahipti. Bitkisel ürünleri en çok solunum (%40.9) sistemi hastalıklarında tercih etmişlerdi ve en sık kullanım amacı tedaviye yardımcı olmaktı (%33.7). Ürünler yaygın olarak aktarlardan alınmakta (%26.6), genellikle ürünlerin yaprakları kullanılmakta ve kullanırken kaynatma yöntemi tercih edilmekteydi. Katılımcılar solunum yolu rahatsızlıkları için nane (%28.4) ve kekiği (%16.0) cilt rahatsızlıklarında ise sarı kantaron (%40.8) ve aloe verayı (%22.4) tercih etmişlerdi. Çalışmanın sonuçlarına göre demografik özelliklerin bitkisel ilaç kullanımıyla anlamlı bir ilişkisi bulunamamıştır. Katılımcılar bitkisel ürünlerin yan etkileri ve bitki ilaç etkileşimleri hakkında yeterli bilgiye sahip değildi. Bitkisel ürünlerin doğru kullanımını açıklamak ve bunların potansiyel olumsuz etkilerine karşı uyarıda bulunmak için eğitim ve bilinçlendirme programlarına ihtiyaç vardır.

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 20.07.2024

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Anahtar Kelimeler

Bitkisel ürün

Üniversite öğrencileri

Aileler

Solunum yolu enfeksiyonları

Yaralar

Investigation of Vocational School of Health Services Students and Their Families' Use of Herbal Products in Respiratory Tract Infections and Various Wounds

ABSTRACT

Medicinal herbal products have been used in the prophylaxis and treatment of various diseases since ancient times. In this study, it was aimed to investigate the use of herbal products by our student group and their families in respiratory tract infections and various wounds. The sample of this cross-sectional research, conducted between May and July 2023, consists of students studying at a public university in Turkey and their families (n=164). In addition to sociodemographic

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data, a questionnaire form prepared by the researchers by reviewing the literature and the Attitude Towards Complementary and Alternative Medicine Scale with 11 questions were used in the study. The questions were made available to the participants via an online survey method. IBM SPSS version 22 program was used to analyze the data. More than half of the participants were female, aged between 18-30 years and 38.4% of them used herbal products frequently. The most frequently used way of obtaining information about herbal products was the internet (30.1%). While 34.1% of the participants found herbal products more effective than other drugs, 63.4% recommended them to others. Only 18.9% of the participants had sufficient knowledge about the side effects of medicinal herbals and herb-drug interactions. Herbal products were mostly preferred for respiratory system diseases (40.9%) and the most common purpose of use was to help treatment (33.7%). The products were commonly purchased from herbalists (26.6%), the leaves of the products were generally used and the boiling method was preferred. Participants preferred peppermint (28.4%) and thyme (16.0%) for respiratory ailments and St John's wort oil (40.8%) and aloe vera (22.4%) for skin conditions. According to the results of the study, no significant relationship was found between demographic characteristics and herbal medicine use. Participants did not have sufficient knowledge about the side effects of herbal products and herb-drug interactions. Education and awareness-raising programs are needed to explain the correct use of herbal products and to warn against their potential negative effects.

Keywords

Herbal product
University students
Families
Respiratory tract infections
Wounds

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GİRİŞ

Astım, bronşit ve kronik obstrüktif akciğer hastalığı (KOAH) gibi çeşitli formlarda bulunan solunum yolu hastalıkları, milyonlarca insanı etkileyerek ciddi mortalite ve morbiditeye yol açmaktadır (Younis ve ark., 2018; Chen ve ark., 2023). Küresel Hastalık, Yaralanma ve Risk Faktörleri Çalışması'na (GBD) göre, zatürre gibi alt solunum yolu enfeksiyonları dünya genelinde yüksek ölüm ve hastalık oranlarına yol açmaktadır. 2016'da yaklaşık 2.38 milyon ölüm bu enfeksiyonlardan kaynaklanmış, bu da alt solunum yolu enfeksiyonlarını tüm yaş gruplarında altıncı, 5 yaş altı çocuklarda ise birinci ölüm nedeni yapmıştır (GBD, 2017). Avrupa'da ise solunum yolu hastalıkları, insidans, prevalans, ölüm oranı ve ekonomik maliyet açısından en yaygın ikinci hastalık grubudur (Brennan, 2017). Solunum yolu hastalıklarına yönelik sağlık bakım maliyetleri, ülkelerin ekonomileri üzerinde artan bir yük oluşturmaktadır. Avrupa Birliği'nin 28 ülkesinde, solunum yolu hastalıklarının yıllık toplam maliyetinin yaklaşık 380 milyar Euro'yu aştığı bildirilmiştir (European Respiratory Society, 2013). Türkiye İstatistik Kurumu'nun (TÜİK) 2020 verilerine göre, ölüm nedenleri arasında solunum yolu hastalıkları %15.8 ile ikinci sıradadır. Solunum yolu hastalıklarından ölümlerin %54.8'i zatürre, %34.5'i kronik alt solunum yolu hastalıkları, %10.8'i ise diğer solunum yolu hastalıklarından kaynaklanmaktadır (TÜİK, 2020). TÜİK 2022 verilerine göre, 0-14 yaş arası çocuklarda en yaygın hastalık üst solunum yolu enfeksiyonları olup, özellikle 0-6 yaş grubunda %31.3 oranıyla en sık görülen hastalıktır (TÜİK, 2022).

Deri yaraları, yanık, ameliyat, travma ve damar hastalıkları gibi çeşitli nedenlerle vücut yüzeyinin bütünlüğünün bozulmasıyla oluşur (Hosseinkhani ve ark., 2017; Zhang ve ark., 2024). Yaralar, dünya genelinde sağlık sistemleri, sağlık çalışanları, hastalar ve aileler üzerinde önemli bir yük oluşturmaktadır. Ayrıca milyonlarca insanın fiziksel ve ruhsal sağlığını etkileyip ciddi maliyetler doğurmaktadır (Kumar ve ark., 2007). Gelişmiş ülkelerde, yaralar nedeniyle ortaya çıkan enfeksiyonlar, iyileşme güçlükleri ve dirençli bakteriler, her yıl milyonlarca dolara mal olmaktadır (Albahri ve ark., 2023; Srivastava ve ark., 2024). Mevcut tahminlere göre dünya genelinde yaklaşık 6 milyon kişi kronik yaralarla mücadele etmektedir (Kumar ve ark., 2007).

Geleneksel ilaçlar bakteriyel enfeksiyonların tedavisinde etkili olsa da ilaç direnci önemli bir sorun yaratmaktadır. Bu nedenle, bitkisel ürünler dünya çapında alternatif tedavi olarak kullanılmakta ve özellikle gelişmekte olan ülkelerde birincil sağlık kaynağı haline gelmektedir (Chaughule & Barve, 2024). 2022'de bitki ekstraktları pazarı 34.4 milyar ABD doları olup, 2027'de 61.5 milyar dolara ulaşması beklenmektedir (Markets and Markets, 2023). Bitkisel özler, antimikrobiyal ve antiviral etkileriyle geleneksel tıpta yaygın kullanılmaktadır (Clercq, 2004; Chaughule & Barve, 2024). Asya, Afrika, Orta Doğu ve Latin Amerika'daki milyonlarca insan için yerel bitkilerden elde edilen geleneksel ilaçlar, yara bakımında önemli bir rol oynamaktadır (Shedoeva ve ark., 2019). Bitkisel ürünlerin ölüme veya hastaneye yatışa yol açması nadir olsa da yan etkiler ve toksisite riski mevcuttur (Singh ve ark., 2012).

Tıbbi bitkisel ürünler, tarih boyunca birçok hastalığın tedavisinde ve önlenmesinde kullanılmıştır. Günümüzde, özellikle tamamlayıcı ve alternatif tıp uygulamalarına olan ilginin artmasıyla birlikte, toplumda bitkisel ürün kullanımına yönelik eğilim de yükselmektedir. Ancak, bu ürünlerin bilinçsiz kullanımı ve ilaç-bitki etkileşimleri ciddi sağlık riskleri doğurabilmektedir. Bu çalışma, Sağlık Hizmetleri Meslek Yüksekokulu (SHMYO) öğrencileri ve ailelerinin solunum yolu enfeksiyonları ve cilt rahatsızlıklarında bitkisel ürün kullanım durumlarını araştırmayı amaçlamaktadır. Ayrıca, bireylerin bitkisel ürünler hakkındaki bilgi düzeyleri, kullanım alışkanlıkları ve kaynaklarına ilişkin veriler toplanarak, bilinçli kullanımın yaygınlaştırılması için önemli noktalar belirlenmeye çalışılacaktır.

Mevcut literatürde bitkisel ürün kullanımına dair birçok araştırma bulunmasına rağmen, özellikle sağlık hizmetleri alanında eğitim gören bireyler ve onların ailelerinin bu konudaki tutumları, bilgi düzeyleri ve kullanım eğilimleri sınırlı bir şekilde ele alınmıştır. Bu çalışma, Türkiye'deki SHMYO öğrencileri ve aileleri üzerinde yapılan nadir çalışmalardan biri olup, bitkisel ürün kullanımına dair güncel ve kapsamlı veriler sunmaktadır. Çalışma, sağlık alanında eğitim gören bireylerin alternatif tedavi yöntemlerine olan yaklaşımlarını ortaya koyarak, gelecekte bu alanda yapılacak bilinçlendirme ve eğitim çalışmalarına katkı sağlayacaktır.

MATERYAL ve METOD

Çalışma Tasarımı ve Katılımcılar

Araştırma tanımlayıcı kesitsel özelliktedir. Araştırma evrenini SHMYO öğrencileri ve aileleri oluşturmaktadır. Evreni belirli örneklem yöntemi kullanılarak %95 güven aralığında 0.05 hata ile minimum örneklem büyüklüğü 150 kişi olarak hesaplanmıştır. Örneklem büyüklüğünü hesaplamak için G power programı 3.1 kullanılmıştır (Faul ve ark., 2007). Veriler toplanmadan önce araştırmacılar tarafından, 10 üniversite öğrencisi ve ailesi üzerinde ön deneme yapılmış; anket üzerinde gerekli düzeltmelerden sonra veriler toplanmaya başlanmıştır.

Örneklem formülü

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{d^2}$$

n: Gerekli minimum örneklem büyüklüğü

Z: Güven düzeyine karşılık gelen z değeri

p: Popülasyondaki olayın gerçekleşme olasılığı

d: Hata payı (bu çalışmada $p < 0.05$ olarak belirtilmiştir)

Veri Toplama Araçları

Araştırmada kullanılan veri toplama formu; sosyo-demografik özellikler, tamamlayıcı ve alternatif tedaviye ilişkin özellikler, bitkisel ürünlerin temin, hazırlanma ve muhafaza yöntemleri ile solunum yolu ve cilt rahatsızlıklarında bitkisel ürün kullanımını içeren soruları kapsamaktadır. Form, literatür taramasıyla hazırlanmıştır. "Tamamlayıcı ve Alternatif Tıbbi (TAT) Karşı Tutum Ölçeği", Hyland ve arkadaşları tarafından 2003 yılında geliştirilmiş olup, Türkiye'de Erci tarafından geçerlilik ve güvenilirliği sağlanmıştır (Hyland ve ark., 2003; Erci, 2007). Bu ölçek, 11 soru içeren ve 6 dereceli Likert tipi (1: Kesinlikle katılıyorum, 2: Katılıyorum, 3: Kısmen katılıyorum, 4: Kısmen katılmıyorum, 5: Katılmıyorum, 6: Kesinlikle katılmıyorum) bir ölçektir. Ölçek iki alt boyuttan oluşmaktadır: TAT alt boyutu ve Bütüncül Sağlık (BS) alt boyutu. Ölçeğin Cronbach alfa güvenilirlik katsayısı 0.72, alt boyutlarının güvenilirlik katsayıları ise 0.60-0.62 arasında değişmektedir (Erci, 2007).

Veri Toplama Araçlarının Uygulanması

Veriler, Mayıs-Temmuz 2023 tarihleri arasında online anket yöntemiyle toplanmıştır. Araştırma formu, öğrenci WhatsApp gruplarına gönderilmiş ve çalışmaya katılmaya gönüllü olan öğrencilerden yanıtlar alınmıştır. Yanıtları toplamak için kartopu tekniği kullanılmış; bu yöntemle bir kişi anketi doldurup, bağlantıyı akrabaları

ve arkadaşlarıyla paylaşmaya davet edilmiştir. Katılımcılara çalışmanın amaçları hakkında bilgi verilmiş ve anketlerle birlikte onam formu da dağıtılmıştır. Ankete Kahramanmaraş Sütçü İmam Üniversitesi SHMYO'nun dokuz farklı bölümünde eğitim görmekte olan ve aydınlatılmış onamı alınan 164 öğrenci ve aileleri katılmıştır.

Çalışmanın yapılabilmesi için Kahramanmaraş Sütçü İmam Üniversitesi klinik araştırmalar etik kurulundan (tarih: 30.05.2023 karar no:10) onay alınmıştır.

İstatistiksel Analizler

Verilerin değerlendirilmesinde, nicel değişkenlerin normal dağılıma uygunluğu Kolmogorov Smirnov testi ile incelenmiştir. Normal dağılıma uygunluk gösteren iki gruplu sosyodemografik değişkenler açısından ölçek puanlarının karşılaştırılması için Independent samples t test, normal dağılıma uygunluk gösteren üç veya daha fazla gruba sahip sosyodemografik değişkenler açısından ölçek puanlarının karşılaştırılması için Tek Yönlü Varyans Analizi (One Way ANOVA) yöntemi kullanılmıştır. Normal dağılıma uygunluk göstermeyen iki gruplu sosyodemografik değişkenlerde ölçek puanlarının karşılaştırılması için Mann-Whitney U testi, üç veya daha fazla gruba sahip sosyodemografik değişkenler açısından ölçek puanlarının karşılaştırılmasında Kruskal-Wallis H testi uygulanmıştır. İstatistiksel parametreler ortalama, standart sapma, medyan (Q1-Q3), oran (%) ve frekans (n) ile belirtilmiş, anlamlılık $p < 0.05$ olarak kabul edilmiştir. Veriler IBM SPSS 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) programı ile analiz edilmiştir.

BULGULAR

SHMYO öğrenci ve ailelerinin sosyo demografik özellikleri Çizelge 1'de verilmiştir.

Araştırmaya katılmayı kabul eden 164 bireyin %84.8'i 18-30 yaş aralığında, %73.2'si öğrenci ve %84.1'i bekarıdır. Katılımcıların beşte dördü (%79.9) kadın olup, yarısından fazlası (%60.4) şehirde ikamet etmektedir. Ayrıca, %82.3'ü önlisans programından mezun ya da hala bu programda eğitim görmektedir. Katılımcıların %83.3'ü herhangi bir sağlık sorunu olmadığını belirtirken, %51.2'si sağlık durumunu "orta", %37.2'si ise "iyi" olarak değerlendirmiştir. Sigara kullananların oranı %14.6'dır. Katılımcıların %51.2'si gelir düzeyini çok düşük olarak belirtmiş, %39.6'sının sosyal güvencesi bulunmadığı tespit edilmiştir. Ayrıca, yarısından fazlası (%56.7) bir sağlık sorunu yaşadıklarında hem geleneksel hem de modern tıp yöntemlerini birlikte tercih ettiklerini ifade etmiştir (Çizelge 1).

Araştırmaya katılan bireylerin %38.4'ü sık sık, %43.9'u ise ara sıra bitkisel ürün kullandıklarını belirtmiştir. Katılımcıların %30.1'i bitkisel ürünlerle ilgili bilgiyi internetten, %20.9'u büyüklerinden ve %14.5'i doktorlardan edindiğini ifade etmiştir. Katılımcıların yarısından fazlası (%59.1), bitkisel ürünlerin tüm hastalıkları önleyemeyeceğini düşündüğünü, %37.2'si ise bitkisel ürünlerin diğer ilaçlardan daha etkili ve güvenli olmadığını savunmuştur. Ayrıca, dörtte üçünden fazlası (%78.7), bitkisel ürünlerin her yaş grubu için uygun olmadığını bildirmiştir. Katılımcıların %63.4'ü bitkisel ürünleri başkalarına da önerdiğini ifade etmiştir. Basın ve yayın yoluyla yapılan haberlerin tıbbi bitkilere olan ilgilerini arttırdığını belirten katılımcıların oranı %24.4 iken, %40.9'u bu konuda "kısmen" etkilenmiş olduğunu söylemiştir. Katılımcıların yalnızca %18.9'u, kullandığı bitkisel ürünlerin yan etkileri ve bitkilerin ilaçlarla etkileşim riski hakkında bilgi sahibi olduğunu belirtmiştir. Bitkisel ürünler en sık solunum yolu hastalıklarında (%40.9) kullanılmış olup, %33.7'si bu ürünleri tedaviye yardımcı olmak amacıyla, %18.9'u ise doğrudan hastalığı tedavi etmek amacıyla kullanmıştır. Katılımcıların %47.6'sı reçeteli ilaçlarla birlikte tıbbi bitkisel ürünler kullanmış, %47.0'si doktora bitkisel ürün kullandığını bildirmiş ve %63.6'sı doktor onayı almıştır (Çizelge 2).

Çalışmaya katılan bireylerin %26.6'sı bitkisel ürünleri aktarlardan satın aldıklarını, %19.8'i doğadan topladıklarını, %17.4'ü ise eczaneden temin ettiklerini ifade etmiştir. Katılımcıların %37.5'inin bitkisel ürünleri kaynatarak tükettiği, %18.6'sının herhangi bir tatlandırıcı kullanmadığı, %18.1'inin ise bal ile tatlandığı belirlenmiştir. Ayrıca, %39'u bitkisel ürünleri hazırlarken cam veya porselen kap kullandığını, %34.7'si bitkisel ürünleri buzdolabı ya da mutfak dolabında sakladığını ve yarıdan fazlası (%55.4) ürünleri cam kavanozda muhafaza ettiğini belirtmiştir (Çizelge 3).

Katılımcılar en sık soğuk algınlığı (%34.5), öksürük (%19.3) ve nezle (%16.8) gibi rahatsızlıklarda bitkisel ürün kullanmışlardır. Solunum yolu hastalıklarında ise en çok nane (%28.4), kekik (%16.0) ve zencefil (%8.6) tercih edilmiştir. Katılımcıların dörtte birinden fazlası (%30.7) bitkilerin yapraklarını kullanırken, yarısından fazlası (%53.5) kaynatma yöntemini tercih etmiştir. Bitkisel ürün kullandıktan sonra sağlık durumlarında iyileşme olduğunu belirten katılımcıların oranı %67.7 olarak tespit edilmiştir (Çizelge 4).

Çizelge 1. Öğrencilerin ve ailelerinin sosyo demografik özellikleri

Table 1. Socio-demographic characteristics of students and their families

Yaş	n	%	Herhangi bir hastalık varlığı*	n	%
18-30	139	84.8	Yok	145	83.3
31-40	4	2.4	Solunum	4	2.2
41-50	14	8.5	Şeker	3	1.8
51-60	7	4.3	Kalp	1	0.6
Cinsiyet			Tansiyon	3	1.8
Kadın	131	79.9	Eklem	3	1.8
Erkek	33	20.1	Mide	3	1.8
Yaşanılan yer			Diğer	12	6.7
Şehir merkezi	99	60.4	Kişisel sağlığı algılama durumu		
İlçe	34	20.7	Kötü	1	0.6
Köy	31	18.9	Orta	84	51.2
Eğitim durumu			İyi	61	37.2
Yok	2	1.2	Çok iyi	13	7.9
İlkokul	9	5.5	Mükemmel	5	3.0
Ortaokul	2	1.2	Sigara kullanımı		
Lise	8	4.9	Evet	24	14.6
Önlisans	135	82.3	Hayır	131	79.9
Lisans	7	4.3	Ara sıra	9	5.5
Lisans üstü	1	0.6	Gelir düzeyi		
Meslek			Çok düşük	84	51.2
İşsiz	12	7.3	Düşük	11	6.7
Memur	8	4.9	Orta	22	13.4
İşçi	7	4.3	İyi	37	22.6
Çiftçi	3	1.8	Çok iyi	10	6.1
Öğrenci	120	73.2	Soyal güvence var mı?		
Ev hanımı	9	5.5	Evet	99	60.4
Emekli	1	0.6	Hayır	65	39.6
Diğer	4	2.4	Hastalıklarda tercih edilen tıp		
Medeni hal			Modern tıp	57	34.8
Evli	25	15.2	Geleneksel tıp	14	8.5
Bekar	138	84.1	Her ikisi de	93	56.7
Dul	1	0.6			

* Birden fazla seçenek işaretlenmiştir.

Çizelge 2. Katılımcıların, tamamlayıcı ve alternatif tedavi ile ilgili bazı özellikleri

Table 2. Some characteristics of the participants regarding complementary and alternative treatment

Değişkenler ve Cevaplar	n	%
Tıbbi bitkisel ürün kullanıyor musunuz?		
Hayır	29	17.7
Evet sık sık	63	38.4
Evet ara sıra	72	43.9
Tıbbi bitkiler ile ilgili bilgiyi nereden öğreniyorsunuz? *		
Büyüklerimden	72	20.9
Televizyondan	22	6.4
İnternette	104	30.1
Eş, dost, akraba arkadaşlarımdan	29	8.4
Doktorlardan	50	14.5
Eczacılardan	37	10.7
Aktarlardan	19	5.5
Diğer (dergi, gazete, broşür vb.)	12	3.5

Tıbbi bitkisel ürünler tüm hastalıkları önleyebilir veya tedavi edebilir mi?		
Evet	27	16.5
Hayır	97	59.1
Fikrim yok	40	24.4
Tıbbi bitkisel ürünleri diğer ilaçlardan daha etkili ve daha güvenli buluyor musunuz?		
Evet	56	34.1
Hayır	61	37.2
Fikrim yok	47	28.7
Kullanılan tüm tıbbi bitkisel ürünlerin her yaş için uygun olduğunu düşünüyor musunuz?		
Evet	16	9.8
Hayır	129	78.7
Fikrim yok	19	11.6
Tıbbi bitkileri başkalarına kullanması için önerir misiniz?		
Evet	104	63.4
Hayır	60	36.6
Basın-yayın yoluyla yapılan haberler tıbbi bitkilere olan ilginizi artırdı mı?		
Evet	40	24.4
Hayır	57	34.8
Kısmen	67	40.9
Kullandığınız tıbbi bitkilerin yan etkileri ve bitki ilaç etkileşimler hakkında yeterli bilgiye sahip olduğunuzu düşünüyor musunuz?		
Evet	31	18.9
Hayır	75	45.7
Kısmen	58	35.4
Tıbbi bitkisel ürünleri en çok hangi rahatsızlıklarınızda kullanıyorsunuz?		
Bitkisel ürün kullanmıyorum	29	17.7
Solunum sistemi hastalıklarında	67	40.9
Sindirim sistemi hastalıklarında	24	14.6
Yaralarda	12	7.3
Yanıklarda	2	1.2
Çeşitli ağrılarda	12	7.3
Diğer (Konstipasyon, uykusuzluk, demir eksikliği vb.)	18	11.0
Tıbbi bitkisel ürün kullanma nedeniniz/nedenleriniz nelerdir? *		
Hastalıklardan korunmak için	51	15.1
Tedaviye yardımcı olmak için	114	33.7
Hastalığı tedavi etmek için	64	18.9
Maliyeti düşük olduğu için	30	8.9
Yan etkisi daha az olduğu için	4	1.2
Geçmişten belli ailemiz kullandığı için	25	7.4
İlaçlardan daha etkili olduğu için	16	4.7
Diğer	4	1.2
Tıbbi bitkisel ürünleri hangi sıklıkla kullanıyorsunuz? *		
Her gün	8	4.9
Haftada 3-4 kez	7	4.3
Ayda 3-4 kez	18	11.0
Sadece kendimi kötü hissettiğimde	108	65.9
Nadiren	1	0.6
Ciddi rahatsızlıklarda	1	0.6
Reçetelenmiş ilaçlarla birlikte tıbbi bitkisel ürün kullandınız mı?		
Evet	78	47.6
Hayır	86	52.4
Doktor reçetesi olmadan ilaç kullanıyor musunuz?		
Evet	33	20.1
Hayır	131	79.9

Doktora gittiğinizde bitkisel ürün kullandığınızı hekime söylüyor musunuz? **		
Evet	77	47.0
Hayır	58	35.4
Cevap evet ise doktorun cevabı ne oldu? ***		
Karşı çıktı	18	23.4
Onayladı	49	63.6
Diğer (bir şey demedi, başka ürün önerisinde bulundu)	10	13.0

* Birden fazla seçenek işaretlenmiştir.

** TAT yöntemi kullananlar üzerinden hesaplanmıştır.

*** Evet cevabı verenler üzerinden hesaplanmıştır.

Çizelge 3. Tıbbi bitkisel ürünlerin temini, hazırlanması ve muhafazası

Table 3. Supply, preparation and preservation of medicinal herbal products

Değişkenler	Cevaplar	n	%
Tıbbi bitkisel ürünleri nereden temin ediyor sunuz? *			
Aktardan		78	26.6
İnternette		20	6.8
Eczaneden		51	17.4
Marketten		15	5.1
Eş, dost, akrabadan		31	10.6
Doğadan topluyorum		58	19.8
Tıbbi bitkisel ilaç hazırlamada en sık kullandığınız yöntem/yöntemler hangisidir? *			
Kaynatma		90	37.5
Ezerek lapa yapma		13	5.4
Kompres yapma		9	3.8
Zeytin yağında bekletme		27	11.3
Yağını çıkarma		17	7.1
Toz şeklinde		15	6.3
Buharı		10	4.2
Tıbbi bitkisel ürünleri tatlandırmak için yardımcı bir madde kullanıyor musunuz? *			
Hayır, tatlandırıcı kullanmıyorum		41	18.6
Evet, şeker ile tatlandırıyorum		28	12.7
Evet, limon ile tatlandırıyorum		37	16.7
Evet bal ile tatlandırıyorum		40	18.1
Evet, pekmez ile tatlandırıyorum		16	7.2
Evet, diğer (reçel, marmelat, meyve suyu vb.)		12	5.4
Bitkisel ilaç hazırlamak için kullandığınız materyalin cinsine dikkat eder misiniz?			
Hayır, her tür hazırlama materyalini kullanırım		55	33.5
Evet, cam veya porselen olmalarına dikkat ederim		64	39.0
Evet, emaye veya teflon vb. olmalarına dikkat ederim		16	9.8
Tıbbi bitkileri nerede muhafaza ediyorsunuz? *			
Mutfak dolabında		68	34.7
Buz dolabında		68	34.7
Diğer		14	7.1
Tıbbi bitkileri hangi ambalaj tipinde muhafaza ediyorsunuz? *			
Cam kavanoz		108	55.4
Metal kavanoz		3	1.5
Bez torba		23	11.8
Kağıt torba		9	4.6
Plastik kap		3	1.5

* Birden fazla seçenek işaretlenmiştir.

Çizelge 4. Solunum yolu hastalıklarında bitkisel ürün kullanımı

Table 4. Use of herbal products in respiratory diseases

Değişkenler	Cevaplar	n	%
Özellikle hangi solunum yolu hastalığında bitkisel ürünü kullanıyor sunuz? *			
Soğuk algınlığı		109	34.5
Üst solunum yolu enfeksiyonu		26	8.2
Nezle		53	16.8
Bademcik iltihabı		15	4.7
Astım		2	0.6
Faranjit		12	3.8
Öksürük		61	19.3
Bronşit		9	2.8
Zatürre		1	0.3
Larenjit		1	0.3
Hangi bitkiyi kullanıyorsunuz? *			
Nane		126	28.4
Rezene		20	4.5
Ada çayı		35	7.9
Dağ çayı		29	6.5
Zencefil		38	8.6
Zerdeçal		18	4.1
Lavanta		4	0.9
Çörek otu		26	5.9
Papatya		29	6.5
Tarçın		21	4.7
Melisa		4	0.9
Kekik		71	16.0
Bitkinin genellikle hangi kısmını sık kullanırsınız? *			
Yaprağını		107	30.7
Çiçeğini		56	16.0
Gövdesini		35	10.0
Tohumunu		26	7.4
Kabuğunu		16	4.6
Kökünü		13	3.7
Tüm bitkiyi		22	6.3
Diğer (yağını, reçinesini)		2	0.6
Bitkisel ürünü en sık nasıl kullanıyor sunuz? *			
Kaynatma		136	53.5
Hiçbir işlem yapmadan		13	5.1
direk çiğneme			
Suda bekletme		39	15.4
Lapa yapma		5	2.0
Buharı		31	12.2
Yağı		10	3.9
Bitkisel ürün aldıktan sonra hastalık durumunuzda iyileşme fark ettiniz mi?			
Evet		111	67.7
Hayır		1	0.6
Kısmen		23	14.0
Bitkisel ürün aldıktan sonra herhangi bir zarar gördünüz mü?			
Evet		3	1.8
Hayır		130	79.3
Kısmen		2	1.3

* Birden fazla seçenek işaretlenmiştir.

Katılımcıların cilt rahatsızlıklarında en sık tercih ettikleri bitkisel ürün sarı kantaron olup, bunu aleovera takip etmiştir (sırasıyla %40.8 ve %22.4). Katılımcıların %9.3'ü bitkilerin yaprak kısımlarını, %19.1'i ise kaynatma yöntemini kullandıklarını belirtmiştir. Bitkisel ürün kullanımının ardından sağlık durumunda iyileşme olduğunu ifade edenlerin oranı %22.6 olarak tespit edilmiştir (Çizelge 5).

Çizelge 5. Çeşitli cilt rahatsızlıklarında bitkisel ürün kullanımı

Table 5. Use of herbal products in various skin disorders

Değişkenler	Cevaplar	n	%
Şu anda veya daha önce cildinizde herhangi bir yanık, yara vs. oldu mu? *			
Hayır		114	63.7
Yara		30	16.8
Çıban		32	17.9
Diğer (yanık, egzema vs.)		3	1.7
Cildinizdeki rahatsızlık için hangi bitkiyi/bitkisel ürünü kullandınız? **			
Sarı kantaron		20	40.8
Zeytin yağı		5	10.2
Çam sakızı		2	4.1
Aloe vera		11	22.4
Isırgan otu		2	4.1
Bamya		1	2.0
Ebe gömeci		1	2.0
Sinir otu		2	4.1
Patates		1	2.0
Elma		1	2.0
Diğer		3	6.1
Cildinizdeki rahatsızlık için bitkinin hangi kısmını kullandınız? *			
Yaprağını		18	9.3
Çiçeğini		11	5.7
Gövdesini		6	3.1
Tohumunu		4	2.1
Yağını		11	5.7
Kabuğunu		7	3.6
Kökünü		4	2.1
Diğer (tüm bitkiyi, reçinesini)		9	4.6
Cildinizdeki rahatsızlık için bitkiyi nasıl kullandınız? *			
Kaynatma		35	19.1
Hiçbir işlem yapmadan direk çiğneme		4	2.2
Suda bekletme		9	4.9
Lapa yapma		10	5.5
Direk yaraya sürme		3	1.6
Buhar		3	1.6
Bitkisel ürün kullandıktan sonra yara, yanık veya çıbanda iyileşme oldu mu?			
Evet		37	22.6
Hayır		1	0.6
Kısmen		11	6.7
Bitkisel ürün kullandıktan sonra yara, yanık veya çıbanda daha kötüye gitme durumu oldu mu?			
Evet		2	1.2
Hayır		46	28.0
Kısmen		1	0.6

* Birden fazla seçenek işaretlenmiştir.

Sosyo-demografik özelliklerin grupları arasında ölçek puanları ve alt boyut puanları arasındaki farklılıklar incelenmiştir. Analiz sonuçlarına göre, BS ve TAT alt boyutları ile ölçeğin toplam puanları açısından sosyo-demografik gruplar arasında anlamlı bir fark bulunmamıştır. Yaş, cinsiyet, ikamet edilen yer, eğitim durumu, meslek, medeni hal, sosyal güvence, sigara kullanımı, tercih edilen tıp yöntemi ve gelir düzeyi değişkenlerinin grupları arasında BS, TAT ve ölçek toplam puanları bakımından farklılık olmadığı saptanmıştır (Çizelge 6).

Çizelge 6. BS ve TAT'a karşı tutum ölçeği puan ortalamaları ve etkileyen faktörler
Table 6. Attitude scale average scores towards HH and CAM and affecting factors

BS		TAT			TOPLAM		
Median(Q1-Q3)		Mean±SD			Mean±SD		
15.00(12.00-17.00)		25.88±2.92			40.35±4.78		
Sosyo demografik özellikler		BS	TAT		TOPLAM		
		Median	(Q1	-Q3)	Mean	±SD	Mean ±SD
Yaş	18-30	15.00	(12.00	-17.00)	25.78	±2.92	40.27 ±4.73
	31-40	13.00	(11.00	-14.00)	27.00	±2.00	39.67 ±2.89
	41-50	14.50	(12.00	-17.00)	26.93	±3.50	41.50 ±5.57
	51-60	15.00	(15.00	-17.00)	25.33	±1.03	40.00 ±5.37
	p	0.585 ^b			0.455 ^a		0.817 ^a
Cinsiyet	Kadın	15.00	(12.00	-17.00)	26.02	±2.79	40.33 ±4.81
	Erkek	15.00	(14.00	-17.00)	25.33	±3.36	40.42 ±4.73
	p	0.222 ^d			0.227 ^c		0.923 ^c
Yaşanılan yer	Şehir merkezi	15.00	(12.00	-17.00)	25.66	±2.95	39.87 ±4.53
	İlçe	15.00	(12.00	-17.00)	26.32	±2.97	41.24 ±5.23
	Köy	15.00	(12.00	-17.00)	26.10	±2.77	40.93 ±5.00
	p	0.444 ^b			0.476 ^a		0.272 ^a
Eğitim durumu	Yok	11.50	(6.00	-17.00)	24.50	±0.71	36.00 ±8.49
	İlkokul	13.00	(12.00	-15.00)	26.14	±1.57	40.43 ±4.54
	Ortaokul	15.00	(13.00	-17.00)	25.50	±7.78	40.50 ±10.61
	Lise	14.00	(10.00	-16.50)	25.38	±3.25	39.38 ±5.21
	Önlisans	15.00	(13.00	-17.00)	25.81	±2.94	40.41 ±4.67
	Lisans	15.00	(12.00	-18.00)	28.14	±1.95	42.71 ±3.64
	p	0.645 ^b			0.541 ^a		0.273 ^a
Meslek	İşsiz	12.50	(10.00	-15.00)	25.08	±3.12	37.00 ±5.24
	Memur	16.00	(13.50	-17.00)	25.88	±3.68	41.13 ±2.36
	İşçi	16.00	(13.00	-19.00)	26.17	±3.31	42.67 ±4.27
	Çiftçi	17.00	(10.00	-17.00)	26.67	±3.79	41.33 ±7.02
	Öğrenci	15.00	(12.50	-17.00)	25.92	±2.89	40.54 ±4.65
	Ev hanımı	14.00	(12.00	-16.50)	25.87	±2.90	40.38 ±5.73
	Diğer	12.50	(8.50	-14.00)	26.25	±2.87	37.50 ±5.80
	p	0.084 ^b			0.990 ^a		0.151 ^a
Medeni Hal	Evli	15.00	(13.00	-17.00)	26.00	3.25	40.70 ±4.75
	Bekar	15.00	(12.00	-17.00)	25.88	2.88	40.37 ±4.73
	Dul	6.00	(6.00	-6.00)	24.00	.	30.00 .
	p	0.884 ^b			0.799 ^a		0.089 ^a
Sosyal güvence	Evet	15.00	(13.00	-17.00)	25.85	±2.67	40.66 ±4.51
	Hayır	15.00	(12.00	-17.00)	25.94	±3.28	39.89 ±5.15
	p	0.217 ^d			0.843 ^c		0.318 ^c
Sigara kullanımı	Evet	14.50	(12.50	-17.00)	25.63	±2.78	40.54 ±4.42
	Hayır	15.00	(12.00	-17.00)	25.96	±2.95	40.47 ±4.86
	Arasıra	13.00	(9.00	-16.00)	25.44	±3.13	38.11 ±4.34
	p	0.384 ^b			0.787 ^a		0.352 ^a
Hastalıklarda Tercih Edilen tıp	Modern tıp	15.00	(12.00	-17.00)	25.80	±3.22	40.45 ±5.38
	Geleneksel tıp	14.00	(10.00	-15.00)	27.21	±1.67	39.93 ±3.89
	Her ikisi de	15.00	(13.00	-17.00)	25.73	±2.85	40.35 ±4.56
	p	0.249 ^b			0.202 ^a		0.935 ^a
Gelir düzeyi	Çok düşük	15.00	(12.00	-16.00)	25.67	±3.02	39.70 ±4.89
	Düşük	15.00	(12.00	-18.00)	26.64	±3.35	41.73 ±4.94
	Orta	15.00	(13.00	-17.00)	26.76	±2.19	42.05 ±4.82
	İyi	15.00	(12.00	-17.00)	25.50	±2.68	40.53 ±3.92
	Çok iyi	14.50	(13.00	-16.00)	26.40	±3.63	40.10 ±5.97
	p	0.657 ^b			0.405 ^a		0.270 ^a

BS: Bütüncül sağlık, TAT: Tamamlayıcı ve alternatif tıp, SD: Standart sapma

^aTek Yönlü (One Way) ANOVA; ^bKruskal Wallis H test; ^cIndependent samples t test; ^dMann Whitney U test

TARTIŞMA

Çalışmada, bitkisel ürün kullanım yaygınlığı %38.4'dür (Çizelge 1). Bu sonuç üniversite öğrencileri arasında %16.5-79 arasında değişen oranlarda bitki ve besin takviyesi kullanımını bildiren önceki çalışmalarla uyumludur (Ayrancı ve ark., 2005; Johnson & Blanchard, 2006; Boparai ve ark., 2017; Sekhri ve ark., 2013). Katılımcıların %79.9'u kadın, %20.1'i erkek olup, benzer çalışmalarda da kadın katılımcıların çoğunlukta olduğu görülmüştür (Ayrancı ve ark., 2005; Sekhri ve ark., 2013; Ali-Shtayeh ve ark., 2013; Boparai ve ark., 2017; Pranskuniene ve ark., 2019; El-Dahiyat ve ark., 2020; Pranskuniene ve ark., 2022). Bu bulgu, geleneksel bilginin nesiller arası aktarımında kadınların oynadığı önemli rolü vurgulamaktadır. Katılımcıların %84.8'i 18-30 yaş aralığındadır ve bu bulgu, benzer çalışmalarda da gözlemlenen genç katılımcı profiliyle örtüşmektedir (Kaner ve ark., 2017).

Çalışmada katılımcıların %30.1'i bitkisel ürünlerle ilgili bilgiyi internetten edinmiştir (Çizelge 2). Bu bulgu Boparai ve ark. (2017) ile Cumhuriyet ve ark. (2023) çalışmasıyla uyumludur. Bu durum, internetin geleneksel tıp konularındaki güncel gelişmeleri takip etme aracı olarak kullanıldığını göstermektedir. Yanlış bilginin önlenmesi için internet üzerinden doğru kaynaklara yönlendirme önemlidir. İkinci sırada ise %20.9 oranıyla aile büyükleri bilgi kaynağı olarak öne çıkmıştır (Çizelge 2). Bu bulgu da önceki çalışmalardaki geleneksel bilgi aktarımı sonuçlarıyla uyumludur (Sekhri ve ark., 2013; Pranskuniene ve ark., 2019; Pranskuniene ve ark., 2022). Sağlık profesyonelleri ise bilgi kaynakları arasında üçüncü ve dördüncü sırada yer almıştır (Çizelge 2). Ancak, Litvanya'da yapılan çalışmalarda katılımcıların sağlık profesyonellerine başvurma oranı daha düşük bulunmuştur. Katılımcılar bitkisel ürünlerle ilgili bilgi için bir doktora ve/veya eczacıya başvurmamış ve bu konuda sağlık uzmanlarına güvenmemişlerdir (Pranskuniene ve ark., 2018; Pranskuniene ve ark., 2019).

Bitkisel ürünleri diğer ilaçlardan daha etkili ve güvenli bulan katılımcıların oranı %34.1'dir (Çizelge 2), ancak Sekhri ve ark. (2013) çalışmasında bu oran %82.5 olarak bildirilmiştir. Her doğal ürünün güvenli olduğu düşüncesi yanıltıcıdır; bazı bitkiler ciddi toksik etkilere ve istenmeyen yan etkilere yol açabilmektedir (Ernst, 2007).

Sonuçlar, katılımcıların %63.4'ünün başkalarına şifalı bitki kullanımını tavsiye ettiğini göstermiştir (Çizelge 2). Benzer çalışmalarda da katılımcıların şifalı bitkileri başkalarına önerdiği tespit edilmiştir; Al-Zahim ve ark. (2013), Suudi Arabistan'daki karaciğer hastalarının %40.7'sinin diğer hastalara bitkisel tedavi önerdiğini belirtirken, Abdelmola ve ark. (2021), katılımcıların %70.2'sinin benzer şekilde tavsiyede bulunduğunu saptamışlardır.

Bitkisel ürünler medya tarafından abartılı bir şekilde sunulmakta ve tamamen zararsız olduğu iddia edilmektedir. Türkiye'de sağlık hizmetlerinin maliyeti nedeniyle birçok kişi koruyucu veya tedavi edici amaçlarla bitkisel ürünlere yönelmektedir. Ancak bu ürünlerin, özellikle hamileler, emziren anneler ve çocuklar için ciddi sağlık riskleri taşıyabileceği bilinmelidir (Erdem & Ata Eren, 2009). Çalışmada katılımcıların %24.4'ü medyada yer alan haberlerin tıbbi bitkilere olan ilgilerini artırdığını, %40.9'u ise kısmen artırdığını ifade etmiştir (Çizelge 2). Nur (2010), çalışmasında katılımcıların %45.1'inin medya etkisiyle bitkisel ilaç kullanmaya karar verdiğini bildirmiştir.

Katılımcıların sadece %18.9'u bitkilerin yan etkileri ve ilaç-bitki etkileşimleri hakkında yeterli bilgiye sahip olduklarını belirtmiştir (Çizelge 2). Bu sonuç, tıp asistanları ve öğrenciler üzerinde yapılan iki çalışmayla desteklenmektedir; bu çalışmalarda tıp asistanları ve öğrencileri, örgün eğitimleri sırasında bitkisel ilaçlar konusunda oldukça sınırlı bir eğitim aldıklarını belirtmişlerdir (Sekhri ve ark., 2013; Xu & Levine, 2008). Her ne kadar bazı bileşikler için klinik kanıtlar bulunsun da toksik etkilerinin ve diğer ilaçlarla olan etkileşimlerinin daha ayrıntılı bir şekilde araştırılması gerekmektedir (Timalsina ve ark., 2021).

Bitkisel ilaçlar, biyoaktif içerikleri sayesinde uzun yıllardır tıbbi rahatsızlıkları tedavi etmek ve sağlığı geliştirmek amacıyla kullanılmaktadır (Albahri ve ark., 2023). Araştırmada katılımcıların bitkisel materyalleri en sık solunum (%40.9) ve sindirim sistemi hastalıklarının (%14.6) tedavisinde tercih ettikleri belirlenmiştir (Çizelge 2). Pranskuniene ve ark. (2019) ise katılımcıların tıbbi bitkileri en çok sindirim (%21.5) ve solunum sistemi (%21.3) hastalıklarında kullandıklarını bildirmiştir.

Tüm dünyada ve Avrupa'da tıbbi bitkisel hammaddelerin hem tüketime hazır sebzeler, reçeller veya baharatlar olarak, hem de tedavi amacıyla kullanıldığı belirtilmiştir (Svanberg, 2012; Yiğitkan ve ark., 2024). Çalışma sonuçlarına göre, şifalı bitkilerin kullanım amaçları arasında tedaviye yardımcı olmak, hastalıkları tedavi etmek ve sağlığı korumak öne çıkmıştır (Çizelge 2). Bu bulgu, Al Akeel ve ark. (2018) ve Abdelmola ve ark. (2021) tarafından Suudi Arabistan'da yapılan araştırmalarla da uyumludur. Ekonomik nedenler ve bitkilerin kimyasal ilaçlara alternatif olarak görülmesi bu tercihi açıklayabilir.

Katılımcıların %47.6'sı reçeteli ilaç tedavisinin yanı sıra bitkisel ilaç kullandıklarını belirtmiştir (Çizelge 2). Marignani ve ark. (2010) da benzer şekilde, katılımcıların %67'sinin reçeteli ilaçlarla birlikte bitkisel ilaç kullandığını saptamıştır. Bu durum, katılımcıların bitkilerin yan etkileri ve ilaç etkileşimleri konusundaki bilgi eksikliklerinden kaynaklanıyor olabilir. Çalışmada katılımcıların %47.0'ı doktora bitkisel ürün kullandıklarını

belirtmiş ve bu kişilerin %63.6'sı hekimin bitkisel ürünü onayladığını ifade etmiştir (Çizelge 2). Renda ve ark. (2018) tarafından yapılan çalışmada ise doktorların %42.5'inin bitkisel ürünleri önerdiği saptanmıştır. Türkiye'de yapılan bir çalışmada ise katılımcıların %79.71'inin, doktora bitkisel ürün kullanım durumları ile ilgili bilgi vermedikleri saptanmıştır. Bunun sebebi olarak ise %50.91'i doktorlarının bunu sormadığı için, %43.64'ü bitkisel ürünleri doktorlarına gitmedikleri bir dönemde kullandıkları için, %5.45'i ise doktorlarının bitkisel ürün kullanımını onaylamayacağını düşündükleri için bilgi vermediklerini belirtmiştir (Cumhur ve ark., 2023).

Katılımcılar bitkisel ürünleri genellikle aktarlardan (%26.6) temin etmektedir (Çizelge 3). Bu bulgu, diğer çalışmalarla uyumludur (Kaner ve ark., 2017; Özkan & Deniz, 2023). Çalışmada eczaneden bitkisel ürün temin etme oranı %17.4 olup, bu oran Türkiye'deki diğer çalışmalardan daha yüksektir (Kaner ve ark., 2017; Özkan & Deniz, 2023). Türkiye'de bitkisel ürünler çoğunlukla internet ve aktarlar aracılığıyla satışa sunulmakta olup, bu durum bilgi eksikliği ve satış esaslı yaklaşımlar nedeniyle riskler taşıyabilir. Bu nedenle, bitkisel ürünlerin güvenilir yerlerden, özellikle eczanelerden temin edilmesi büyük önem taşımaktadır.

Bulgular, bitkisel ürünlerin solunum sistemi rahatsızlıkları, özellikle soğuk algınlığı, nezle, öksürük ve üst solunum yolu enfeksiyonları için sıkça kullanıldığını göstermiştir. En yaygın kullanılan bitkiler sırasıyla nane (%28.4), kekik (%16.0), zencefil (%8.6), adaçayı (%7.9), dağ çayı (%6.5), papatya (%6.5) ve çörek otu (%5.9) olarak belirlenmiştir (Çizelge 4). Pranskuniene ve ark. (2022) nane ve diğer bitkilerin öksürük ve burun tıkanıklığını hafifletmek için kullanıldığını bildirmiştir. Yücel ve Yücel (2022) de benzer şekilde, solunum yolu rahatsızlıklarında bu bitkisel ürünlerin tercih edildiğini saptamıştır. Araştırma, şifalı bitkilerin genellikle yaprak ve çiçek kısımlarının kullanıldığını ortaya koymuştur; bu kısımlar sırasıyla %30.7 ve %16 oranında tercih edilmiştir (Çizelge 4). Yapraklar ve çiçekler, dünya genelinde ilaç olarak yaygın bir şekilde kullanılmaktadır (Pranskuniene ve ark., 2019; Abdelmola ve ark., 2021; Pranskuniene ve ark., 2022; Mrabti ve ark., 2022). Bu materyallerin yaygın kullanılmasının nedeni, bol bulunmaları ve hasadının kolay olmasıdır (Söukand ve Pieroni, 2016). En popüler hazırlama yöntemi kaynatmadır (Çizelge 3, 4, 5). Bu sonucumuz diğer çalışmalarla uyumludur (Phumthum ve ark., 2018; Younis ve ark., 2018). Bunun sebebi kaynatmanın daha kolay, basit ve çok zaman almaması olabilir.

Doğal bitkisel ürünler, önemli farmakolojik özellikleri sayesinde yara tedavisi ve enfeksiyon önlenmesinde etkili bir şekilde kullanılmaktadır (Albahri ve ark., 2023). Araştırmada sarı kantaron (%40.8) ve aloe vera (%22.4) cilt rahatsızlıklarında en sık tercih edilen bitkisel ürünler olarak belirlenmiştir (Çizelge 5). Sarı kantaron yağı ve aloe vera'nın cilt tedavisindeki etkinliği önceki çalışmalarda da gösterilmiştir (Süntar ve ark., 2010; Akgun ve ark., 2017).

Çalışmada tamamlayıcı ve alternatif tıp kullanımının demografik faktörlerle anlamlı bir ilişkisi bulunmamıştır (Çizelge 6). El-Dahiyat ve ark. (2020) benzer şekilde yaptıkları bir çalışmada, bitkisel ürün kullanımının yaş ile ilişkili olduğunu belirtmiştir. İbrahim ve ark. (2018) ise eğitim düzeyi ve medeni durum gibi bazı demografik değişkenlerle ilişki bulmuştur. Diğer bir çalışma, tamamlayıcı ve alternatif tıp kullanımının 50 yaş üstü ve şehirde yaşayan kişilerle ilişkili olduğunu ortaya koymuştur (Ali-Shtayeh ve ark., 2013).

SONUÇ ve ÖNERİLER

Bu çalışma, Sağlık Hizmetleri Meslek Yüksekokulu öğrencileri ve ailelerinin bitkisel ürün kullanımına yönelik eğilimlerini, bilgi kaynaklarını ve bilinç düzeylerini incelemiştir. Araştırma bulgularına göre: Katılımcıların büyük bir kısmı bitkisel ürünleri solunum yolu hastalıkları ve cilt rahatsızlıklarında kullanmaktadır. Bitkisel ürünlerin güvenilirliği ve ilaç-bitki etkileşimleri konusunda bilgi eksikliği bulunmaktadır. Katılımcılar bitkisel ürünleri çoğunlukla aktar ve internetten temin etmekte, ancak bu durum kontrolsüz ve yanlış kullanım riskini artırmaktadır. Medya ve internet, bitkisel ürünler hakkında bilgi edinmede en yaygın kaynaklar arasında yer almakta, ancak bu kaynaklar çoğu zaman bilimsel veriye dayalı olmayan bilgileri içermektedir. Katılımcıların bir kısmı reçeteli ilaçlarla birlikte bitkisel ürün kullanmakta, ancak bu tür kombinasyonların doktor bilgisi dahilinde olup olmadığı net değildir. Bu sonuçlardan hareketle;

- Bitkisel ürünlerin güvenli kullanımı, yan etkileri ve ilaçlarla etkileşimleri konusunda halkı ve sağlık profesyonellerini bilinçlendirecek eğitim programları düzenlenmelidir.
- Özellikle sağlık alanında eğitim gören öğrencilerin tamamlayıcı ve alternatif tıp konularında daha fazla bilgi edinmesini sağlayacak ders içerikleri ve eğitim programları oluşturulmalıdır.
- Bitkisel ürünlerin kontrollü ve güvenilir satışını sağlamak için aktar, internet ve diğer satış noktalarına yönelik daha sıkı düzenlemeler getirilmelidir.
- Hastaların, reçeteli ilaçlarla birlikte bitkisel ürün kullanımı konusunda doktorlarına danışma oranı artırılmalı, sağlık çalışanları bu konuda daha fazla yönlendirici olmalıdır.

-Medya ve internet üzerinden yayılan yanlış bilgilerin önüne geçebilmek için bilimsel dayanaklı, güvenilir kaynakların yaygınlaştırılması ve toplumun bu kaynaklara yönlendirilmesi sağlanmalıdır.

Bu çalışma, bitkisel ürün kullanımına dair mevcut durumu analiz ederek, bilinçli ve güvenli kullanımın teşvik edilmesine yönelik stratejiler geliştirilmesine katkıda bulunmaktadır. Gelecekte yapılacak araştırmalar, farklı demografik gruplar, farklı hastalık türleri ve bitkisel ürünlerin etkinliği üzerine odaklanarak, daha kapsamlı bir perspektif sunabilir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

- Abdelmola, A.O., Bahri, A., Abuallut, I., Refaei, B.A., Hakami, W.K., Abutaleb, A.K., Mahzari, S.M., Mashragi, M.A., Es'hag S.A., & Aldarbi, K.F. (2021). Prevalence, knowledge, and perception about the use of herbal medicines japan-Saudi Arabia. *Journal of Family Medicine and Primary Care*, 10(6), 2386-2393.
- Akgun, S.G., Aydemir, S., Ozkan, N., Yuksel, M., & Sardas, S. (2017). Evaluation of the wound healing potential of Aloe vera-based extract of Nerium oleander. *Northern Clinics of Istanbul*, 4(3), 205-212.
- Al Akeel, M.M., Al Ghamdi, W.M., Al Habib, S., Koshm, M., & Al Otaib, F. (2018). Herbal Medicines: Saudi population knowledge, attitude, and practice at a glance. *Journal of Family Medicine and Primary Care*, 7(5), 865-875.
- Albahri G, Badran A, Hijazi A, Daou, A., Baydoun, E., Nasser, M., & Merah, O. (2023). The therapeutic wound healing bioactivities of various medicinal plants. *Life (Basel)*, 13(2), 317.
- Ali-Shtayeh, M.S., Jamous, R.M., Jamous, R.M., & Salameh, N.M. (2013). Complementary and alternative medicine (CAM) use among hypertensive patients in Palestine. *Complementary Therapies in Clinical Practice*, 19(4), 256-263.
- Al-Zahim, A.A., Al-Malki, N.Y., Al-Abdulkarim, F.M., Al-Sofayan, S.A., Abunab, H.A., & Abdo, A.A. (2013). Use of alternative medicine by Saudi liver disease patients attending a tertiary care center: Prevalence and attitudes. *Saudi Journal of Gastroenterology*, 19(2), 75-80.
- Ayrancı, U., Son, N., & Son, O. (2005). Prevalence of nonvitamin, nonmineral supplement usage among students in a Turkish university. *BMC Public Health*, 5, 47.
- Boparai, J.K., Singh, A., Gupta, A.K., Matreja, P.S., Khanna, P.M.L., Gupta, V., & Gautam, R.K. (2017). A study to determine the knowledge and level of awareness of medical undergraduates about herbal medicines and herb-drug interactions. *International Journal of Basic & Clinical Pharmacology*, 6(1), 17-24.
- Brennan, N.J. (2017). Respiratory Diseases: Overview. In: S.R. Quah, (Ed.), *International Encyclopedia of Public Health*, 2nd ed, (pp. 328-331).: Academic Press, Massachusetts.
- Chaughule, R.S., & Barve, R.S. (2024). Role of herbal medicines in the treatment of infectious diseases. *Vegetos*, 37, 41-51.
- Chen, S., Kuhn, M., Prettnner, K., Yu, F., Yang, T., Bärnighausen, T., Bloom, D., & Wang, C. (2023). The global economic burden of chronic obstructive pulmonary disease for 204 countries and territories in 2020-50: a health-augmented macroeconomic modelling study. *The Lancet Global Health*, 11(8), e1183-e1193.
- Cumhur, H., Aydin, A., Oturak, G., Ekerbicer, H. C., & Etcioğlu, E. (2023). Assessment of Herbal Product Use Among Infertile Women: A Survey-Based Study in Türkiye. *Eurasian Journal of Family Medicine*, 12(3), 117-124.
- Clercq, E.D. (2004). Antiviral drugs in current clinical use. *Journal of Clinical Virology*, 30(2), 115-133.
- El-Dahiyat, F., Rashrash, M., Abuhamdah, S., Abu Farha, R., & Babar, Z.U.D. (2020). Herbal medicines: a cross-sectional study to evaluate the prevalence and predictors of use among Jordanian adults. *Journal of Pharmaceutical Policy and Practice*, 13(1), 1-9.
- Erci, B. (2007). Attitudes towards holistic complementary and alternative medicine: a sample of healthy people in Turkey. *Journal of Clinical Nursing*, 16(4), 761-768.
- Erdem, S., & Ata Eren, P. (2009). Tedavi amacıyla kullanılan bitkiler ve bitkisel ürünlerin yan etkileri. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, 66(3), 133-141.
- Ernst, E. (2007). Herbal medicines: balancing benefits and risks. *Novartis Found Symp* 282, 154-167.
- European Respiratory Society. (2013). The economic burden of lung disease. In: *European Lung White Book*. <https://www.ersnet.org/the-european-lung-white-book/>. (Erişim tarihi: 12.12.2023).

- Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39(2), 175-191.
- GBD. (2017). 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, 390(10100), 1151–1210.
- Hosseinkhani, A., Falahatzadeh, M., Raoofi, E., & Zarshenas, M.M. (2017). An evidence-based review on wound healing herbal remedies from reports of traditional Persian medicine. *Evidence-Based Complementary and Alternative Medicine*, 22(2), 334-343.
- Hyland, M.E., Lewith, G.T., & Westoby, C. (2003). Developing a measure of attitudes: the holistic complementary and alternative medicine questionnaire. *Complementary Therapies in Medicine*, 11(1), 33–38.
- İbrahim, I.R., Hassali, M.A., Saleem, F., Al Tukmagi, H.F., & Dawood, O.T. (2018). Use of complementary and alternative medicines: a cross-sectional study among hypertensive patients in Iraq. *Journal of Pharmaceutical Health Services Research*, 9(1), 59–65.
- Johnson, S.K., & Blanchard, A. (2006). Alternative medicine and herbal use among university students. *Journal of American College Health*, 55(3), 163-168.
- Kaner, G., Karaalp, C., & Seremet-Kürklü, N. (2017). Üniversite öğrencileri ve ailelerinde bitkisel ürün kullanım sıklığının ve bitkisel ürün kullanımını etkileyen faktörlerin belirlenmesi. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, 74(1), 37-54.
- Kumar, B., Vijayakumar, M., Govindarajan, R., & Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing: Exploring medicinal plants of India. *Journal of Ethnopharmacology*, 114(2), 103-113.
- Marignani, M., Gallina, S., Di Fonzo, M., Deli, I., Begini, P., Gigante, E., Epifani, M., Angeletti, S., & Fave, G.D. (2010). Use and safety perception of herbal remedies in patients with liver/biliary tract disorders: an Italian study. *Journal of Clinical Gastroenterology*, 44, 54-57.
- Markets & Markets (2023). Plant Extracts Market by Type (Oleoresins, Essential Oils, Flavonoids, Alkaloids, Carotenoids, Pharmaceuticals, Dietary Supplements) From, source and Region - Global Forecast to 2027. Plant Extracts Market size Trend Overview. https://www.marketsandmarkets.com/Market-Reports/plant-extracts-market-942.html?gad_source=1&gclid=Cj0KCQjwZmwbBhD8ARIsAH4v1gUxHj4E--EzMiBZeSzcTYlLrZbeROdzoUx32lJOqorZVhp_KpYutlkaAkazEALw_wcB. (Erişim tarihi:18.02.2024).
- Mrabti, H.N., Doudach, L., Mekkaoui, M., Khalil, Z., Harraoui, K., Fozia, F., Mrabti, N.N., El-Shazly, M., Alotaibi, A., Ullah, R., El Abbes Faouzi, M., & Bouyahya, A. (2022). Profile of medicinal plants traditionally used for the treatment of skin burns. *Evidence Based Complementary and Alternative Medicine*, 2022, Article ID 3436665, 10 pages.
- Nur, N. (2010). Knowledge and behaviours related to herbal remedies: a cross-sectional epidemiological study in adults in Middle Anatolia, Turkey. *Health and Social Care in the Community*, 18(4), 389-395.
- Özkan, O., & Deniz, T. (2023). An assessment of consumer demand for medicinal plants: A case of Istanbul. *Eurasian Journal of Forest Science*, 11(1), 1-13.
- Phumthum, M., Srithi, K., Inta, A., Junsongduang, A., Tangjitman, K., Pongamornkul, W., Trisonthi, C., & Balslev, H. (2018). Ethnomedicinal plant diversity in Thailand. *Journal of Ethnopharmacology*, 214, 90–98.
- Pranskuniene, Z., Balciunaite, R., Simaitiene, Z., & Bernatoniene, J. (2022). Herbal medicine uses for respiratory system disorders and possible trends in new herbal medicinal recipes during COVID-19 in pasvalys district, Lithuania. *International Journal of Environmental Research and Public Health*, 19(15), 8905.
- Pranskuniene, Z., Dauliute, R., Pranskunas, A., & Bernatoniene, J. (2018). Ethnopharmaceutical knowledge in Samogitia region of Lithuania: Where old traditions overlap with modern medicine. *Journal of Ethnobiology and Ethnomedicine*, 14, 70.
- Pranskuniene, Z., Ratkeviciute, K., Simaitiene, Z., Pranskunas, A., & Bernatoniene, J. (2019). Ethnobotanical Study of Cultivated Plants in Kaisiadorys District, Lithuania: Possible Trends for New Herbal Based Medicines. *Evidence-Based Complementary and Alternative Medicine*, 2019, Article ID 3940397, 15 pages.
- Renda, G., Yaşar, Y.K., Yılmaz, E., Sanrı, H., Dilaver, İ., Demirtaş, Y., Çan, G., & Sezen, F.S. (2018). Aile hekimleri ve eczacıların bitkisel ürün kullanımına yaklaşımları: Trabzon ilinde pilot çalışma. *Türkiye Aile Hekimliği Dergisi*, 22(3), 141-156.
- Sekhri, K., Bhanwra, S., & Nandha, R. (2013). Herbal products: a survey of students' perception and knowledge about their medicinal use. *International Journal of Basic Clinical Pharmacology*, 2(1), 71-76.
- Singh, D., Gupta, R., & Saraf, S.A. (2012). Herbs-are they safe enough? An overview. *Critical Reviews in Food Science and Nutrition*, 52(10), 876–898.
- Shedoeva, A., Leavesley, D., Upton, Z., & Fan, C. (2019). Wound healing and the use of medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2019(1), 2684108.

- Sõukand, R., & Pieroni, A. (2016). The importance of a border: medical, veterinary, and wild food ethnobotany of the Hutsuls living on the Romanian and Ukrainian sides of Bukovina. *Journal of Ethnopharmacology*, 185, 17-40.
- Srivastava, G. K., Martinez-Rodriguez, S., Md Fadilah, N. I., Looi Qi Hao, D., Markey, G., Shukla, P., Fauzi, M.B., & Panetsos, F. (2024). Progress in Wound-Healing Products Based on Natural Compounds, Stem Cells, and MicroRNA-Based Biopolymers in the European, USA, and Asian Markets: Opportunities, Barriers, and Regulatory Issues. *Polymers*, 16(9), 1280.
- Süntar, I.P., Akkol, E.K., Yilmazer, D., Baykal, T., Kirmizibekmez, H., Alper, M., & Yeşilada, E. (2010). Investigations on the in vivo wound healing potential of *Hypericum perforatum* L. *Journal of Ethnopharmacology*, 127(2), 468-477.
- Svanberg, I. (2012). The use of wild plants as food in pre-industrial Sweden. *Acta Societatis Botanicorum Poloniae*, 81(4), 317-327.
- Timalsina, D., Pokhrel, K.P., & Bhusal, D. (2021). Pharmacologic Activities of Plant-Derived Natural Products on Respiratory Diseases and Inflammations. *BioMed Res Int* 2021, Article ID 1636816, 23 pages.
- TÜİK. (2020). Ölüm ve Ölüm Nedeni İstatistikleri, <https://data.tuik.gov.tr/Bulten/Index?p=%C3%96l%C3%BCm-ve-%C3%96l%C3%BCm-Nedeni-%C4%B0statistikleri-2020-37243&dil=1> (Erişim tarihi: 08.01.2024).
- TÜİK. (2022). <https://data.tuik.gov.tr/Bulten/Index?p=Turkiye-Saglik-Arastirmasi-2022-49747#:~:text=Bunu%20s%C4%B1ras%C4%B1yla%2C%20%29%2C4,ve%20di%C5%9F%20sa%C4%9Fl%C4%B1%C4%9F%C4%B1%20sorunlar%C4%B1%20izledi> (Erişim tarihi:08.01.2024).
- Xu, S., & Levine, M. (2008). Medical residents' and students' attitudes towards herbal medicines: a pilot study. *The Canadian journal of Clinical Pharmacology*, 5(1), e1-4.
- Yiğitkan, S., Çavuşoğlu, M., Çağlayan, M. V., Yener, İ., Fırat, M., Kaya, E. Ç., Yılmaz, M. A., Ertas, A. (2024). LC-MS/MS Analysis and Biological Activities of Different Parts of *Ziziphora capitata* L. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 27(Suppl 2), 307-315.
- Younis, W., Asif, H., Sharif, A., Riaz, H., Bukhari, I.A., & Assiri, A.M. (2018). Traditional medicinal plants used for respiratory disorders in Pakistan: a review of the ethno-medicinal and pharmacological evidence. *Chinese Medicine*, 13, 48.
- Yücel, D., & Yücel, E. (2022). Plants used in complementary medicine in the treatment of respiratory tract diseases in Türkiye. *Antolian Journal of Botany*, 6(1), 18-26.
- Zhang, W., Liu, L., Cheng, H., Zhu, J., Li, X., Ye, S., & Li, X. (2024). Hydrogel-based dressings designed to facilitate wound healing. *Materials Advances*, 5(4), 1364-1394.



Molecular Docking Studies and Biological Activities of Chalcones Targeting Acetylcholinesterase, and Carbonic Anhydrase Isoenzymes

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ABSTRACT

Chalcone molecules are important pharmacophores in medicinal chemistry and have various biological functions, including inhibitory effects on the enzymes carbonic anhydrase (CA) and acetylcholinesterase (AChE). Carbonic anhydrase I and II inhibitors are used in the treatment of disorders such as retinal and cerebral edema (CAI), epilepsy, and glaucoma (CA II). Furthermore, acetylcholinesterase inhibitors, which were originally created to treat Alzheimer's disease, have proven useful for patients suffering from Parkinson's disease-related memory problems, behavioral disorders, and cognitive decline. The drugs on the market have adverse effects. Therefore, new drug candidates are required to address the issues raised. In this study, chalcone compounds were synthesized to investigate their CA and AChE inhibitory effects and their chemical structures were confirmed using NMR. The inhibitory effects of the synthesized compounds on carbonic anhydrase and acetylcholine esterase enzymes were presented for the first time in this study. Carbonic anhydrases and AChE inhibitory effects of 1-21 were investigated using described methodologies. As a result of the studies, it was determined that the compounds were in the inhibition range of 2.65-82.33 μ M for hCA I and 2.63-74.89 μ M for hCA II, while the IC₅₀ values of the reference AZA were 46.75 μ M (hCA I) and 38.25 μ M (hCA II). Moreover, these compounds inhibited AChE in the range of 15.53-177.46, while the IC₅₀ value of the reference drug Tacrin was measured as 25.78 nM. Among the synthesized chalcone derivatives, compound **5** emerged as the most potent inhibitor for hCA I and AChE, while compound **13** was the strongest for hCA II. AutoDock Vina docking results showed that compound **5** had the strongest affinity for hCA I (-8.0 kcal mol⁻¹) and AChE (-7.0 kcal mol⁻¹), while compound **13** was most potent for hCA II (-8.1 kcal mol⁻¹). Key interactions with catalytic residues suggest that halogen and methoxy groups enhance enzyme binding, stability, and hydrogen bonding. These findings suggest that these compounds hold promise as potential drug candidates for CA and AChE related disorders.

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Asetilkolinesteraz ve Karbonik Anhidraz Izoenzimlerini Hedef Alan Şalkonların Moleküler Yerleştirme Çalışmaları ve Biyolojik Aktiviteleri

ÖZET

Şalkon molekülleri, medisinale kimyada önemli farmakoforlar olup, karbonik anhidraz (CA) ve asetilkolinesteraz (AChE) enzimleri üzerindeki inhibitör etkiler de dahil çeşitli biyolojik fonksiyonlara sahiptir. Karbonik anhidraz I ve II inhibitörleri, retina ve serebral ödem (CAI), epilepsi ve glokom (CA II) gibi bozuklukların tedavisinde

Medisinal Kimya

Araştırma Makalesi

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kullanılır. Ayrıca, başlangıçta Alzheimer hastalığını tedavi etmek için oluşturulan asetilkolinesteraz inhibitörlerinin Parkinson hastalığıyla ilişkili hafıza sorunları, davranış bozuklukları ve bilişsel gerileme çeken hastalar için de yararlı olduğu kanıtlanmıştır. Piyasadaki ilaçların olumsuz etkileri bulunmaktadır. Bu nedenle, ortaya çıkan sorunları ele almak için yeni ilaç adaylarına ihtiyaç vardır. Bu çalışmada, CA ve AChE inhibitör etkilerini araştırmak için şalkon bileşikler sentezlenmiş ve kimyasal yapıları NMR kullanılarak doğrulanmıştır. Bu çalışma ile sentezlenen bileşiklerin karbonik anhidraz ve asetilkolin esteraz enzimleri üzerindeki inhibitör etkileri ilk kez sunulmuştur. Bileşik 1-21'in CA ve AChE inhibitör etkileri açıklanan metodolojiler kullanılarak araştırılmıştır. Yapılan araştırmalar sonucunda bileşiklerin hCA I için 2.65-82.33 μM ve hCA II için 2.63-74.89 μM inhibisyon aralığında olduğu tespit edilirken, referans AZA'nın IC_{50} değerlerinin ise 46.75 μM (hCA I) ve 38.25 μM (hCA II) olduğu görülmüştür. Dahası, bu bileşikler AChE'yi 15.53-177.46 aralığında inhibe ederken, referans ilaç Tacrin'in IC_{50} değeri ise 25.78 nM olarak ölçülmüştür. Sentezlenen şalkon türevleri arasında, bileşik 5 hCA I ve AChE için en etkili inhibitör olarak ortaya çıkarken, bileşik 13 hCA II için en güçlüsüydü. AutoDock Vina ile yapılan molecular docking sonuçlarına göre, bileşik 5 hCA I (-8.0 kcal mol^{-1}) ve AChE (-7.0 kcal mol^{-1}) için en güçlü afiniteyi gösterirken, bileşik 13 hCA II (-8.1 kcal mol^{-1}) için en güçlü inhibitör olarak belirlendi. Kilit katalitik kalıntılarla etkileşimleri, halojen ve metoksi gruplarının enzim bağlanmasını, stabilitesini ve hidrojen bağlarını güçlendirdiğini göstermektedir. Bu bulgular, bu bileşiklerin CA ve AChE ile ilişkili bozukluklar için potansiyel ilaç adayları olarak umut vadettiğini göstermektedir.

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Anahtar Kelimeler

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Karbonik Anhidraz
Asetilkolinesteraz
Moleküler Doking
Şalkon

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INTRODUCTION

The human lifespan has increased due to advancements in medicine that improve living conditions. Conditions such as dementia are increasingly imposing a significant burden on society due to the rising longevity of individuals. Among the most prevalent illnesses affecting the elderly is dementia. Alzheimer's disease is the most common cause of dementia (AD). (Ferri et al., 2005). Alzheimer's disease is a neurological disease that causes cognitive processes like memory, attention, executive functions, visuospatial skills, and language skills to decrease (Tierney et al., 1996; Petersen et al., 1999; Galvin et al., 2005; Petersen et al., 2006). Currently, research focuses on early detection and effective treatment options. Alzheimer's disease etiology hypotheses include the cholinergic, amyloid, glutamergic, metal, oxidative stress, genetic, and inflammatory hypotheses. The development and activity of the cerebral cortex, cerebral blood flow, sleep cycle, learning, memory, and perception are all significantly influenced by cholinergic transmission. Acetylcholine is a neurotransmitter that causes the smooth muscles in blood vessels to relax and the smooth muscles in the gastrointestinal, urinary, and ocular tracts to contract. The cholinergic hypothesis states that either an increase in the activity of the acetylcholinesterase (AChE) enzyme or a decrease in the brain's generation of acetylcholine, one of the key neurotransmitters, results in a decrease in the amount of acetylcholine. Acetylcholine's carboxylic acid ester group is hydrolyzed by AChE, resulting in the formation of acetic acid and choline. Acetylcholine hydrolyzes in synapses and neuromuscular junctions, preventing nerve transmission. Hence, in the autonomic and somatic nervous systems, AChE is essential for cholinergic neuron transmission. Acetylcholine concentrations in synapses rise as a result of AChE enzyme inhibition, allowing receptors to engage with acetylcholine more intensely and for longer periods of time. Tacrine is an AChE inhibitor first produced for clinical use in 1986. Later, second-generation AChE inhibitors with improved tolerance were developed, including donepezil (1996), rivastigmine (1998), and galantamine (2000) (Knopman et al., 2021; Marucci et al., 2021; Poyraz et al., 2024). In all living organisms, the conversion of carbon dioxide and bicarbonate ions, which are crucial in biochemical processes, to one another is of extreme importance.

Metalloenzymes such as carbonic anhydrase (CAs, EC 4.2.1.1) can utilize certain ions or simple molecules as substrates. Fundamentally, it is understood to catalyze the reaction between bicarbonate and carbon dioxide. To meet the metabolic needs in a shorter amount of time, carbonic anhydrases catalyze this reaction, even though it can occur spontaneously at physiological pH. The Zn^{2+} ion, which is typically tightly bound and interacts with water and histidine molecules in the active site of the enzyme, serves to bind and activate the water molecule, thereby catalyzing the reversible hydration reaction of carbon dioxide to bicarbonate and proton ions through a metal hydroxide nucleophilic mechanism. Humans are known to possess 16 distinct CA isoenzymes (α -hCA) that are members of the α -class. Among them, CA I and CA II are cytosolic isoenzymes. CA I is responsible for acid-base balance and control of respiration. Sweat glands, adipose tissue, the gastrointestinal tract, and the corneal epithelium are among the tissues and cells that contain CA I isoenzyme. One of the isoenzymes that has been examined the most in inhibition studies is CA II. It regulates intraocular pressure by delivering sodium to the eye. This enzyme deficiency is associated with bone resorption, brain calcification, and renal acidity. Furthermore, carbonic anhydrase enzymes play an important role in many physiological and pathological processes. CA inhibitors and CA activators are considered possible treatments for disorders such as edema, glaucoma, obesity, cancer, epilepsy, and osteoporosis due to their role in physiological and pathological processes. Furthermore, CA isoenzymes could be possible targets for Alzheimer's disease (AD) (Supuran et al., 2008; Temperini et al., 2008; Le Duc, et al., 2017).

Chalcones are substances with the chemical formula 1,3-diaryl-2-propen-1-one. They have an α , β -unsaturated ketone structure. Chalcones are naturally present in many plants due to their simple structure, ease of availability, broad bioactivity spectrum, and tendency to cyclize to produce compounds with therapeutic properties (Ouyang et al., 2021). It has often been taken into consideration when developing new lead compounds with medical potential. Chalcones have a wide range of biological activities, including antibacterial, antifungal, antidiabetic, anti-inflammatory, cytotoxic, and anticancer properties (Mazumder et al., 2024) (Figure 1)

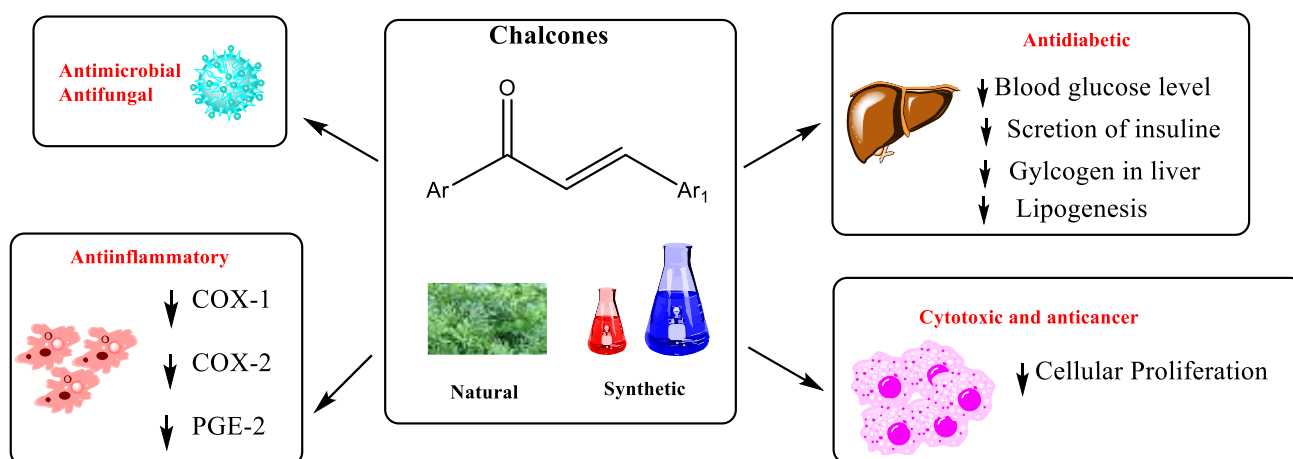


Figure 1 Summarized scheme of some important pharmacological properties of chalcones

Şekil 1 Şalkonların bazı önemli farmakolojik özelliklerinin özet şeması

Due to promising effects obtained according to the works of literature and also as part of our ongoing research survey, in this work, chalcone derivatives (Scheme 1) were investigated in their CAs and AChE inhibition as well as molecular docking was applied.

MATERIAL and METHOD

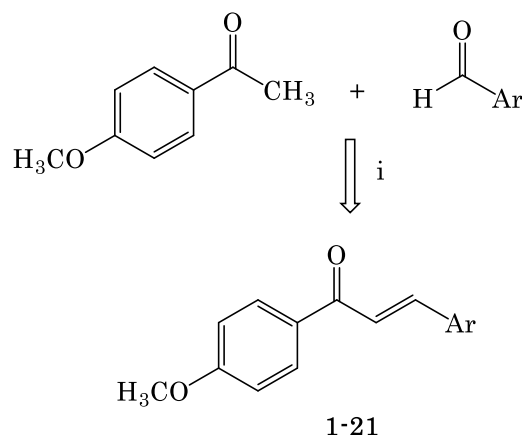
Chemistry

^1H NMR (400 MHz) spectra in $DMSO-d_6$ (Merck) were taken using Varian Mercury Plus spectrometer, Varian Inc., Palo Alto, California, U.S. 9100/IA9100 instrument (Bibby Scientific Limited, Staffordshire, UK) was used to determine melting points. Reactions were monitored by Thin Layer Chromatography (TLC) using silica gel HF254 (Merck Art 5715).

General synthesis procedure of 1-21, Scheme 1

To the mixture of 4-methoxyacetophenone (1 m mol) and a suitable aldehyde (1 m mol) in EtOH (20 ml), NaOH (in aqua 10%, 4 ml) was added. The contents of the flask were stirred at room temperature for 24 hours. The formed

substance is poured into cold water (100 ml) and then HCl (in aqua 10%, w v⁻¹) was added until mixture neutralized. The solid formed was filtered and washed with water. H₂O / Ethanol mixture was used for crystallization (Tugrak et al., 2016; Tugrak et al., 2017; Tugrak et al., 2018) (Scheme 1)



Ar: 1= Phenyl
2= 4-Methylphenyl
3= 4-Methoxyphenyl
4= 4-Chlorophenyl
5= 4-Bromophenyl
6= 4-Fluorophenyl
7= 2-Chlorophenyl
8= 3-Chlorophenyl
9= 2-Fluorophenyl
10= 3-Fluorophenyl
11= 2,4-Dimethoxyphenyl

Ar: 12= 2,3-Dimethoxyphenyl
13= 2,3,4-Trimethoxyphenyl
14= 2,4,5-Trimethoxyphenyl
15= 3,4,5-Trimethoxyphenyl
16= 3-Bromophenyl
17= 3-Hydroxy-4-methoxyphenyl
18= Thiophenone-2-yl
19= Benzyloxyphenyl
20= Naphthalen-2-yl
21= Benzo[d][1,3]dioxol-5-yl

Reagents: (i) 10%

NaOH, EtOH, r.t., 24 h

Scheme 1 Synthesis of the compounds 1-21

Şema 1 Bileşik 1-21'in sentezi

(E)-1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-one (1)

Yield: 90%. m. p.: 98-100°C, Lit m. p.: 99-102°C (Shinichi et al., 1990). ¹H-NMR (CDCl₃) δ 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.80 (d, 1H, *J* = 15.7 Hz, =CH), 7.65-7.63 (m, 2H, Ar-H), 7.54 (d, 1H, *J* = 15.7 Hz, =CH), 7.43-7.37 (m, 3H, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(*p*-tolyl)-prop-2-en-1-one (2)

Yield: 88%. m. p.: 96-98°C, Lit m. p.: 95-97°C (Umesha et al., 2015). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.78 (d, 1H, *J* = 15.6 Hz, =CH), 7.55-7.48 (m, 3H, Ar-H, =CH), 7.25-7.21 (m, 2H, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.88 (s, 3H, CH₃O), 3.85 (s, 3H, CH₃O)

(E)-1,3-Bis(4-methoxyphenyl)-prop-2-en-1-one (3)

Yield: 91%. m. p.: 96-98°C, Lit m. p.: 99°C (Vaidya et al., 2012). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.78 (d, 1H, *J* = 15.5 Hz, =CH), 7.60 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.43 (d, 1H, *J* = 15.5 Hz, =CH), 6.99-6.92 (m, 4H, Ar-H), 3.88 (s, 3H, CH₃O), 3.85 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(4-chlorophenyl)-prop-2-en-1-one (4)

Yield: 90%. m. p.: 112-114°C, Lit m. p.: 115°C (Vaidya et al., 2012). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.74 (d, 1H, *J* = 15.6 Hz, =CH), 7.58-7.49 (m, 3H, Ar-H, =CH), 7.38 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(4-bromophenyl)-prop-2-en-1-one (5)

Yield: 85%. m. p.: 155-156°C, Lit m. p.: 152-154°C (Bale et al., 2018). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.72 (d, 1H, *J* = 15.6 Hz, =CH), 7.55-7.48 (m, 5H, Ar-H, =CH), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(4-fluorophenyl)-prop-2-en-1-one (6)

Yield: 80%. m. p.: 113-115°C, Lit m. p.: 115-118°C (Kumar et al., 2012). ¹H-NMR (CDCl₃) δ 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.76 (d, 1H, *J* = 15.6 Hz, =CH), 7.65-7.61 (m, 2H, Ar-H), 7.47 (d, 1H, *J* = 15.6 Hz, =CH), 7.13-7.08 (m, 2H, Ar-H), 6.94 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(2-chlorophenyl)-prop-2-en-1-one (7)

Yield: 81%. m. p.: 117-119°C, Lit m. p.: 118-120°C (Kazi et al., 2017). ¹H-NMR (CDCl₃) δ 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.76 (d, 1H, *J* = 15.6 Hz, =CH), 7.65-7.61 (m, 2H, Ar-H), 7.47 (d, 1H, *J* = 15.6 Hz, =CH), 7.13-7.08 (m, 2H, Ar-H), 6.94 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(3-chlorophenyl)-prop-2-en-1-one (8)

Yield: 82%. m. p.: 95-97°C, Lit m. p.: 97-99°C (Abdel-Halim et al., 2013). ¹H-NMR (CDCl₃) δ 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.75 (d, 1H, *J* = 15.8 Hz, =CH), 7.62-7.47 (m, 2H, Ar-H, =CH), 7.35 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.08-6.97 (m, 2H, Ar-H), 6.83 (d, 1H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(2-fluorophenyl)-prop-2-en-1-one (9)

Yield: 80%. m. p.: 100-102°C, Lit m. p.: 105-106°C (Choi et al., 2015). ¹H-NMR (CDCl₃) δ 8.04 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.98 (d, 1H, *J* = 15.9 Hz, =CH), 7.66 (d, 1H, *J* = 15.9 Hz, =CH), 7.62-7.35 (m, 1H, Ar-H), 7.21-7.10 (m, 2H, Ar-H), 6.97 (d, 1H, *J* = 8.9 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(3-fluorophenyl)-prop-2-en-1-one (10)

Yield: 85%. m. p.: 100-102°C, Lit m. p.: 104.5-105.5 °C (Roman et al., 2015). ¹H-NMR (CDCl₃) δ 8.04 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.77 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.53 (d, 1H, *J* = 15.6 Hz, =CH), 7.40-7.35 (m, 1H, Ar-H), 7.16-7.10 (m, 2H, Ar-H, =CH), 6.98 (d, 1H, *J* = 8.9 Hz, Ar-H), 6.83 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(2,4-dimethoxyphenyl)-prop-2-en-1-one (11)

Yield: 91%. m. p.: 85-87°C, Lit m. p.: 82°C (Bandgar et al., 2010). ¹H-NMR (CDCl₃) δ 8.06-8.01 (m, 3H, Ar-H, =CH), 7.58-7.53 (m, 2H, Ar-H, =CH), 6.97 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.54-6.47 (m, 2H, Ar-H), 3.90 (s, 3H, CH₃O), 3.88 (s, 3H, CH₃O), 3.85 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(2,3-dimethoxyphenyl)-prop-2-en-1-one (12)

Yield: 86%. m. p.: 100-102°C, Lit m. p.: 99.0-99.5°C (Wu et al., 2011). ¹H-NMR (CDCl₃) δ 8.10-8.03 (m, 3H, Ar-H, =CH), 7.61 (d, 2H, *J* = 15.8 Hz, =CH), 7.29-7.25 (m, 2H, Ar-H), 7.13-7.06 (m, 1H, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.90 (s, 3H, CH₃O), 3.89 (s, 3H, CH₃O), 3.88 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(2,3,4-trimethoxyphenyl)-prop-2-en-1-one (13)

Yield: 85%. m. p.: 91-92°C, Lit m. p.: 94°C (Russell, 1934). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.97 (d, 1H, *J* = 15.8 Hz, =CH), 7.57 (d, 1H, *J* = 15.8 Hz, =CH), 7.38 (d, 1H, *J* = 8.8 Hz, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.72 (d, 1H, *J* = 8.8 Hz, Ar-H), 3.94 (s, 3H, CH₃O), 3.91 (s, 3H, CH₃O), 3.90 (s, 3H, CH₃O), 3.89 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)-prop-2-en-1-one (14)

Yield: 86%. m. p.: 120-122°C, Lit m. p.: 124°C (Roman et al., 2015). ¹H-NMR (CDCl₃) δ 8.04 (d, 1H, =CH, *J* = 15.5 Hz), 7.93 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.48 (d, 1H, *J* = 15.7 Hz, =CH), 6.84 (s, 1H, Ar-H), 6.77 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.21 (s, 1H, Ar-H), 3.80 (s, 6H, 2 x CH₃O), 3.75 (s, 3H, CH₃O), 3.57 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (15)

Yield: 81%. m. p.: 132-134°C, Lit m. p.: 131.1-132.5°C (Kovar et al., 2020). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.71 (d, 1H, *J* = 15.6 Hz, =CH), 7.42 (d, 1H, *J* = 15.6 Hz, =CH), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.86 (s, 2H, Ar-H), 3.92 (s, 6H, 2xCH₃O), 3.90 (s, 3H, CH₃O), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(3-bromophenyl)-prop-2-en-1-one (16)

Yield: 75%. m. p.: 121-123°C, Lit m. p.: 126°C (Patil et al., 2007). ¹H-NMR (CDCl₃) δ 7.72 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.32-7.26 (m, 1H, Ar-H, =CH), 7.18-7.11 (m, 4H, Ar-H, =CH), 6.98 (d, 1H, *J* = 8.9 Hz, Ar-H), 6.83 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (17)

Yield: 70%. m. p.: 148-150°C, Lit m. p.: 150-152°C (Raghavan et al., 2015). ¹H-NMR (DMSO-*d*₆) δ 9.12 (bs, OH), 8.13 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.69 (d, 1H, *J* = 15.4 Hz, =CH), 7.58 (d, 1H, *J* = 15.4 Hz, =CH), 7.32-7.27 (m, 2H, Ar-H), 7.07 (d, 2H, *J* = 8.8 Hz, Ar-H) 6.98 (d, 1H, *J* = 8.3 Hz, Ar-H), 3.86 (s, 3H, CH₃O), 3.83 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(thiophen-2-yl)-prop-2-en-1-one (18)

Yield: 72%. m. p.: 103-105°C, Lit m. p.: 106-107°C (Shibata et al., 1991). ¹H-NMR (CDCl₃) δ 8.02 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.93 (d, 1H, *J* = 15.3 Hz, =CH), 7.41-7.33 (m, 3H, Ar-H, =CH), 7.09-7.07 (m, 1H, Ar-H), 6.98 (d, 2H, *J* = 8.8 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(4-(benzyloxy)phenyl)-prop-2-en-1-one (19)

Yield: 76%. m. p.: 132-133°C, Lit m. p.: 135-137°C (Bale et al., 2018). ¹H-NMR (CDCl₃) δ 8.03 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.84 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.77 (d, 1H, *J* = 15.3 Hz, =CH), 7.59 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.45-7.34 (m, 6H, Ar-H, =CH), 7.09-6.96 (m, 3H, Ar-H), 5.11 (s, 2H, OCH₂), 3.88 (s, 3H, CH₃O)

(E)-1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)-prop-2-en-1-one (20)

Yield: 84%. m. p.: 151-152°C, Lit m. p.: 155°C (Mai et al., 2014). ¹H-NMR (CDCl₃) δ 8.65 (d, 1H, *J* = 15.4 Hz, =CH), 8.27 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.09 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.93-7.88 (m, 3H, Ar-H), 7.65-7.50 (m, 4H, Ar-H, =CH), 6.98 (d, 2H, *J* = 8.9 Hz, Ar-H), 3.89 (s, 3H, CH₃O)

(E) 1-(4-Methoxyphenyl)-3-(benzo[d][1,3]dioxol-5-yl)-prop-2-en-1-one (21)

Yield: 78%. m. p.: 132-133°C, Lit m. p.: 134-135°C (Schwartz et al., 1977). ¹H-NMR (CDCl₃) δ 8.02 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.72 (d, 1H, *J* = 15.5 Hz, =CH), 7.38 (d, 1H, *J* = 15.5 Hz, =CH), 7.17-7.10 (m, 2H, Ar-H), 6.97 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.84 (d, 1H, *J* = 8.0 Hz, Ar-H), 6.02 (s, 2H, OCH₂O), 3.88 (s, 3H, CH₃O)

PHARMACOLOGICAL/BIOLOGICAL ASSAYS

AChE inhibition study

The effects of compounds on AChE enzyme were investigated. Two substrates which are 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylcholiniodate (AChI) were used in the inhibition tests. In addition, Tris / HCl (100 ml, 1M, pH = 8) buffer was used in this method for AChE enzyme. The final volume was completed to 100 μL and changes in the absorption were followed at 412 nm. The IC₅₀ values of the compounds were calculated via activity (%) versus compounds concentration plots (Tugrak et al., 2021c, Yurt et al., 2024; El-Sayed et al., 2019).

hCA Inhibition studies

Human CA isoforms (hCA I and hCA II) were isolated using the sepharose - 4B-L- tyrosine sulfanilamide affinity segregation process, as previously reported. Protein concentrations were measured using the Bradford method at 595 nm. The compounds' inhibitory effects were studied by measuring esterase activity in accordance with Verpoorte et al., as described in prior investigations. The hCA activity was evaluated by monitoring the conversion of the p-nitrophenyl acetate substrate to p-nitrophenolate at 348 nm using a spectrophotometer (UV-VIS Spectrophotometer, UV mini-1240, Shimadzu Corporation). Acetazolamide (AZA) was utilized as the control medication. (Kocyigit et al., 2017; Bradford, 1976; Verpoorte et al., 1967; Guney et al., 2014; Tugrak et al., 2020; Tugrak et al., 2021a; Tugrak et al., 2021b).

Computation Method

In this study, *in silico* docking analyses were performed using the AutoDock Vina (Trott et & Olson, 2010) program. The molecular structures (1-21) were optimized in their ground state and gas phase employing Density Functional Theory (DFT) with the B3LYP functional and the 6-311++G(d,p) basis set, utilizing the Gaussian 09W software package (Frisch et al., 2009). Three-dimensional representations of the optimized structures were generated using the GaussView program (Dennington et al., 2009), without imposing symmetry constraints.

RESULTS and DISCUSSION

Chemistry

Chalcones **1-21** were synthesized by reacting suitable aldehyde with 4-methoxyacetophenone under alkaline conditions at room temperature *via* Claisen-Schmidt Condensation (Scheme 1 and Figure 2). The progress of the

reaction was monitored by TLC. After the reaction, the mixture was cooled in ice water and acidified with 10% HCl until pH = 7. The crystals were obtained by crystallization in an ethanol-water mixture. Confirmation of compound structures was done via melting points and ^1H NMR spectra. Experimental part details the chalcones' (1-21) characterization by ^1H NMR. Chalcones are open chain flavonoids. Chemically they consist of α , β -unsaturated carbonyl system, and two aromatic rings. They are available in both cis (*Z*) and trans (*E*) forms. In this work, the compounds were seen as E isomer on the basis of coupling constants. Olefinic carbon-carbon bond of each chalcone was established by their large trans coupling constants (*J*) in the range of 15-16 Hz in the ^1H NMR spectra.

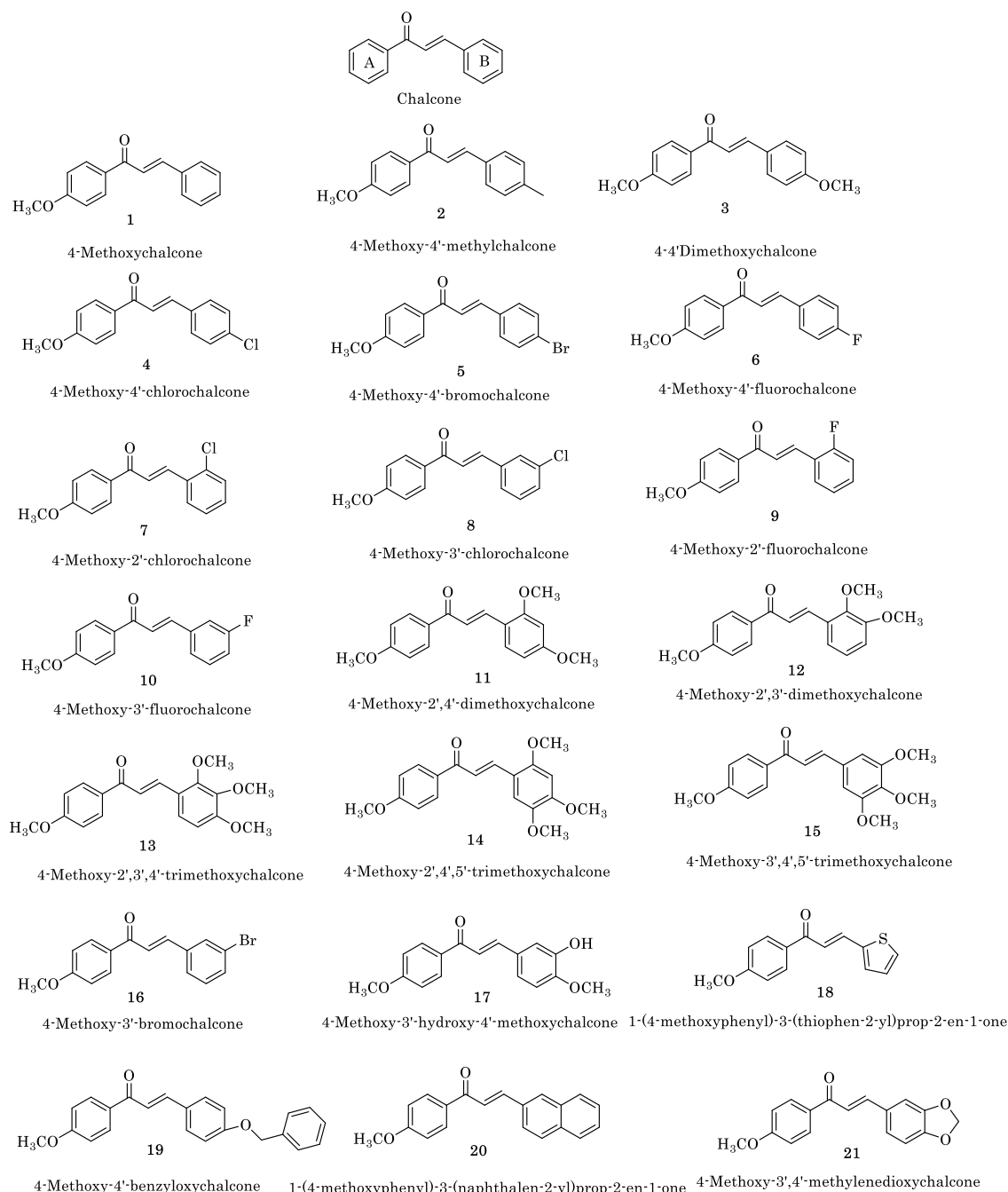


Figure 2 Chemical structure of chalcones used in this study.

Şekil 2 Bu çalışmada kullanılan şalkonların kimyasal yapısı

Bioactivity results

The inhibitory effects of 4-methoxychalcone derivatives on hCA I, hCA II, and AChE were investigated in this study. The IC_{50} values of the chalcones were calculated using Activity (%) - [Chalcone]. The inhibitory data are compiled in Table 1, alongside those pertaining to acetazolamide (AZA), utilized as a standard inhibitor for both hCA isoenzymes. To assess the impact of chalcone derivatives (1-21) on the specified metabolic enzymes, the

subsequent results have been presented:

(i) Physiologically significant hCA I is predominantly present in erythrocytes and is also expressed in normal colonic mucosa (Mikus et al., 2018). Regarding CA I, all chalcone derivatives (**1-21**) exhibited IC_{50} values in the low nanomolar range, specifically between 2.65 μ M and 82.33 μ M. Among the synthesized chalcone derivatives (**1-21**), chalcone **5**, with a bromo group in the meta position, exhibited the highest inhibition (IC_{50} : 2.65 μ M), followed by chalcone **4** (IC_{50} : 3.08 μ M) in comparison to AZA (Table 1). AZA, serving as a positive control and utilized as a therapeutic medication, exhibited a IC_{50} value of 46.75 μ M. The analysis of inhibitory results indicated that halogenated compounds exhibited greater inhibition. All chalcones exhibited superior hCA I inhibition compared to AZA, with the exception of compounds **17** (IC_{50} : 82.33), **18** (IC_{50} : 77.91), **19** (IC_{50} : 56.62), and **21** (IC_{50} : 48.43).

(ii) The chalcones **1-21** demonstrated IC_{50} values ranging from 2.63 to 74.89 μ M against the predominant cytosolic hCA II isoform (Table 1). Many chalcones were found to have strong inhibitory effects on hCA II isoenzyme. AZA also revealed an IC_{50} of 38.25 μ M against the hCA II isoenzyme. Based on the IC_{50} values, compound **13** exhibited a potency 14.5 times greater than AZA against hCA II. Additionally, compound **20** may be regarded as the second most powerful drug, exhibiting an IC_{50} value of 6.32 μ M against hCA II, as indicated in Table 1. The IC_{50} data indicated that lead compound **13** inhibited hCA II with 7.2 times more selectivity than hCA I.

Table 1 The enzyme inhibition parameters of the compounds 1-21 against carbonic anhydrase isoenzyme I and II (CA I, II), acetylcholinesterase (AChE) enzyme

Çizelge 1 Bileşik 1-21'in karbonik anhidraz izoenzim I ve II (CA I, II), asetilkolinesteraz (AChE) enzimine karşı enzim inhibisyon parametreleri

Compound No	CA-I (IC_{50} , μ M)	r^2	CA-II (IC_{50} , μ M)	r^2	AChE (IC_{50} , μ M)	r^2
1	24.0	0.9522	48.15	0.8423	177.46	0.871
2	28.73	0.94	48.02	0.872	53.72	0.8901
3	6.90	0.9377	64.23	0.8518	18.0	0.9301
4	3.08	0.8704	30.83	0.9899	52.46	0.9158
5	2.65	0.9614	21.98	0.865	15.53	0.9932
6	11.74	0.9925	29.61	0.8914	80.97	0.9144
7	15.10	0.9927	30.97	0.8545	53.47	0.9508
8	12.0	0.9502	26.79	0.8832	70.41	0.9752
9	33.13	0.8865	16.62	0.861	89.95	0.9504
10	9.44	0.8894	48.02	0.8933	81.18	0.8936
11	18.67	0.874	9.37	0.979	143.0	0.8584
12	21.18	0.9164	22.11	0.9577	96.62	0.9829
13	18.99	0.8133	2.63	0.974	135.19	0.8858
14	32.69	0.916	52.69	0.8989	160.27	0.8506
15	36.0	0.9556	51.41	0.8415	143.98	0.8804
16	31.85	0.8948	43.69	0.9825	62.88	0.8891
17	82.33	0.9135	74.89	0.9163	61.44	0.9863
18	77.91	0.8144	39.58	0.9945	94.21	0.8325
19	56.62	0.8451	9.38	0.9283	98.73	0.9505
20	24.63	0.86	6.32	0.8576	69.94	0.9759
21	48.43	0.8998	16.58	0.9219	94.81	0.967
AZA	46.75	0.955	38.25	0.833	-	-
Tacrine	-	-	-	-	25.78	0.989

*TAC: nm

(iii) The AChE inhibitory effects of the synthesized compounds were assessed using Ellman's technique, consistent with prior research employing the reference drug tacrine (Ellman et al., 1961). Tacrine, the first AChE inhibitor used in clinical trials, was withdrawn from the market due to hepatotoxicity. Even while drugs like donepezil, galantamine, and rivastigmine are available, their efficacy remains poor. As a result, research into new anti-AD drug possibilities remains popular. Table 1 indicates that the chemicals evaluated exhibited IC_{50} values ranging from 15.53 to 177.46 μ M against the AChE enzyme. Compounds **3** and **5** exhibited significant inhibitory effects with low IC_{50} values of 18.0 μ M and 15.53 μ M, respectively. Considering the IC_{50} value, compound **5** was deemed the most effective acetylcholinesterase inhibitor in the series.

Molecular Docking

Molecular docking is a computational technique used to predict the interaction between a ligand and a receptor, often within the context of drug discovery. This approach is frequently used in drug design and discovery because it helps scientists find possible therapeutic candidates by modeling molecular interactions and forecasting how well a drug might attach to its target. In order to further the development of therapeutic drugs, scientists can optimize the design of novel compounds with enhanced efficacy, selectivity, and decreased adverse effects by examining these interactions (Karagac et al., 2024; Demir Y, 2020). It involves simulating how a small molecule, such as a drug candidate, fits into the active site of a target protein, evaluating binding affinity and potential interactions. By predicting how well the molecule binds and interacts with the protein, docking helps in identifying promising drug candidates before experimental testing (Erdogan et al., 2024; Demir et al., 2024). The process typically employs algorithms to explore the conformational space of both the ligand and the receptor, aiming to identify the most energetically favorable binding pose. Scoring functions are integral to docking, quantifying the strength of interactions such as hydrogen bonding, hydrophobic effects, and electrostatic forces. Advances in molecular docking have incorporated machine learning to improve accuracy, though challenges such as receptor flexibility and water-mediated interactions remain significant. This method continues to play a crucial role in streamlining the drug discovery process, reducing the cost and time associated with experimental screening (Taylor et al., 2002; Ferreira et al., 2015; Meng et al., 2011). At this stage of the study, the molecular docking or interactions of **1-21** molecules with hCA I (PDB: 2CAB), hCA II (PDB: 5AML), and AChE (PDB: 1EVE) targets were examined with the help of Autodock Vina (Trott et & Olson, 2010), a free program that allows its users. First of all, molecules **1-21** were quantitatively optimized using DFT/B3LYP theory/functional and 6-311++G (d,p) basis set with the help of Gaussian 09W package program (Frisch et al., 2009) and Gauss View 5.0 interface (Dennington et al., 2009) programs. Based on both experimental and theoretical results, the three-dimensional optimization structures of only compounds **5** and **13** were given in Figure 3. The sum of electronic and zero-point energies for these compounds were calculated as -3325.54720857 and -1106.02428896 a.u, respectively.

The ligands (**1-21**) were subsequently converted into PDB and PDBQT formats, with all ligand-related processes conducted using the Discovery Studio Visualizer 4.0 (DSV 4.0) software (Scientific Software). Based on experimental protocols and relevant literature, the target proteins selected for this study were hCA I (PDB ID: 2CAB) (Kannan et al., 1984; Mahmudov et al., 2022), hCA II (PDB ID: 5AML) (Mahmudov et al., 2022; Ivanova et al., 2015), and AChE (PDB ID: 1EVE) (Mahmudov et al., 2022; Kryger et al., 1999). The three-dimensional PDB structures of these receptors were obtained from the RCSB Protein Data Bank (PDB) (Protein Data Bank). Similarly, heteroatoms present in the three target proteins were removed using Discovery Studio Visualizer 4.0 (DSV 4.0) (Scientific Software). Polar hydrogen atoms and their corresponding charges were subsequently added, and the processed data were saved again in PDB and PDBQT formats. To enhance the precision of the docking analysis, the active sites and key residues of the target proteins were identified as follows: HIS119, HIS96, and HIS94 for hCA I (PDB ID: 2CAB); THR200, THR199, LEU198, PHE131, VAL121, HIS119, HIS96, HIS94, GLN92, ASN67, and ASN62 for hCA II (PDB ID: 5AML); and HIS440, PHE330, GLU327, TRP279, SER200, and TRP84 for AChE (PDB ID: 1EVE) (Mert et al., 2025). To incorporate these active zones, the following grid parameters were developed: 40 x 40 x 40 Å³ x, y, z dimensions, 0.375 Å space and 39.041, - 10.534, 10.394 x, y, z centers for hCA I / PDB: 2CAB; 32 x 40 x 32 Å³ x, y, z dimensions, 0.375 Å space and -5.915, 5.359, 16.391 x, y, z centers for hCA II / PDB: 5AML; 44 x 44 x 38 Å³ x, y, z dimensions, 0.375 Å space and -0.39, 63.448, 67.71 x, y, z centers for AchE / PDB: 1EVE (Mert et al., 2025). Among the tested ligands, compound **5** demonstrated the strongest binding affinity to hCA I (-8.0 kcal mol⁻¹) and AChE (-7.0 kcal mol⁻¹), while compound **13** exhibited the highest binding affinity to hCA II (-8.1 kcal mol⁻¹). The binding modes and interactions of these compounds were visualized to elucidate key stabilizing interactions such as hydrogen bonds and hydrophobic effects. Detailed molecular docking results for all compounds are presented in Table 2, highlighting the varying affinities across the three targets. The findings reveal that the compounds exhibit diverse binding profiles, with compounds **5** and **13** emerging as potent inhibitors, hence the interactions between these molecules and their respective proteins were given in Figures. 4-6. Figure 4 illustrates the docking pose and molecular interactions of ligand **5** with human carbonic anhydrase isozyme I (hCA I). Panel (a) provides a three-dimensional visualization of ligand **5** within the active site of hCA I, highlighting its strategic positioning to interact with critical residues. Panel (b) offers a detailed two-dimensional interaction map, showcasing the specific types of interactions. Ligand **5** forms hydrogen bonds with GLN92 and HIS119, which play a pivotal role in stabilizing the ligand-protein complex.

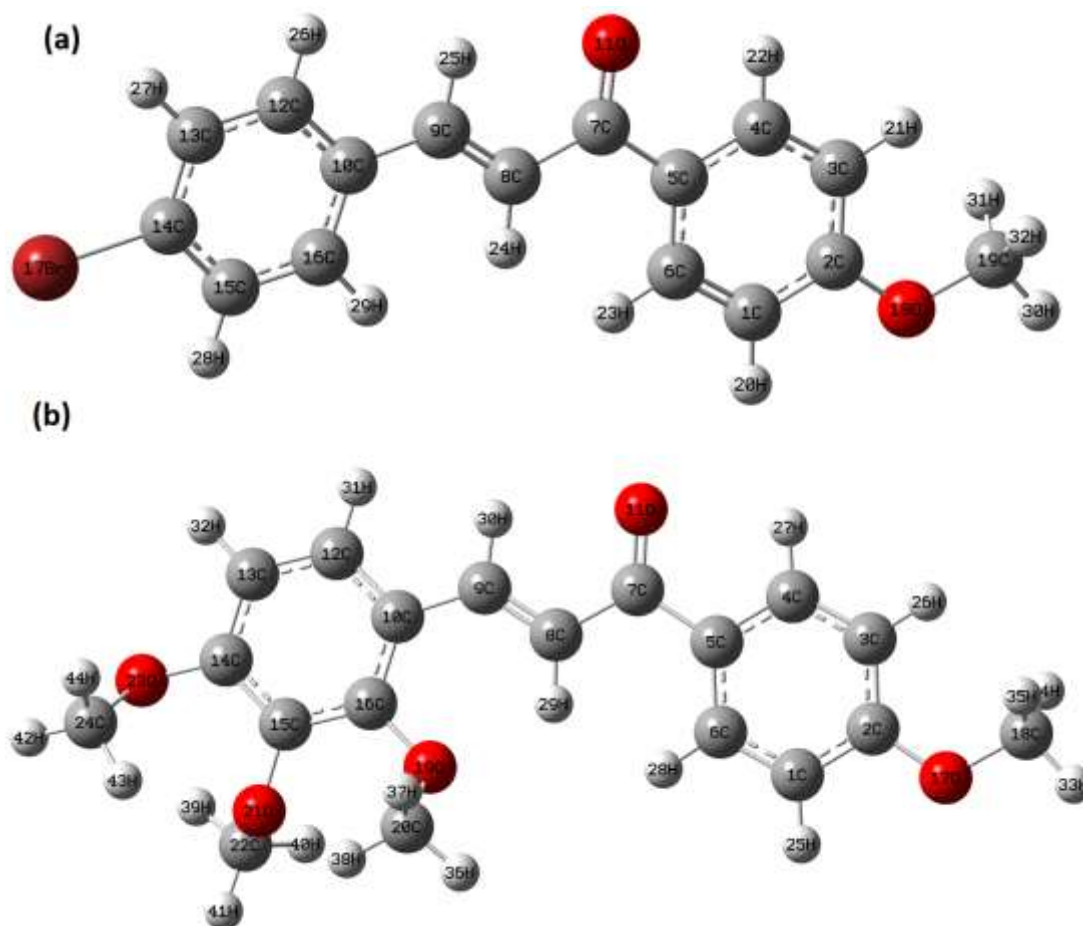


Figure 3 The optimized structures of **5** (a) and **13** (b) molecules.
Şekil 3 5 (a) ve 13 (b) moleküllerinin optimize edilmiş yapıları.

Additionally, Pi - Pi stacking interactions with HIS94 and HIS200, and alkyl interactions with LEU198, contribute to the compound's binding stability. The close proximity of ligand **5** to the catalytic zinc ion suggests potential competitive inhibition, rendering it a potent inhibitor of hCA I activity. This comprehensive interaction profile underscores ligand **5**'s high binding affinity ($-8.0 \text{ kcal mol}^{-1}$) as reported in the docking analysis, confirming its potential as a selective inhibitor. Figure 5 demonstrates the binding interaction of ligand **13** within the active site of hCA II. In panel (a), the three-dimensional docking visualization highlights the ligand's orientation relative to the catalytic pocket. Panel (b) elaborates on the molecular interactions, showing critical hydrogen bonds with residues such as GLN92 and THR200. These interactions are complemented by carbon hydrogen bonds with HIS94 and van der Waals interactions with VAL121 and LEU198, ensuring ligand stability within the active site. π -donor hydrogen bonding and π -Sigma interactions with THR200 further enhance binding specificity. The reported binding affinity of ligand **13** ($-8.1 \text{ kcal mol}^{-1}$) aligns with these interactions, confirming its superior inhibitory potential against hCAII compared to other compounds in the study. The high specificity and stability of ligand **13** make it a promising candidate for targeting hCA II-related pathologies. Figure 6 presents the molecular docking results of ligand **5** with acetylcholinesterase (AChE). Panel (a) shows the ligand's three-dimensional positioning within the enzyme's active site, while panel (b) provides a detailed interaction map. Ligand **5** forms π - π stacking interactions with TRP84 and PHE330, which are critical for substrate recognition and inhibition. Additional hydrogen bonds with HIS440 and van der Waals interactions with GLU199 and ILE444 further stabilize the ligand within the catalytic pocket. The compound also engages in alkyl interactions with TYR334, contributing to its binding affinity. The docking score of ligands **5** ($-7.0 \text{ kcal mol}^{-1}$) reflects these diverse and strong interactions, demonstrating its potential as an effective AChE inhibitor. These results support the hypothesis that ligand **5** may act as a competitive inhibitor, interfering with substrate binding and enzymatic activity.

Table 2 Molecular docking scores of **1-21** compounds.

Çizelge 2 1-21 bileşiklerin moleküler yerleştirme puanları.

Compounds	hCA I / PDB: 2CAB + ligands Affinity (kcal mol ⁻¹)	hCA II / PDB: 5AML + ligands Affinity (kcal mol ⁻¹)	AChE / PDB: 1EVE + ligands Affinity (kcal mol ⁻¹)
1	-6.8	-6.6	-6.9
2	-7.0	-6.8	-6.7
3	-6.7	-6.8	-6.6
4	-6.4	-6.5	-6.7
5	Ki =1.36759 µM H. B = 1	-6.6	Ki =7.39542 µM H. B =0
6	-7.3	-6.5	-6.5
7	-6.7	-6.8	-6.5
8	-6.9	-6.9	-6.4
9	-7.0	-6.8	-5.9
10	-6.8	-7.0	-6.8
11	-6.2	-6.8	-6.9
12	-7.0	-7.3	-6.9
13	-6.6	-8.1	-6.9
14	-5.9	Ki =1.1552 µM H. B = 1	-6.0
15	-6.1	-6.7	-6.4
16	-7.0	-6.1	-6.9
17	-6.4	-7.1	-6.3
18	-6.4	-6.5	-5.8
19	-6.1	-6.4	-6.9
20	-6.7	-7.4	-6.8
21	-7.9	-7.9	-6.8
21	-7.9	-7.7	-6.9

Ki: Inhibition constant

H. B: Hydrogen Bonding

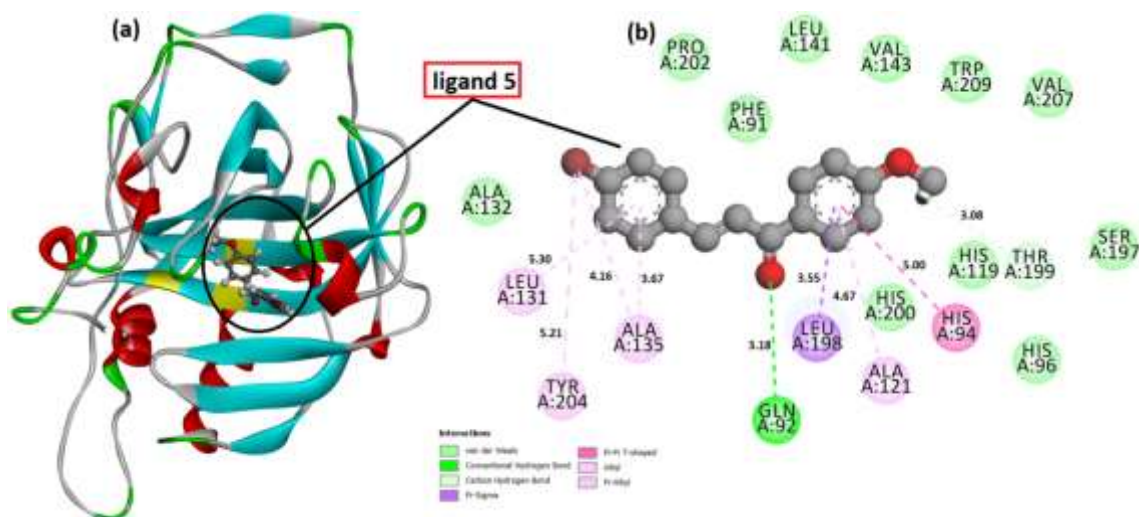


Figure 4 The localization of the molecule to the active site of the protein 2CAB (a) and 2D (b) molecular docking results for compound **5** and PDB: 2CAB.

Şekil 4 Molekülün protein 2CAB'nin aktif bölgesine lokalizasyonu (a) ve 2D (b) bileşik 5 ve PDB:2CAB için moleküler yerleştirme sonuçları

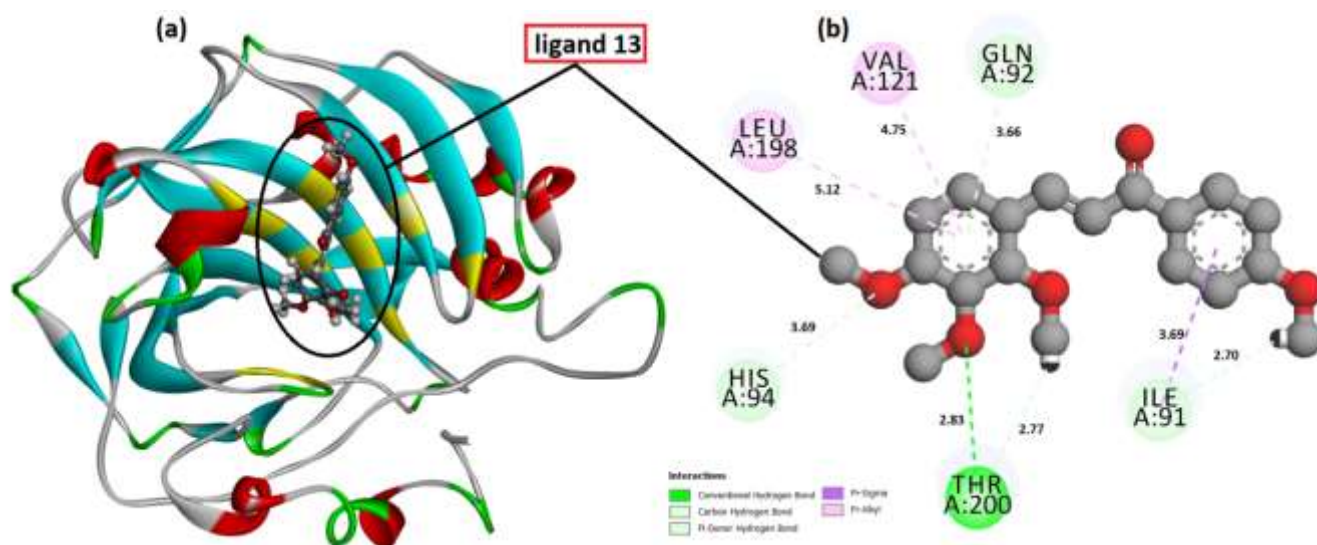


Figure 5 The localization of the molecule to the active site of the protein 5AML (a) and 2D (b) molecular docking results for compound 13 and PDB: 5AML.

Şekil 5 Molekülün protein 5AML'nin aktif bölgesine lokalizasyonu (a) ve 2D (b) bileşik 13 ve PDB: 5AML için moleküler yerleştirme sonuçları

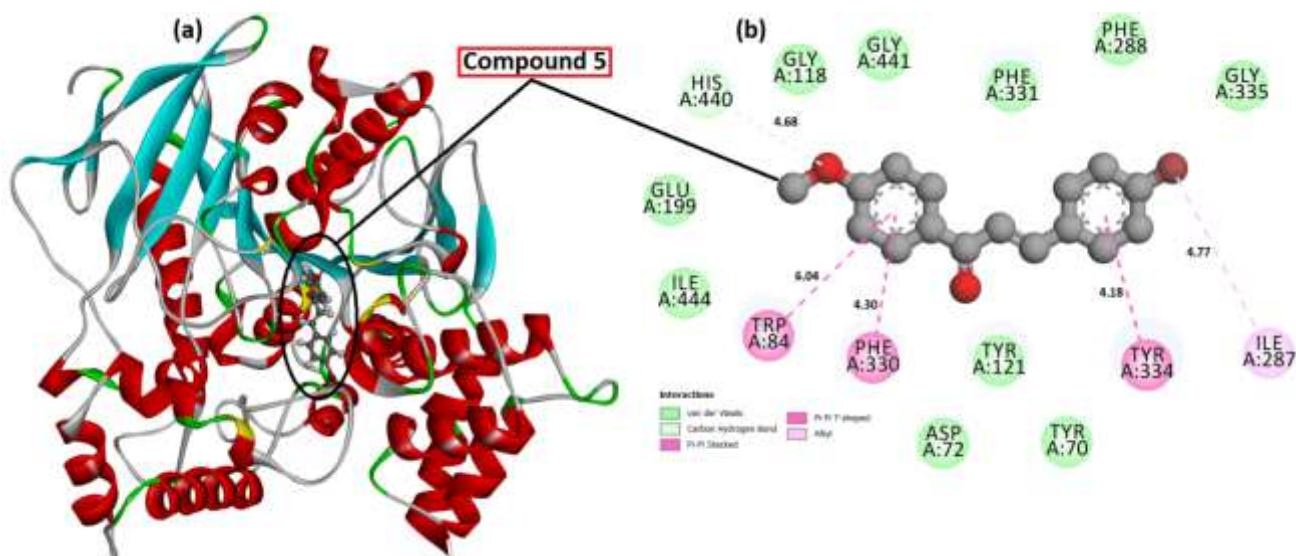


Figure 6 The localization of the molecule to the active site of the protein 1EVE (a) and 2D (b) molecular docking results for compound 5 and PDB: 1EVE.

Şekil 6 Molekülün protein 1EVE'nin aktif bölgesine lokalizasyonu (a) ve 2D (b), bileşik 5 ve PDB: 1EVE için moleküler yerleştirme sonuçları

CONCLUSION

In conclusion, chalcone derivatives were successfully produced with high yields and they were evaluated for their inhibitory potentials on the hCA I, hCA II, and AChE enzymes. The computational evaluation of current enzyme inhibitors and molecular docking analysis was also performed. According to the IC₅₀ values, compound 5 and compound 13 were 17.6 and 14.5 times more potent than the reference acetazolamide against hCA I and hCA II, respectively. Compound 5 significantly inhibited AChE at micromolar doses. The inhibitory potential of the newly synthesized compounds (1-21) against hCA I (PDB: 2CAB), hCA II (PDB: 5AML), and AChE (PDB: 1EVE) was

evaluated through *in silico* molecular docking simulations utilizing the AutoDock Vina platform. Compound **5** showed the highest docking score for hCA I ($-8.0 \text{ kcal mol}^{-1}$) and AChE ($-7.0 \text{ kcal mol}^{-1}$), indicating a favorable interaction with key catalytic residues such as HIS119, HIS94, and HIS96 in hCA I, and TRP84 and PHE330 in AChE. Compound **13** displayed the best docking score for hCA II ($-8.1 \text{ kcal mol}^{-1}$), interacting with critical residues GLN92, THR200, and HIS94, suggesting a strong stabilizing effect within the enzyme's active site. These results demonstrate that the halogen and methoxy substituents in compounds **5** and **13** likely contribute to their enhanced enzyme-ligand interactions, hydrogen bonding potential, and overall stability within the binding pocket. Additionally, the agreement between computational predictions and experimental IC₅₀ values highlights the reliability of molecular docking as a predictive tool. A strong concordance between the computational predictions and experimental data was observed, underscoring the reliability of the applied approach. These findings indicate that these compounds, alongside existing market agents, hold significant promise as inhibitors targeting AChE, hCA I, and hCA II proteins. Overall, prospective investigations have identified possible compounds **5** and **13** for CAs and compound **5** for AChE. Compounds **5** and **13** can be considered as remarkable candidates for further studies. In other words, given their potent inhibition profiles, compounds **5** and **13** are strong candidates for further optimization and preclinical evaluation in the development of novel inhibitors targeting carbonic anhydrase and acetylcholinesterase-related disorders. At the same time, compounds synthesized by molecular modifications can be supported by *in vivo* studies in future studies.

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Contribution Rate Statement Summary of Researchers

Mehtap Tugrak Sakarya, investigation, methodology, writing – original draft, review & editing, Halise Inci Gul and Mustafa Gul, review & editing, supervision, Yusuf Sert, carried out the molecular docking studies, Hulya Akıncioglu and İlhami Gulcin were conducted biological activity tests. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

This article to be published does not require ethics committee approval and/or legal/special permission.

REFERENCES

- Abdel-Halim, M., Keeton, A. B., Gurpinar, E., Gary, B. D., Vogel, S. M., Engel, M., & Abadi, A. H. (2013). Trisubstituted and tetrasubstituted pyrazolines as a novel class of cell-growth inhibitors in tumor cells with wild type p53. *Bioorganic & Medicinal Chemistry*, 21(23), 7343-7356.
- Bale, A. T., Khan, K. M., Salar, U., Chigurupati, S., Fasina, T., Ali, F., & Perveen, S. (2018). Chalcones and -chalcones: As potential α -amylase inhibitors; synthesis, screening, and molecular modelling studies. *Bioorganic Chemistry*, 79, 179-189.
- Bandgar, B. P., Gawande, S. S., Bodade, R. G., Totre, J. V., & Khobragade, C. N. (2010). Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. *Bioorganic & Medicinal Chemistry*, 18(3), 1364-1370.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
- Choi, J. W., Jang, B. K., Cho, N. C., Park, J. H., Yeon, S. K., Ju, E. J., & Park, K. D. (2015). Synthesis of a series of unsaturated ketone derivatives as selective and reversible monoamine oxidase inhibitors. *Bioorganic & Medicinal Chemistry*, 23(19), 6486-6496.
- Demir, Y. (2020). Naphthoquinones, benzoquinones, and anthraquinones: Molecular docking, ADME and inhibition studies on human serum paraoxonase-1 associated with cardiovascular diseases. *Drug Development Research*, 81(5), 628-636.
- Demir, Y., Ozturk N., Isiyel, M., Ceylan, H. (2024). Effects of Carnosic and Usnic Acid on Pentose Phosphate Pathway Enzymes: An Experimental and Molecular Docking Study. *ChemistrySelect*, 9, e202401067
- Dennington, R., Keith, T., Millam, J. (2009). GaussView, version 5, Semichem Inc.: Shawnee Mission, KS.
- Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharma*, 7(2), 88-90.

- El-Sayed, N. A., Farag, A. E., Ezzat, M. A. F., Akincioglu, H., Gulcin, I., Abou-Seri, S. M. (2019). Design, synthesis, in vitro and in vivo evaluation of novel pyrrolizine-based compounds with potential activity as cholinesterase inhibitors and anti-Alzheimer's agents. *Bioorg. Chem*, 93, 103312
- Erdogan, M., Onder, A., Demir Y., Onder, F. C. (2024). Novel Dibenzoazepine-Substituted Triazole Hybrids as Cholinesterase and Carbonic Anhydrase Inhibitors and Anticancer Agents: Synthesis, Characterization, Biological Evaluation, and In Silico Studies. *ACS Omega*, 9, 46860–46878.
- Ferreira, L. G., Dos Santos, R. N., Oliva, G., Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies, *Molecules*, 20(7), 13384-13421.
- Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., & Intl, A. D. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet*, 366(9503), 2112-2117.
- Frisch, M.J., Trucks, G., Schlegel, H., Scuseria, G., Robb, M., Cheeseman, J., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. (2009). Gaussian 09, Revision D. 01, Gaussian, Inc.: Wallingford, CT.
- Galvin, J. E., Powlishta, K. K., Wilkins, K., McKeel, D. W., Xiong, C. J., Grant, E., & Morris, J. C. (2005). Predictors of preclinical Alzheimer disease and dementia - A clinicopathologic study. *Archives of Neurology*, 62(5), 758-765.
- Guney, M., Coskun, A., Topal, F., Dastan, A., Gulcin, I., Supuran, C.T. (2014). Oxidation of cyanobenzocycloheptatrienes: Synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives. *Bioorganic & Medicinal Chemistry*, 22(13), 3537-3543.
- Ivanova, J., Leitans, J., Tanc, M., Kazaks, A., Zalubovskis, R., Supuran, C. T., Tars, K. (2015). X-ray crystallography-promoted drug design of carbonic anhydrase inhibitors. *Chemical Communications*, 51(33), 7108-7111.
- Kannan, K., Ramanadham, M., Jones, T. (1984). Structure, refinement, and function of carbonic anhydrase isozymes: refinement of human carbonic anhydrase I. *Annals of the New York Academy of Sciences*, 429(1), 49-60.
- Karagac, S. M., Yesilkent, E. N., Kizir, D., Ozturk, N., Isiyel, M., Karadas, H., Tosun, H., Karaman, M., Ceylan, H., Demir, Y. (2024). Esculetin improves inflammation of the kidney via gene expression against doxorubicin-induced nephrotoxicity in rats: *In vivo* and *in silico* studies. *Food Bioscience*, 62, 105159.
- Kazi, I., Guha, S., & Sekar, G. (2017). CBr as a halogen bond donor catalyst for the selective activation of benzaldehydes to synthesize α,β -unsaturated ketones. *Organic Letters*, 19(5), 1244-1247.
- Knopman, D. S., Amieva, H., Petersen, R. C., Chételat, G., Holtzman, D. M., Hyman, B. T., & Jones, D. T. (2021). Alzheimer disease. *Nature Reviews Disease Primers*, 7(33), 1-21.
- Kocuyigit, U. M., Budak, Y., Gurdere, M. B., et al. (2017). Synthesis, characterization, anticancer, antimicrobial and carbonic anhydrase inhibition profiles of novel (3aR,4S,7R,7aS)-2-(4-((E)-3-(3-aryl)acryloyl) phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisindole-1,3(2H)-dione derivatives. *Bioorganic Chemistry*, 70, 118-125.
- Kryger, G., Silman, I., Sussman, J. L. (1999). Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. *Structure*, 7(3), 297-307.
- Kovar, S. E., Fourman, C., Kinstedt, C., Williams, B., Morris, C., Cho, K. J., & Ketcha, D. M. (2020). Chalcones bearing a 3,4,5-trimethoxyphenyl motif are capable of selectively inhibiting oncogenic K-Ras signaling. *Bioorganic & Medicinal Chemistry Letters*, 30(11), 127144.
- Kumar, R., Sharma, P., Shard, A., Tewary, D. K., Nadda, G., & Sinha, A. K. (2012). Chalcones as promising pesticidal agents against diamondback moth (*Plutella xylostella*): microwave-assisted synthesis and structure-activity relationship. *Medicinal Chemistry Research*, 21(6), 922-931.
- Le Duc, Y., Licsandru, E., Vullo, D., Barboiu, M., & Supuran, C. T. (2017). Carbonic anhydrases activation with 3-amino-1H-1,2,4-triazole-1-carboxamides: Discovery of subnanomolar isoform II activators. *Bioorganic & Medicinal Chemistry*, 25(5), 1681-1686.
- Mahmudov, I., Demir, Y., Sert, Y., Abdullayev, Y., Sujayev, A., Alwasel, S. H., Gulcin, I. (2022). Synthesis and inhibition profiles of N-benzyl-and N-allyl aniline derivatives against carbonic anhydrase and acetylcholinesterase—A molecular docking study. *Arabian Journal of Chemistry*, 15(3), 103645
- Mai, C. W., Yaeghoobi, M., Abd-Rahman, N., Kang, Y. B., & Pichika, M. R. (2014). Chalcones with electron-withdrawing and electron-donating substituents: Anticancer activity against TRAIL resistant cancer cells, structure-activity relationship analysis and regulation of apoptotic proteins. *European Journal of Medicinal Chemistry*, 77, 378-387.
- Marucci, G., Buccioni, M., Dal Ben, D., Lambertucci, C., Volpini, R., & Amenta, F. (2021). Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology*, 1(190), 108352.
- Mazumder, R., Ichudaule, Ghosh, A., Deb, S., & Ghosh, R. (2024). Significance of chalcone scaffolds in medicinal chemistry. *Topics in Current Chemistry*, 382(3), 22.

- Meng, X. Y., Zhang, H. X., Mezei, M., Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-aided Drug Design*, 7(2), 146-157.
- Mert, S., Demir, Y., Sert, Y., Kasımoğulları, R., Gulcin I. (2025). Synthesis, biological evaluation and molecular docking of novel pyrazole derivatives as multitarget acetylcholinesterase and carbonic anhydrase inhibitors. *Journal of Molecular Structure*, 1319, 139472.
- Mikus, P., Krajciová, D., Mikulová, M., Horváth, B., Pecher, D., Garaj, V., & Supuran, C. T. (2018). Novel sulfonamides incorporating 1,3,5-triazine and amino acid structural motifs as inhibitors of the physiological carbonic anhydrase isozymes I, II and IV and tumor-associated isozyme IX. *Bioorganic Chemistry*, 81, 241-252.
- Ouyang, Y., Li, J. J., Chen, X. Y., Fu, X. Y., Sun, S., & Wu, Q. (2021). Chalcone Derivatives: Role in Anticancer Therapy. *Biomolecules*, 11(6), 894.
- Patil, P. S., Dharmaprakash, S. M., Ramakrishna, K., Fun, H. K., Kumar, R. S. S., & Rao, D. N. (2007). Second harmonic generation and crystal growth of new chalcone derivatives. *Journal of Crystal Growth*, 303(2), 520-524.
- Petersen, R. C., Parisi, J. E., Dickson, D. W., Johnson, K. A., Knopman, D. S., Boeve, B. F., & Kokmen, E. (2006). Neuropathologic features of amnesic mild cognitive impairment. *Archives of Neurology*, 63(5), 665-672.
- Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild cognitive impairment - Clinical characterization and outcome. *Archives of Neurology*, 56(3), 303-308.
- Poyraz, S., Döndas, H. A., Yamali, C., Belveren, S., Demir, Y., Aydinoglu, S., & Sansano, J. M. (2024). Design, synthesis, biological evaluation and docking analysis of pyrrolidine-benzenesulfonamides as carbonic anhydrase or acetylcholinesterase inhibitors and antimicrobial agents. *Journal of Biomolecular Structure & Dynamics*, 42(7), 3441-3458.
- Protein data Bank. <https://www.rcsb.org/>.
- Raghavan, S., Manogaran, P., Kuppuswami, B. K., Venkatraman, G., & Narasimha, K. K. G. (2015). Synthesis and anticancer activity of chalcones derived from vanillin and isovanillin. *Medicinal Chemistry Research*, 24(12), 4157-4165.
- Roman, B. I., De Ryck, T., Patronov, A., Slavov, S. H., Vanhoecke, B. W. A., Katritzky, A. R., & Stevens, C. V. (2015). 4-Fluoro-3',4',5'-trimethoxychalcone as a new anti-invasive agent. From discovery to initial validation in an metastasis model. *European Journal of Medicinal Chemistry*, 101, 627-639.
- Russell, A. (1934). The constitution of tannins. Part I. Reduction products of chalkones and the synthesis of a typical phlobatannin. 218-221.
- Scientific Software <http://www.3dsbiovia.com/>.
- Schultze, S., & Grubmüller, H. (2021). Time-lagged independent component analysis of random walks and protein dynamics. *Journal of Chemical Theory and Computation*, 17(9), 5766-5776.
- Schwartz, M. A., Rose, B. F., Holton, R. A., Scott, S. W., & Baburao, V. (1977). Intramolecular oxidative coupling of diphenolic, monophenolic, and nonphenolic substrates. *Journal of the American Chemical Society*, 99(8), 2571-2577.
- Shibata, K., Katsuyama, I., Matsui, M., & Muramatsu, H. (1991). Synthesis of 3-cyano-2-methylpyridines substituted with heteroaromatics. *Journal of Heterocyclic Chemistry*, 28(1), 161-165.
- Shinichi, I., Minako, S., & Ito, K. (1990). Polymer-supported poly(amino acids) as new asymmetric epoxidation catalyst of α -unsaturated ketones. *Journal of Organic Chemistry*, 55(24), 6047-6049.
- Supuran, C. T. (2008). Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nature Reviews: Drug Discovery*, 7(2), 168-181.
- Taylor, R. D., Jewsbury, P. J., Essex, J. W. (2002). A review of protein-small molecule docking methods. *Journal of Computer-aided Molecular Design*, 16, 151-166.
- Temperini, C., Scozzafava, A., & Supuran, C. T. (2008). Carbonic anhydrase activation and the drug design. *Current Pharmaceutical Design*, 14(7), 708-715.
- Tierney, M. C., Szalai, J. P., Snow, W. G., Fisher, R. H., Nores, A., Nadon, G., & StGeorgeHyslop, P. H. (1996). Prediction of probable Alzheimer's disease in memory-impaired patients: A prospective longitudinal study. *Neurology*, 46(3), 661-665.
- Trott, O., Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455-461.
- Tugrak, M., Gul, H. I., Akincioglu, H., & Gulcin, I. (2021a). New chalcone derivatives with pyrazole and sulfonamide pharmacophores as carbonic anhydrase inhibitors. *Letters in Drug Design and Discovery*, 18(2), 191-198.
- Tugrak, M., Gul, H. I., Anil, B., & Gülçin, I. (2020). Synthesis and pharmacological effects of novel benzenesulfonamides carrying benzamide moiety as carbonic anhydrase and acetylcholinesterase inhibitors. *Turkish Journal of Chemistry*, 44(6), 1601.

- Tugrak, M., Gul, H. I., Demir, Y., & Gulcin, I. (2021b). Synthesis of benzamide derivatives with thiourea-substituted benzenesulfonamides as carbonic anhydrase inhibitors. *Archiv Der Pharmazie*, 354(2).
- Tugrak, M., Gul, H. I., Demir, Y., Levent, S., & Gulcin, I. (2021c). Synthesis and in vitro carbonic anhydrases and acetylcholinesterase inhibitory activities of novel imidazolinone-based benzenesulfonamides. *Archiv Der Pharmazie*, 354(4).
- Tugrak, M., Gul, H. I., Sakagami, H., Gulcin, I., & Supuran, C. T. (2018). New azafluorenones with cytotoxic and carbonic anhydrase inhibitory properties: 2-Aryl-4-(4-hydroxyphenyl)-5-indeno[1,2-]pyridin-5-ones. *Bioorganic Chemistry*, 81, 433-439.
- Tugrak, M., Gul, H. I., Sakagami, H., & Mete, E. (2017). Synthesis and anticancer properties of mono Mannich bases containing vanillin moiety. *Medicinal Chemistry Research*, 26(7), 1528-1534.
- Tugrak, M., Yamali, C., Sakagami, H., & Gul, H. I. (2016). Synthesis of mono Mannich bases of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one and evaluation of their cytotoxicities. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31(5), 818-823.
- Umesha, B., Basavaraju, Y. B., & Mahendra, C. (2015). Synthesis and biological screening of pyrazole moiety containing analogs of podophyllotoxin. *Medicinal Chemistry Research*, 24(1), 142-151.
- Vaidya, S. S., Vinaya, H., & Mahajan, S. S. (2012). Microwave-assisted synthesis, pharmacological evaluation, and QSAR studies of 1,3-diaryl-2-propen-1-ones. *Medicinal Chemistry Research*, 21(12), 4311-4323.
- Verpoorte, J. A., Mehta, S., Edsall, J. T. (1967). Esterase activities of human carbonic anhydrases B and C. *The Journal of Biological Chemistry*, 242(18), 4221-4229.
- Wu, J. Z., Li, J. L., Cai, Y. P., Pan, Y., Ye, F. Q., Zhang, Y. L., & Liang, G. (2011). Evaluation and discovery of novel synthetic chalcone derivatives as anti-inflammatory agents. *Journal of Medicinal Chemistry*, 54(23), 8110-8123.
- Yurt, B., Saglamtas, R., Demir, Y., İzol, E., Diril, H., Caglayan, C. (2024). Determination of in vitro antioxidant, anticholinergic, and antiepileptic activities of some medicinal and aromatic plant extracts. *KSU Journal of Agriculture and Nature*, 27, (Suppl. 1), 1-15.

Protective Effects of Hesperidin and Salicylic Acid on *Lemna minor* L. Exposed to Evercion Yellow Textile Dye

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ABSTRACT

Hesperidin (HES) is a flavonone glycoside from the flavonoid family that is present in citrus species. It has potent anti-oxidant and anti-cancer properties. In times of stress, the phenolic chemical salicylic acid (SA), also known as a plant hormone, functions as a signal molecule, controlling the plant's reaction and maintaining its survival. For the removal of numerous harmful chemicals, phytoremediation, sometimes referred to as green reclamation, is an efficient, affordable, environmentally benign, and simple procedure. Duckweed (*Lemna minor* L.) is an important bioindicator species in phytoremediation study. Following the application of 75 ppm, 150 ppm, and 300 ppm reactive dye Evercion yellow 1X, the effects of 0.5 mM SA and 0.5 mM hesperidin on duckweed (*L. minor* L.) were examined in this study. The use of 0.5 mM SA against stress boosted the activities of peroxidase (POD), ascorbate peroxidase (APX), glutathione S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT). Additionally, total glutathione (GSH), total chlorophyll, and carotenoid content were altered by SA treatment. Similar to the SA application, the application of HES was effective in lowering stress. Lipid peroxidation content measured as malondialdehyde (MDA) content was found to be higher than the control groups. Results suggest that SA plays a positive role in *L. minor* against Evercion yellow 1X.

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Evercion Sarı Tekstil Boyasına Maruz Kalan *Lemna minor* L. Üzerinde Hesperidin ve Salisilik Asit'in Koruyucu Etkileri

ÖZET

Hesperidin (HES), turuncgöl türlerinde bulunan flavonoid ailesinden bir flavonon glikozittir. Güçlü anti-oksidan ve anti-kanser özelliklere sahiptir. Stres zamanlarında, bitki hormonu olarak da bilinen fenolik kimyasal salisilik asit (SA) bir sinyal molekülü olarak işlev görür, bitkinin tepkisini kontrol eder ve hayatta kalmasını sağlar. Çok sayıda zararlı kimyasalın ortadan kaldırılması için, bazen yeşil ıslah olarak da adlandırılan fitoremediasyon, verimli, uygun maliyetli, çevreye zarar vermeyen ve basit bir prosedürdür. Su mercimeği (*Lemna minor* L.) fitoremediasyon çalışmalarında önemli bir biyoindikatör türüdür. Bu çalışmada, 75 ppm, 150 ppm ve 300 ppm reaktif boya Evercion yellow 1X uygulamasının ardından, 0.5 mM SA ve 0.5 mM HES'in su mercimeği (*L. minor* L.) üzerindeki etkileri incelenmiştir. Strese karşı 0,5 mM SA kullanımı peroksidaz (POD), askorbat peroksidaz (APX), glutatyon S-transferaz (GST), glutatyon redüktaz (GR), süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitelerini artırmıştır. Ayrıca, toplam gutatyon (GSH), toplam klorofil ve karotenoid içeriği SA uygulaması ile değişmiştir. Malondialdehit (MDA) içeriği olarak ölçülen lipid peroksidasyon düzeyi, kontrol gruplarına göre daha yüksek bulunmuştur.

Bitki Fizyolojisi

Araştırma Makalesi

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Anahtar Kelimeler

Lemna minor

Hesperidin

Glutatyon

Reaktif boyar madde

Pigmentasyon

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INTRODUCTION

The textile industry is one of the world's main sources of pollution because it releases undesirable dye effluents (Yaseen & Scholz, 2019). Reactive dyestuffs are widely used in the textile industry because of their ease of use and stability. Both organic and inorganic pollutants can have a considerable negative impact on plant growth (Purwanti et al. 2018; Titah et al. 2018; Tangahu et al., 2019; Imron et al., 2019). Plants as bioremediation agents hold great promise for the breakdown of various hues as well as organic and inorganic contaminants (Ali, 2010; Singh & Singh, 2017).

A member of the Lemnaceae family, duckweed is a little and uncomplicated floating aquatic plant (Driever et al., 2005; Ceschin et al., 2018). *L. minor* is a useful plant for phytoremediation because it can quickly develop, adapt to a variety of aquatic circumstances, and collect pollutants (Ceschin et al., 2020; Can Terzi et al., 2021). Numerous physiological activities, such as gene transcription, signal transduction, redox signal pathways, and immunological response, require reactive oxygen species (ROS). However, excessive ROS generation results in damaging effects on DNA, proteins, and lipids that ultimately cause cell death (Li et al., 2017). Plants have developed defense mechanisms, both enzymatic and non-enzymatic, to scavenge ROS and lessen their harmful effects (Singh & Satheeshkumar, 2024).

Hesperidin is a flavanone that can be found in citrus and other plants naturally. It has been shown to have a variety of pharmacological actions, including antioxidant, anti-inflammatory, anti-hypercholesterolemic, and anticarcinogenic qualities (Tamilselvam et al., 2013; Janigashvili et al., 2024).

SA is a natural plant growth regulator that has a significant impact on a variety of physiological and metabolic processes that affect a plant's ability to grow and develop. Therefore, SA can be used to counteract elemental toxicities, heavy metal stressors, pathogen virulence, and salt stress (Wani et al., 2017).

The purpose of this study was to determine how SA and hesperidin protected *L. minor* from a reactive dye called Evercion Yellow 1X (EY). Antioxidant enzymes, pigment analysis, and lipid peroxidation tests were performed on samples collected on days 1, 4, and 7.

MATERIAL and METHOD

Hesperidin used in this study was originally synthesized in the chemistry laboratory at Inonu University. The hesperidin compound (Figure 1) was synthesized by organic orange fruits and identified by FT-IR spectroscopy.

Hesperidin synthesis from the orange fruits

Although hesperidin is an insoluble flavonoid in water, its solubility varies depending on pH. First, the dried oranges were ground and powdered. 12.5 g of ground orange powder was dispersed in 125 mL distilled water for 1 hour and mixed with a magnetic stirrer. Since Hesperidin was insoluble in water, the water-soluble species were removed by filtration. CaCO_3 was used to adjust the pH of the environment. 25 g of CaCO_3 was dissolved in 250 ml of distilled water, the Ca(OH)_2 solution obtained by removing the insoluble part was used to adjust the pH of the environment. Ca(OH)_2 solution was added dropwise onto the orange mixture and the pH of the medium was adjusted to 10, and it was mixed for 24 hours to solubilize hesperidin. After solubilization, the mixture was filtered with filter paper, the insoluble part was removed and the pH of the solution was neutralized with HCl. Hesperidin, which dissolves in basic pH but does not dissolve in neutral pH, was obtained by precipitation. Efficiency 85% (Figure 2).

In FTIR spectra (Figure 3), hydroxyl tension peaks at 3400 cm^{-1} , C-H tension peaks of the aromatic ring at 2800 cm^{-1} circumference, and aromatic C = C tension peaks at 1600 cm^{-1} .

Experimental Design

L. minor mature plants in good condition were obtained from the Turkish seed firm Erciyes in Kayseri. EY, a reactive dyestuff, was selected for this study. Plants were acclimated to Hoagland medium in the greenhouse (temperature: $23 \pm 2^\circ\text{C}$; light/dark cycle: 16/8 h; light intensity: 10,000 lux) for one week before dye treatment (Hoagland & Arnon, 1950). Healthy fronds (30-40 g) from plants were detached and placed in 250 ml glass beakers

with 1/30-diluted Hoagland culture solution with one of the following treatments; Hoagland medium, 75 ppm EY, 150 ppm EY, 300 ppm EY, 0.5 mM SA, 0.5 mM SA + 75 ppm EY, 0.5 mM SA + 150 ppm EY, 0.5 mM SA + 300 ppm EY, 0.5 mM HES, 0.5 mM HES + 75 ppm EY, 0.5 mM HES + 150 ppm EY and 0.5 mM HES + 300 ppm EY. Each experiment was replicated three times. The fronds were picked on days one, four, and seven.

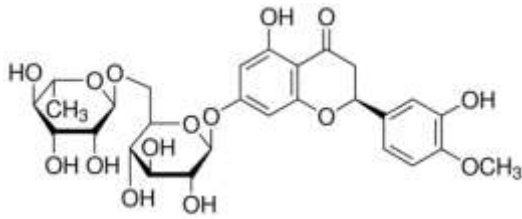


Figure 1. Hesperidin molecule
Şekil 1. Hesperidin molekülü



Figure 2. Hesperidin
Şekil 2. Hesperidin

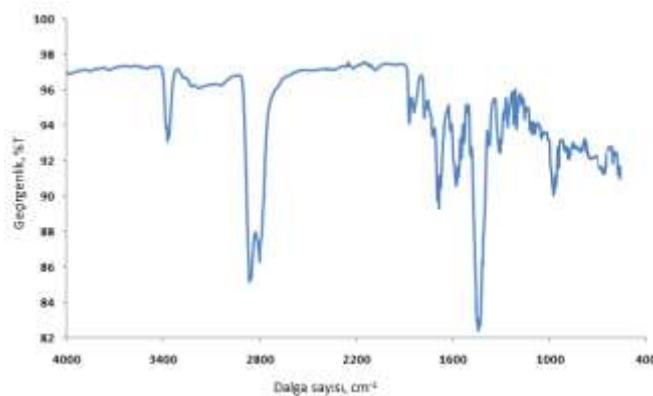


Figure 3. FT-IR spectrum of hesperidin
Şekil 3. Hesperidin FT-IR spektrumu

Enzyme extraction and protein content

According to Huang et al. (2013), enzyme extractions were evaluated. A 5 ml solution of 0.1 M potassium phosphate buffer (pH 7.8) was used to homogenize 0.5 g of *L. minor*. At 4°C and 10000 g, homogenates were centrifuged for 15 minutes. Using bovine serum albumin as the reference protein, the protein content was assessed using a Bradford protein assay (Bradford, 1976).

POD activity

POD activity was calculated in accordance with Peters et al. (1989) and Mac Adam et al. (1992). A solution was prepared by vortexing 3 ml of 0.1 M potassium phosphate buffer (pH 6.0), 0.04 ml of 0.03 M H₂O₂, and 0.05 ml of 0.2 M guaiacol. To 0.9 mL of this solution, 0.1 mL of extract was added. The increase of absorbance at 436 nm was recorded within 1 min. The extinction coefficient of guaiacol is 26.6 mM⁻¹ cm⁻¹.

APX activity

The measurement of APX activity was followed Nakano and Asada's (1981) guidelines. The reaction mixture was prepared by adding 550 µl of phosphate buffer (pH 7.6), 100 µl of 10 mM EDTA, 12 mM H₂O₂, 250 µl of extract, and 100 µl of 0.25 mM AsA. Enzyme activity was determined as the absorbance change obtained in 1 minute at a wavelength of 290 nm. APX activity was calculated with an extinction coefficient of 2.8 mM⁻¹ cm⁻¹.

GST activity

The GST activity was determined using the Habig et al. (1974) technique. The reaction mixture included 400 µl of 0.1 M potassium phosphate buffer, 400 µl of reduced glutathione, 100 µl of sample, and 150 µl of CDNB. The absorbance change over 1 minute at a wavelength of 344 nm was used to determine enzyme activity. CDNB has an extinction coefficient of 9.6 mM⁻¹ cm⁻¹.

GR activity

According to Carlberg & Mannervik (1985), an assessment of GR activity was carried out. A solution containing 2

mM EDTA in 0.2 M potassium phosphate buffer (pH 7), 10 mM Tris-HCl (pH 7), 2 mM NADPH, and 20 mM GSSG in 10 ml distilled water was prepared. The reaction mixture was prepared by combining 500 µl of 0.2 M potassium phosphate buffer, 50 µl of 2 mM NADPH, 50 µl of 20 mM GSSG, 250 µl of distilled water, and 150 µl of the sample. The enzyme activity was measured as the change in absorbance per minute at 340 nm and calculated using the NADPH extinction coefficient of $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$.

GSH content

The amount of GSH was calculated in accordance with Akerboom & Sies (1981). 2.378 mg of DTNB and 0.248 mg of NADPH were prepared in 1 ml of 125 mM sodium diphosphate buffer (pH 7.5) containing 6.3 mM EDTA. 700 µl of NADPH and 100 µl of DTNB were incubated in a water bath at 30 °C, then 100 µL of distilled water, 5 ml of GR, and 100 µl of the extract were added. The reaction was calculated at 412 nm.

SOD activity

The McCord Fridovich (1969) approach was used to assess SOD activity. A solution A was prepared by mixing 100 mL of a 50 mM K_2HPO_4 buffer at pH 7.8 with a solution containing 24.8 mg of cytochrome c and 10 mL of distilled water with 0.76 mg of xanthine. Solution B was prepared separately by dissolving 0.2 U/ml of xanthine oxidase. A reaction mixture was prepared by combining 900 µl of solution A, 50 µl of solution B, and 100 µl of the sample. At 550 nm, enzyme activity was determined.

CAT activity

The Luck (1965) technique was used to evaluate CAT activity. A 1/15 M pH 7 potassium phosphate buffer has been prepared, and 160 µl of H_2O_2 was added to 100 ml of this buffer. Then 200 µl of sample was added to 800 µl of phosphate buffer to which H_2O_2 was added, and absorbance changes over 1 minute were determined by reading at 240 nm. The molar extinction coefficient for H_2O_2 is $0.0396 \text{ cm}^2 \mu\text{mol}^{-1}$.

Pigments analysis

De Kok and Graham's (1980) approach was utilized to determine pigment content. To begin, a 1 g leaf sample was homogenized in 50 ml of acetone and stored at +4°C for 24 hours. After adding 1/5 water to the homogenate, it was homogenized again in a shaking incubator for 15 minutes and kept at +4 °C for 24 hours. At the end of the period, the samples were centrifuged at 5000 g for 10 minutes. The absorbance values of the centrifuged samples were read in a spectrophotometer at 662, 645, 470 nm according to Lichtenthaler and Welburn (1983).

Lipid peroxidation assay

Heath and Packer (1968) used the thiobarbituric acid reactive chemicals approach to measure MDA levels. A 0.5 g leaf sample was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 10,000 g for 10 minutes. From the centrifuged sample, 2 mL of the supernatant was taken and 2 mL of 0.5% thiobarbituric acid (TBA) was added. The mixture was incubated in a water bath at 95°C for 30 minutes. The absorbance was measured using both 532 and 600 nm wavelengths. MDA content was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Statistical Analysis

The results were reported as the mean with standard error (SE). The entire data set from all studies was statistically evaluated using SPSS version 25.0. In the study, the conformity of the data to normal distribution was checked with the Shapiro-Wilk Test, and the homogeneity of the variances was checked with the LEVENE test. Since the data provided normal distribution, the ANOVA test, one of the parametric test methods, was used in the analysis of multi-group variables and the Duncan multiple comparison test was used for pairwise comparisons between groups (Duncan, 1955).

RESULTS and DISCUSSION

Antioxidant Enzyme Activities

The synthetic dyes used in the textile industry pollute a significant amount of water. Textile dyes do not adhere tightly to the fabric and are released as effluent into the aquatic environment (Rania et al., 2022).

In nature, plants are continually exposed to abiotic and biotic stresses. Furthermore, plants have both enzymatic and non-enzymatic antioxidant defense systems that help to minimize ROS production and cellular damage.

Lemnaceae are among the world's tiniest and quickest growing angiosperms, producing massive amounts of biomass. Lemna species have a high economic value and diverse applications in biotechnology and ecology (Alp et

al., 2023). Their morphological and physiological characteristics enable them to perform valuable bioassays in restricted environments in a short amount of time, making them ideal laboratory organisms. *Lemna* species are used as bioindicators for ecotoxicological studies, both in vivo and in vitro (Basiglioni et al., 2018; Beker Akbulut et al., 2020; Beker Akbulut & Özhan Turhan, 2021). HES is one of the most common flavonoids discovered in citrus species. It is an active pharmacological compound having antihypertensive, anti-inflammatory, and anticancer properties (Iranshahi et al., 2015; Tabeshpour et al., 2020; Arikan et al., 2022; Ji et al., 2024).

POD is a common enzyme found in animals, plants, microbes, and cultured cells. It catalyzes the oxidation of phenols and amines with hydrogen peroxide, removing their toxicity. POD activity was shown to be higher in the EY-applied groups than in the control group. It rose with time in those treated with 0.5 mM SA and dye. The 0.5 mM HES and dye groups demonstrated an increase in POD activity over time. In the 0.5 mM SA +300 ppm dye groups, the maximal POD activity was 15.42 U/mg protein. The groups treated with 0.5 mM HES +150 ppm dye showed the highest POD activity (16.33 U/mg protein). The changes in POD activity were found to be statistically significant, $F(11,24)= 2.85$, $p=.015$. (Figure 4).

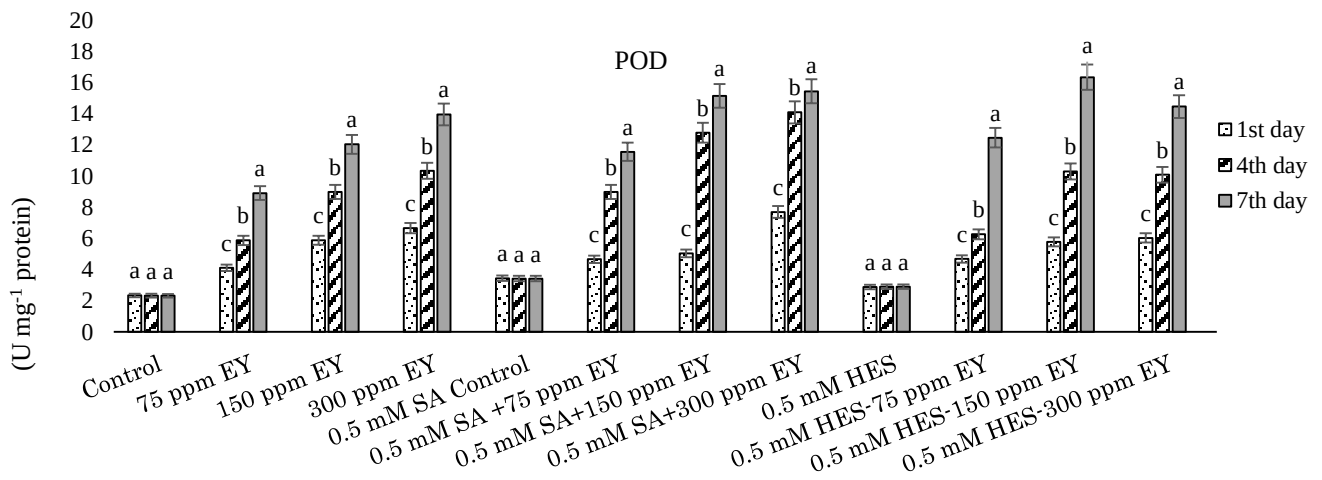


Figure 4. POD activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24) = 2.85$, $p=.015$

Şekil 4. EY, SA ve HES uygulanan *L. minor*'da POD aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24)= 2.85$, $p=.015$

Ascorbate peroxidase enzymes serve a critical function in regulating ROS in cells, particularly H_2O_2 . Following 75, 150, and 300 ppm EY treatment, APX activity increased with the number of days. The 0.5 mM SA and dye administered groups increased as the number of days increased. On the seventh day, the maximum APX activity (15.26 U/mg protein) was identified in the groups treated with 0.5 mM HES (Figure 5).

GSTs are multifunctional proteins that are involved in a variety of intracellular events such as stress metabolism, primary and secondary metabolism, herbicide detoxification, plant defense against heavy metals, ozone destruction, and xenobiotics. GST activity increased with increasing dye concentrations and days. The changes in GST activity were found to be statistically significant, $F(11,24)= 3.57$, $p<.004$. Only in the dye-applied groups did the maximum GST activity result from 300 ppm EY application. GST activation was found to be greater in the 0.5 mM SA-treated groups than in the dye-only groups. GST activity was shown to be decreased in the 0.5 mM HES treated groups compared to the 0.5 mM SA applied groups (Figure 6).

GR reduces glutathione by using NADPH as a substrate. On the fourth and seventh days, dye-only groups had lower GR activity than the control group. (GR activity decreased on the fourth and seventh days in the groups treated with 0.5 mM SA). The lowest GR activity in the 0.5 mM HES treated groups was seen on the seventh day in the 300 ppm EY applied groups. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 1.36$, $p<.254$ (Figure 7).

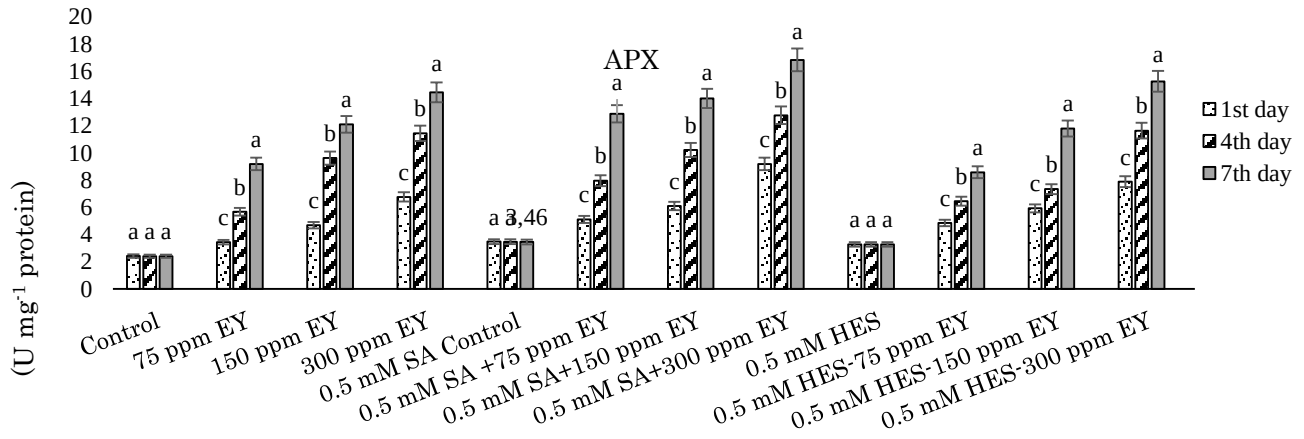


Figure 5. APX activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24) = 3.88$, $p = .003$

Şekil 5. EY, SA ve HES uygulanan *L. minor*'da APX aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24) = 3.88$, $p = .003$

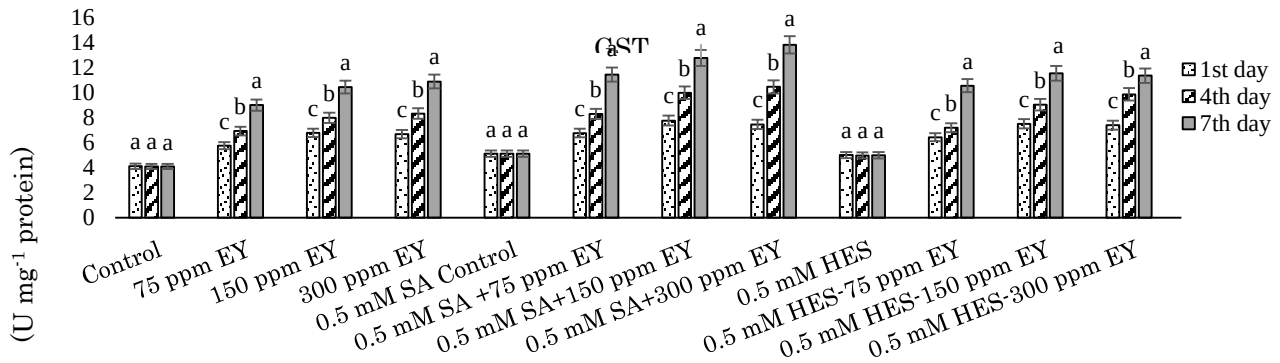


Figure 6. GST activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24) = 3.57$, $p < .004$

Şekil 6. EY, SA ve HES uygulanan *L. minor*'da GST aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24) = 3.57$, $p < .004$

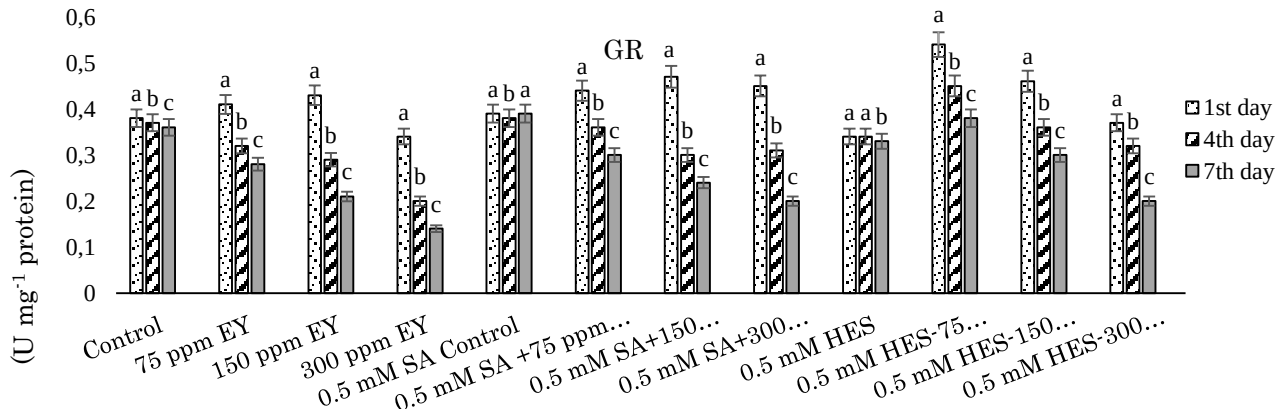


Figure 7. GR activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24) = 1.36$, $p < .254$

Şekil 7. EY, SA ve HES uygulanan *L. minor*'da GR aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24) = 1.36$, $p < .254$

GSH is a key molecule in sulfur metabolism and plant response to oxidative stress. GSH levels increased in response to increasing dye concentrations and days. The dye-applied groups had the highest GSH concentration. The 0.5 mM SA treated groups had higher GSH content than the dye-only groups. On the seventh day, the groups that applied 0.5 mM HES-300 ppm EY had the highest GSH content (8.36 U/mg protein). This is because significant differences exist between the applied dose groups and the control, $F(211,24)= 6.36, p<.000$ (Figure 8).

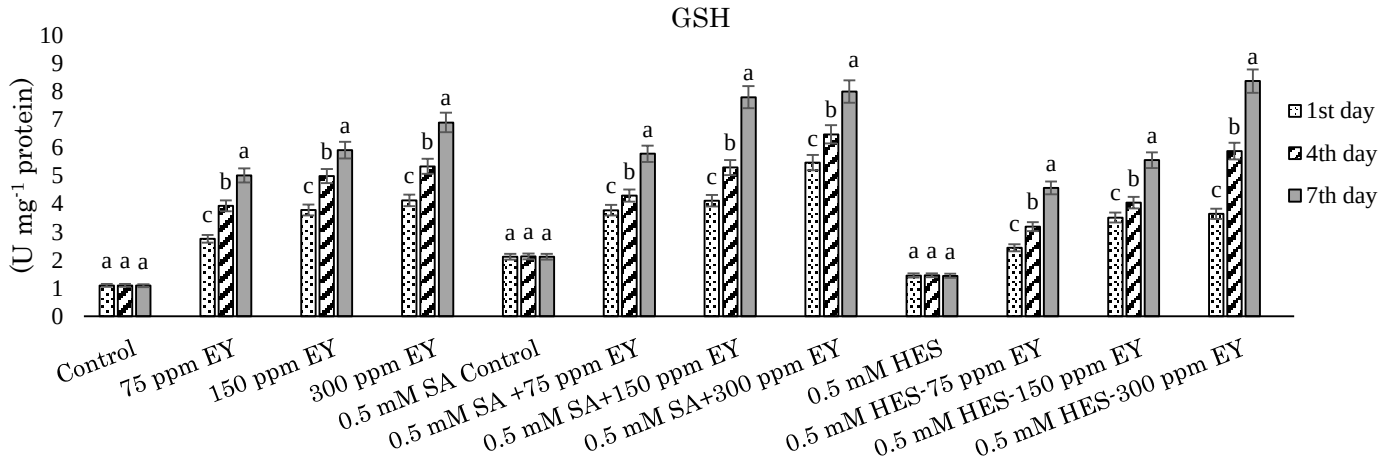


Figure 8. GSH content in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other, $F(11,24)= 6.36, p<.000$

Şekil 8. EY, SA ve HES uygulanan *L. minor*'da GSH içeriği. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24)= 6.36, p<.000$.

SOD defends plants against abiotic stress-induced ROS. SOD activity increased in dye-treated groups compared to the control. The changes in SOD activity was found to be statistically significant, $F(11,24)= 3.10, p<.010$. The group that applied 300 ppm EY on the seventh day showed the highest SOD activity. Increased concentrations in the SA and HES applied groups resulted in an increase in SOD activity (Figure 9).

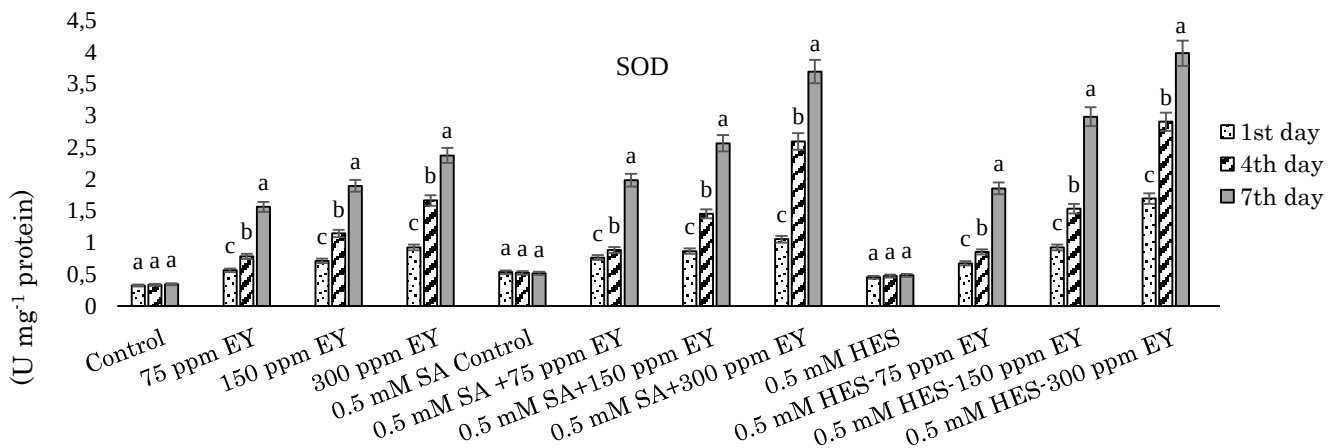


Figure 9. SOD activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24)= 3.10, p<.010$

Şekil 9. EY, SA ve HES uygulanan *L. minor*'da SOD aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır $F(11,24)= 3.10, p<.010$

CAT is a tetrameric heme-containing enzyme, removes H_2O_2 from plants under stress to prevent oxidative damage. CAT activity increased on the fourth day solely in the dye-applied groups, but declined on the seventh day. The

groups that applied 300 ppm EY on the seventh day showed the highest CAT activity. On the fourth day, the groups who received both SA and 300 ppm dye showed the highest CAT activity. CAT activity reduced on the seventh day in HES-applied groups. This is because significant differences exist between the applied dose groups and the control, $F(11,24)= 1.94$, $p<.084$ (Figure 10).

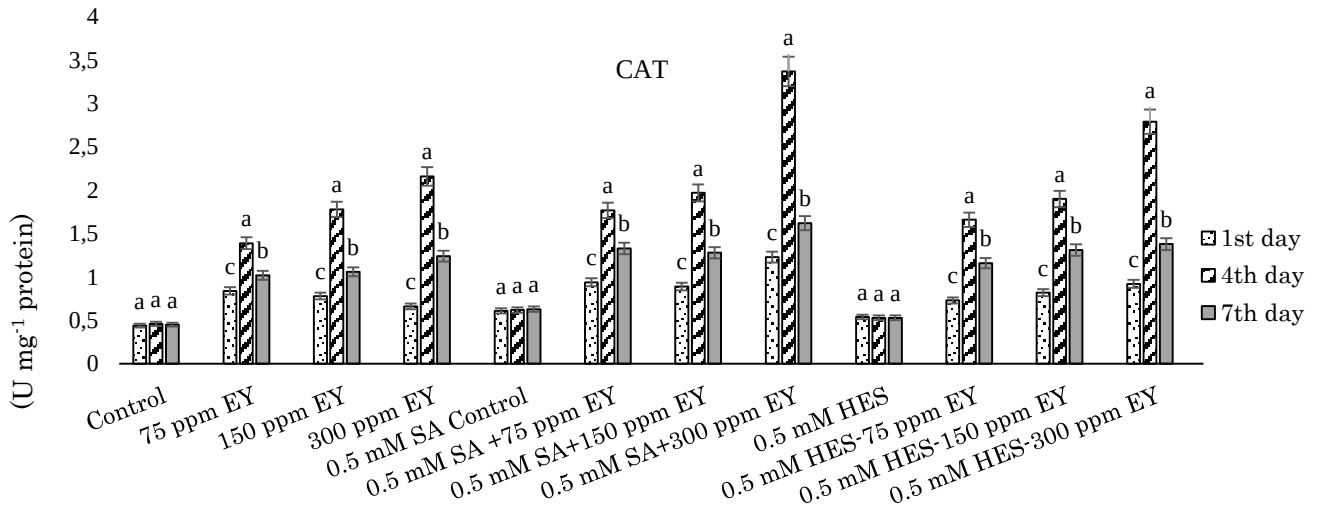


Figure 10. CAT activity in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24)= 1.94$, $p<.084$

Şekil 10. EY, SA ve HES uygulanan *L. minor*'da CAT aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24)= 1.94$, $p<.084$

Photosynthetic Pigment Analysis

Stress conditions negatively affect photosynthetic activity in plants. In comparison to the control group, the overall chlorophyll concentration in the 75 ppm, 150 ppm, and 300 ppm dye treated groups decreased as time passed. The total chlorophyll content increased on the first day compared to the control, but then decreased on consecutive days in the groups treated with 0.5 mM SA and 75, 150, and 300 ppm dyes. On day 7, total chlorophyll content increased compared to day 4 in groups treated with 0.5 mM HES and 75, 150, or 300 ppm dye. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 11.61$, $p<.000$ (Figure 11).

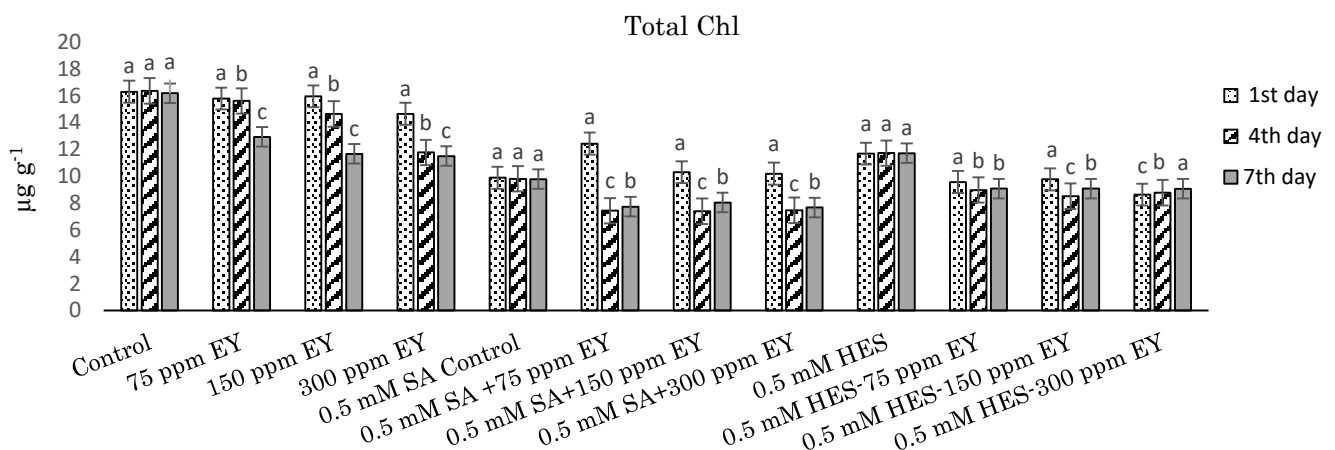


Figure 11. Changes in total chlorophyll content in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24)= 11.61$, $p<.000$

Şekil 11. EY, SA ve HES uygulanan *L. minor*'da total klorofil içeriğindeki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24)= 11.61$, $p<.000$

The dye-treated groups' carotenoid concentration reduced by 75 ppm, 150 ppm, and 300 ppm compared to the control. On the first day, groups receiving 0.5 mM SA and 75 ppm dye had the greatest carotenoid levels (4.07 $\mu\text{g g}^{-1}$). On the seventh day, those treated with 0.5 mM SA and 75 ppm dye had the lowest carotenoid levels (1.78 $\mu\text{g/g}$). The carotenoid content of the HES treated groups reduced in comparison to the control, with the exception of the HES and 75 ppm dye applied groups on the first day. The changes in carotenoid content were found to be statistically significant, $F(11,24)= 2.24$, $p<.048$ (Figure 12).

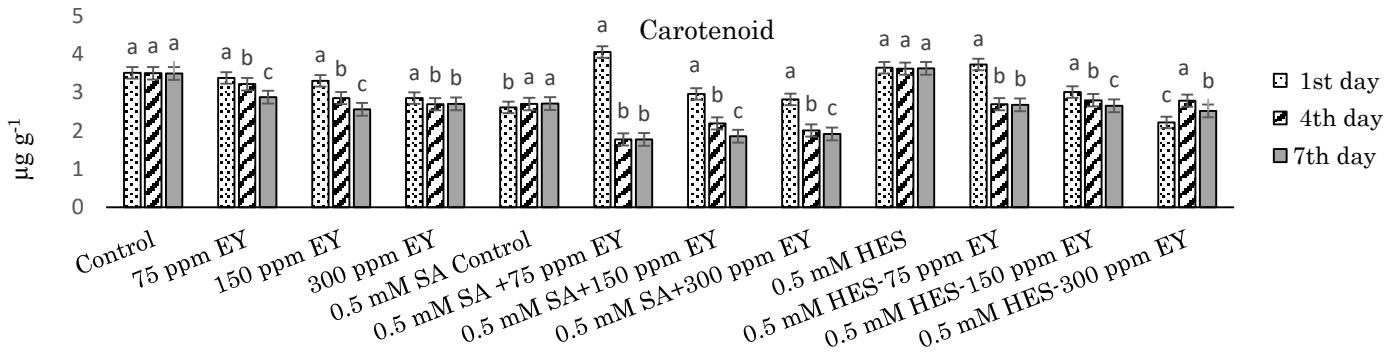


Figure 12. Changes in Carotenoid content in *L. minor* treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, $F(11,24)= 2.24$, $p<.048$

Şekil 12. EY, SA ve HES uygulanan *L. minor*'da karotenoid miktarındaki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, $F(11,24)= 2.24$, $p<.048$

Lipid Peroxidation Analysis

Increased Lipid peroxidation content in membranes weakens their barrier function and increases permeability to organic substances and ions. This causes sulfhydryl group destruction and enzyme inactivation. On the fourth day, groups that received 300 ppm EY had the highest MDA activity. On the seventh day, MDA levels in the dye-applied groups decreased. MDA concentrations peaked on day four in those treated with 0.5 mM SA and 300 ppm EY. MDA levels in the groups treated with 0.5 mM HES and dye increased on day four but decreased on day seven. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 2.60$, $p<.025$ (Figure 13).

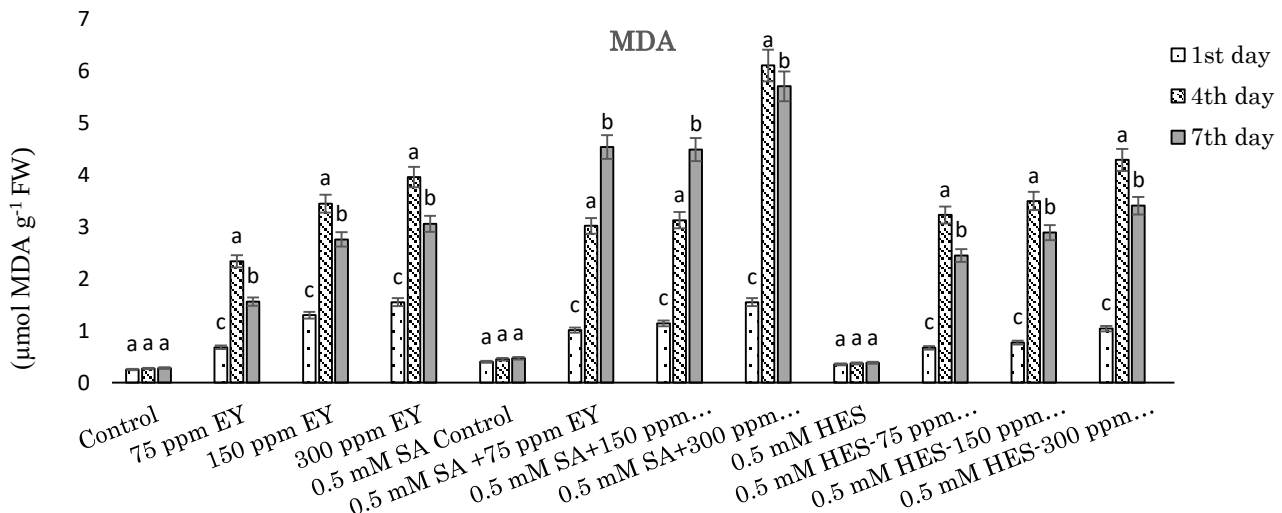


Figure 13. Changes in MDA content in *L. minor* treated with EY, SA and HES. Vertical columns represent standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test $F(11,24)= 2.60$, $p<.025$

Şekil 13. EY, SA ve HES uygulanan *L. minor*'da MDA miktarındaki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır $F(11,24)= 2.60$, $p<.025$

CONCLUSION

In conclusion, when employed in agriculture, water containing reactive dyes can end up in human bodies. Reactive dyes provide a significant risk to human health and may be a primary contributor to cancerous disorders. A very promising species for natural-environment technologies is *L. minor*. Oxidative stress was brought on by Everzol yellow dye (EY) in *L. minor*. Antioxidant activity and pigmentation were significantly decreased. POD, APX, GST, GSH, and SOD were all elevated upon application of SA and hesperidin. Day 7 saw a decline in GR and CAT activity in the hesperidin and SA groups. Day 4 saw an increase in MDA content. The results of this investigation indicate that the combination of hesperidin and exogenous SA helped *L. minor* recover from dye-induced stress.

Contribution Rate Statement Summary of Researchers

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

Conflict of Interests and Ethical Statement

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

REFERENCES

- Akerboom, T.P.M., & Sies, H. (1981). Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. *Method Enzymol*, 77, 373-382. [https://doi.org/10.1016/S0076-6879\(81\)77050-2](https://doi.org/10.1016/S0076-6879(81)77050-2).
- Alkimi, G. D., Daniel, D., Dionísio, R., Soares, A. M. V. M., Barata, C., & Nunes, B. (2019). Effects of diclofenac and salicylic acid exposure on *Lemna minor*: Is time a factor? *Environmental Research*, 177, 1-15. <https://doi.org/10.1016/j.envres.2019.108609>.
- Alp, F. N., Arikan, B., Ozfidan-Konakci, C., Ekim, R., Yildiztugay, E., & Turan, M. (2023). Rare earth element scandium mitigates the chromium toxicity in *Lemna minor* by regulating photosynthetic performance, hormonal balance and antioxidant machinery. *Environmental Pollution*, 316, 1-13. <https://doi.org/10.1016/j.envpol.2022.120636>.
- Al-Snai, A. E., (2019). *Lemna minor*: Traditional uses, chemical constituents and pharmacological effects-A review. *IOSR Journal of Pharmacy*, 9(8), 6-11. (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219.
- Arikan, B., Ozfidan-Konakci, C., Yildiztugay, E., Zengin, G., Alp, F. N., & Elbasan, F. (2022). Exogenous hesperidin and chlorogenic acid alleviate oxidative damage induced by arsenic toxicity in *Zea mays* through regulating the water status, antioxidant capacity, redox balance and fatty acid composition. *Environmental Pollution*, 292, 1-15. <https://doi.org/10.1016/j.envpol.2021.118389>.
- Ashraf, S., Ali, Q., Zahir, Z. A., Ashraf, S., & Asghar, H. N. (2019). Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicology and Environmental Safety*, 174, 714-727. <https://doi.org/10.1016/j.ecoenv.2019.02.068>.
- Aziz, A., Kapoor, D., 2018. Salicylic Acid: It's physiological role and interactions. *Research Journal of Pharmacy and Technology*, 11(7), 3171-3177. <https://doi.org/10.5958/0974-360X.2018.00583.8>.
- Bansal, N., & Kanwar, S. S. (2013). Peroxidase (s) in environment protection. *The Scientific World Journal*. 1-9. <https://doi.org/10.1155/2013/714639>.
- Beker Akbulut, G., Turhan, D. Ö., & Yiğit, E. (2020). Alleviation of Everzol Red LFB toxicity in duckweed (*Lemna minor* L.) by exogenous salicylic Acid. *KSU J. Agric Nat*, 23(4), 876-884. <https://doi.org/10.18016/ksutarimdog.vi.683962>.
- Beker Akbulut, G., Özhan Turhan, D. (2021). Role of salicylic acid in resistance to everzol navy ED in *Lemna minor* L.(Duckweed). *International Journal of Pure and Applied Sciences* 7(1), 185-195. <https://doi.org/10.29132/ijpas.894056>.
- Basiglini, E., Pintore, M., & Forni, C. (2018). Effects of treated industrial wastewaters and temperatures on growth and enzymatic activities of duckweed (*Lemna minor* L.). *Ecotoxicology and Environmental Safety*, 153, 54-59. <https://doi.org/10.1016/j.ecoenv.2018.01.053>.
- Bozbuga, R. (2020). Expressions of pathogenesis related 1 (PR1) gene in *Solanum lycopersicum* and Influence of salicylic acid exposures on host-Meloidogyne incognita interactions. In Doklady. *Biochemistry and Biophysics*. Pleiades Publishing. 494(1), 266-269. <https://doi.org/10.1134/S1607672920050038>.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Buttar, Z. A., Wu, S. N., Arnao, M. B., Wang, C., Ullah, I., & Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. *Plants*, 9(7), 809. <https://doi.org/10.3390/plants9070809>.

- Can-Terzi, B., Goren, A. Y., Okten, H. E., & Sofuoglu, S. C. (2021). Biosorption of methylene blue from water by live *Lemna minor*. *Environmental Technology & Innovation*, 22, 1-12. <https://doi.org/10.1016/j.eti.2021.101432>.
- Carlberg, I. & Mannervik, B. (1985). Glutathione reductase. *Methods in Enzymology*, 113, 484-490. [https://doi.org/10.1016/S0076-6879\(85\)13062-4](https://doi.org/10.1016/S0076-6879(85)13062-4).
- Ceschin, S., Crescenzi, M., & Iannelli, M. A. (2020). Phytoremediation potential of the duckweeds *Lemna minuta* and *Lemna minor* to remove nutrients from treated waters. *Environmental Science and Pollution Research*, 27(13), 15806-15814. <https://doi.org/10.1007/s11356-020-08045-3>.
- Chavoushi, M., Najafi, F., Salimi, A., & Angaji, S. A. (2020). Effect of salicylic acid and sodium nitroprusside on growth parameters, photosynthetic pigments and secondary metabolites of safflower under drought stress. *Scientia Horticulturae*, 259, 1-6. <https://doi.org/10.1016/j.scienta.2019.108823>.
- Ciceralli, N. I. (2004). Effect of stress on antioxidant defense systems of sensitive and resistant cultivars of lentil (*Lens culinaris* M.) (Thesis number 153433). Master's Thesis, ODTÜ, Ankara. Council of High Education Thesis Center.
- Cohen, S. P. & Leach, J. E. (2019). Abiotic and biotic stresses induce a core transcriptome response in rice. *Scientific Reports*, 9(1), 1-11. <https://doi.org/10.1038/s41598-019-42731-8>.
- De Kok, L. & Graham, M. (1989). Levels of pigments, soluble proteins, amino acids and sulhydryl compounds in foliar tissue of *Arabidopsis thaliana* during dark-induced and natural senescence. *Plant Physiology and Biochemistry* (Paris), 27(2), 203-209. ISSN/ISBN: 0981-9428.
- Demirci, T., Asci, Ö. A., & Baydar, N. G. (2021). Influence of salicylic acid and L-phenylalanine on the accumulation of anthraquinone and phenolic compounds in adventitious root cultures of madder (*Rubia tinctorum* L.). *Plant Cell, Tissue and Organ Culture*, 144(2), 313-324. <https://doi.org/10.1007/s11240-020-01952-w>.
- Di, X., Gomila, J. & Takken, F. L. (2017). Involvement of salicylic acid, ethylene and jasmonic acid signalling pathways in the susceptibility of tomato to *Fusarium oxysporum*. *Molecular Plant Pathology*, 18(7), 1024-1035. <https://doi.org/10.1111/mpp.12559>.
- Dorina, S., Judith, S., Björn, W., Julia, S., Andrea, S., Muffler, K., & Roland, U. (2020). A new strategy for a combined isolation of EPS and pigments from cyanobacteria. *Journal of Applied Phycology*, 32, 1-12. <https://doi.org/10.1007/s10811-020-02063-x>.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1-42. <http://dx.doi.org/10.2307/3001478>.
- Feng, J., Zhang, M., Yang, K. N., & Zheng, C. X. (2020). Salicylic acid-primed defence response in octoploid strawberry 'Benihoppe' leaves induces resistance against *Podosphaera aphanis* through enhanced accumulation of proanthocyanidins and upregulation of pathogenesis-related genes. *BMC Plant Biology*, 20, 1-18. <https://doi.org/10.21203/rs.2.16580/v1>.
- Gong, H., Jiao, Y., Hu, W. W., & Pua, E. C. (2005). Expression of glutathione-S-transferase and its role in plant growth and development in vivo and shoot morphogenesis in vitro. *Plant Molecular Biology*, 57(1), 53-66. <https://doi.org/10.1007/s11103-004-4516-1>.
- Habig, W., Pabst, M., & Jakoby, W. (1974). The first enzymatic step in mercapturic acid formation. Glutathione-S-transferase. *J. Biol. Chem.*, 249:7130-7139. [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8).
- Hasanuzzaman, M., Nahar, K., Anee, T. I., & Fujita, M. (2017). Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiology and Molecular Biology of Plants*, 23(2), 249-268. <https://doi.org/10.1007/s12298-017-0422-2>.
- Havaux, M. (2014). Carotenoid oxidation products as stress signals in plants. *The Plant Journal*, 79(4), 597-606. <https://doi.org/10.1111/tpj.12386>.
- Heath, R. L. & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1), 189-198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1).
- Hernández-Ruiz, J. & Arnao, M. B. (2018). Relationship of melatonin and salicylic acid in biotic/abiotic plant stress responses. *Agronomy*, 8(4):33. <https://doi.org/10.3390/agronomy8040033>.
- Hoagland, D. R., Arnon, D. I. (1950). The water culture method for growing plants without soil. UC College of Agriculture, Ag. Exp. Station, Berkeley, CA. *Circular*. 347, 1-32. https://www.nutricaodeplantas.agr.br/site/downloads/hoagland_arnon.pdf
- Hossen, M. S., Karim, M. F., Fujita, M., Bhuyan, M. B., Nahar, K., Masud, A. A. C., Mahmud J. A., & Hasanuzzaman, M. (2022). Comparative physiology of indica and japonica rice under salinity and drought stress: An intrinsic study on osmotic adjustment, oxidative stress, antioxidant defense and methylglyoxal detoxification. *Stresses*, 2(2), 156-178. <https://doi.org/10.3390/stresses2020012>.
- Huang, L., Lu, Y., Gao, X., Du, G., Ma, X., Liu, M., & Chen, Y. (2013). Ammonium-induced oxidative stress on plant growth and antioxidative response of duckweed (*Lemna minor* L.). *Ecological Engineering*, 58, 355-362. <https://doi.org/10.1016/j.ecoleng.2013.06.031>.

- Iatrou, E. I., Kora, E., & Stasinakis, A. S. (2018). Investigation of biomass production, crude protein and starch content in laboratory wastewater treatment systems planted with *Lemna minor* and *Lemna gibba*. *Environmental Technology*, 40(20), 2649-2656. <https://doi.org/10.1080/09593330.2018.1448002>.
- Janigashvili, G., Chkhikvishvili, I., Ratiani, L., Maminaishvili, T., Chkhikvishvili, D., & Sanikidze, T. (2024). Effects and medical application of plant-origin polyphenols: A narrative review. *Bioactive Compounds in Health and Disease-Online* ISSN: 2574-0334; Print ISSN: 2769-2426, 7(8), 375-385. <https://doi.org/10.31989/bchd.v7i8.1414>.
- Ji, Z., Deng, W., Chen, D., Liu, Z., Shen, Y., Dai, J., Zhou H., Zhang, M., Xu, H., & Dai, B. (2024). Recent understanding of the mechanisms of the biological activities of hesperidin and hesperidin and their therapeutic effects on diseases. *Heliyon*. 10(5), <https://doi.org/10.1016/j.heliyon.2024.e26862>.
- Li, Z., Xu, X., Leng, X., He, M., Wang, J., Cheng, S., & Wu, H. (2017). Roles of reactive oxygen species in cell signaling pathways and immune responses to viral infections. *Archives of Virology*, 162(3), 603-610. <https://doi.org/10.1007/s00705-016-3130-2>.
- Li, C., Ji, J., Wang, G., Li, Z., Wang, Y., & Fan, Y. (2020). Over-expression of LcPDS, LcZDS, and LcCRTISO, genes from wolfberry for carotenoid biosynthesis, enhanced carotenoid accumulation, and salt tolerance in tobacco. *Frontiers in Plant Science*, 11:119. <https://doi.org/10.3389/fpls.2020.00119>.
- Lichtenthaler, H. K. & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents: Portland Press Ltd. <https://doi.org/10.1042/bst0110591>.
- Lo, W. J., Chiou, Y. C., Hsu, Y. T., Lam, W. S., Chang, M. Y., Jao, S. C., & Li, W. S. (2007). Enzymatic and nonenzymatic synthesis of glutathione conjugates: application to the understanding of a parasite's defense system and alternative to the discovery of potent glutathione s-transferase inhibitors, *Bioconjugate Chem.*, 18, 109-120. <https://doi.org/10.1021/bc0601727>.
- Luck H. (1965) Catalase. Methods of enzymatic analysis, 885-894. <http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4>.
- MacAdam, J. W., Nelson, C. J., & Sharp, R. E. (1992). Peroxidase activity in the leaf elongation zone of tall fescue: I. Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. *Plant Physiology*, 99(3), 872-878. <http://dx.doi.org/10.1104/pp.99.3.872>.
- McCord J.M. & Fridovich I. (1969). Superoxide dismutase: An enzymic function for erythrocyte superoxide (hemocytin), *J. Biol. Chem*, 244(22), 6049-6055. [https://doi.org/10.1016/S0021-9258\(18\)63504-5](https://doi.org/10.1016/S0021-9258(18)63504-5).
- Nakano, Y. & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22(5), 867-880. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>.
- Parlak, K. U. & Yilmaz, D. D. (2012). Response of antioxidant defences to Zn stress in three duckweed species. *Ecotoxicology and Environmental Safety*, 85, 52-58. <https://doi.org/10.1016/j.ecoenv.2012.08.023>.
- Peters, J. L., Castillo, F. J., & Heath, R. L. (1989). Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutants, ozone and sulfur dioxide. *Plant Physiology*, 89(1), 159-164. <https://doi.org/10.1104/pp.89.1.159>.
- Sackey, L. N., Kočí, V., & van Gestel, C. A. (2020). Ecotoxicological effects on *Lemna minor* and *Daphnia magna* of leachates from differently aged landfills of Ghana. *Science of the Total Environment*, 698, 1-7. <https://doi.org/10.1016/j.scitotenv.2019.134295>.
- Sarker, U. & Oba, S. (2020). The response of salinity stress-induced *A. tricolor* to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. *Frontiers in Plant Science*, 11, 1-14. <https://doi.org/10.3389/fpls.2020.559876>.
- Sharma, G., & Mathur, V. (2020). Modulation of insect-induced oxidative stress responses by microbial fertilizers in *Brassica juncea*. *FEMS Microbiology Ecology*, 96(4), 1-10. <https://doi.org/10.1093/femsec/fiaa040>.
- Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., Murphy M. P., Yamamoto, M. & Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews Molecular Cell Biology*, 23(7), 499-515. <https://doi.org/10.1038/s41580-022-00456-z>.
- Singh, H., Kumar, D., & Soni, V. (2022). Performance of chlorophyll a fluorescence parameters in *Lemna minor* under heavy metal stress induced by various concentration of copper. *Scientific Reports*, 12, 1-14. <https://doi.org/10.1038/s41598-022-14985-2>.
- Singh, A., & Satheeshkumar, P. K. (2024). Reactive Oxygen Species (ROS) and ROS Scavengers in Plant Abiotic Stress Response. In *Stress Biology in Photosynthetic Organisms: Molecular Insights and Cellular Responses* (pp. 41-63). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-1883-2_3
- Souza, L. R. R., Bernardes, L. E., Barbetta, M. F. S., & da Veiga, M. A. M. S. (2019). Iron oxide nanoparticle phytotoxicity to the aquatic plant *Lemna minor*: effect on reactive oxygen species (ROS) production and chlorophyll a/chlorophyll b ratio, *Environmental Science and Pollution Research*, 26(23), 24121-24131. <https://doi.org/10.1007/s11356-019-05713-x>.

- Stephenie, S., Chang, Y. P., Gnanasekaran, A., Esa, N. M., Gnanaraj, C. (2020). An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. *Journal of Functional Foods*, 68, 1-10. <https://doi.org/10.1016/j.jff.2020.103917>.
- Strzałka, K., Kostecka-Gugała, A., & Latowski, D. (2003). Carotenoids and environmental stress in plants: significance of carotenoid-mediated modulation of membrane physical properties. *Russian Journal of Plant Physiology*, 50(2), 168-173. <https://doi.org/10.1023/A:1022960828050>.
- Sun, S., Li, X., Sun, C., Cao, W., Hu, C., Zhao, Y., & Yang, A. (2019). Effects of ZnO nanoparticles on the toxicity of cadmium to duckweed *Lemna minor*. *Science of the Total Environment*, 662, 697-702. <https://doi.org/10.1016/j.scitotenv.2019.01.275>.
- Tamilselvam, K., Braid, N., Manivasagam, T., Essa, M. M., Prasad, N. R., Karthikeyan, S., Thenmozhi, A. J. & Guillemin, G. J. (2013). Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. *Oxidative medicine and cellular longevity*. 1, 1-11. <https://doi.org/10.1155/2013/102741>.
- Teixeira, G. C. M., de Mello Prado, R., Oliveira, K. S., D'Amico-Damião, V., & Junior, G. D. S. S. (2020). Silicon increases leaf chlorophyll content and iron nutritional efficiency and reduces iron deficiency in Sorghum plants. *Journal of Soil Science and Plant Nutrition*, 20, 1311-1320. <https://doi.org/10.1007/s42729-020-00214-0>.
- Wani, A. B., Chadar, H., Wani, A. H., Singh, S., & Upadhyay, N. (2017). Salicylic acid to decrease plant stress. *Environmental Chemistry Letters*, 15(1), 101-123. <https://doi.org/10.1007/s10311-016-0584-0>.
- Wang, Y., Cui, X., Yang, B., Xu, S., Wei, X., Zhao, P., Niu, F., Sun, M., Wang, C., Chieng, H., & Jiang, Y. Q. (2020). WRKY55 transcription factor positively regulates leaf senescence and the defense response by modulating the transcription of genes implicated in the biosynthesis of reactive oxygen species and salicylic acid in Arabidopsis. *Development, The Company of Biologists*, 147, 1-15. <https://doi.org/10.1242/dev.189647>.
- Yu, J., Cang, J., Lu, Q., Fan, B., Xu, Q., Li, W., & Wang, X. (2020). ABA enhanced cold tolerance of wheat 'dn1' via increasing ROS scavenging system. *Plant Signaling and Behavior*, 15(8), 1-11. <https://doi.org/10.1080/15592324.2020.1780403>.

The Impact of Water Deficit on Yield Response Factors: A Case Study of Cotton and Silage-Maize in İslahiye

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ABSTRACT

Understanding the effects of water deficit on crop yield is essential for growers and researchers, as it represents a crucial uncertainty in agriculture, along with factors such as sales price, tenure, input costs, and input quality. This study compares and analyzes the yield response factors of two crops, cotton and silage maize, to water deficit using data from İslahiye town of Gaziantep Province in Türkiye. The primary data source is the 2016 Türkiye Plant Water Consumption Guide, prepared by TAGEM and DSI using the FAO Penman-Monteith method. The yield response factor K_y indicates the sensitivity of the crop yield to the potential evapotranspiration (PET) deficit in ten-day periods, t . Their yield response factors change depending on the growth stage and percentage of water deficit. Our model is based on water production function and the soil moisture balance equation, and the model can be applied to all crops, including herbaceous, trees, and vines. The water shortage rate is the same for each t . The solution of the maximization model by a Mixed-Integer Programming (MIP) Solver revealed that both crop yields are considerably affected by water deficits, with similar sensitivities observed. However, while cotton is more sensitive until a 15 per cent deficit, silage maize becomes more sensitive beyond this value. The findings will have a significant impact on decision-making for water allocation, ensuring optimal use of this essential resource. This model and its solution use optimization algorithms to provide new knowledge and perspectives to manage water deficits better and optimize crop yields.

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Kısıntılı Sulamanın Verim Tepki Faktörleri Üzerindeki Etkisi: İslahiye'de Pamuk ve Silajlık Mısır Üzerine Bir Vaka Çalışması

ÖZET

Su kıtlığının bitki verimi üzerindeki etkilerini anlamak, tarımda satış fiyatı, mülkiyet süresi, girdi maliyetleri ve girdi kalitesi gibi unsurlarla birlikte önemli bir belirsizliği temsil ettiğinden, yetiştiriciler ve araştırmacılar için hayati bir öneme sahiptir. Bu çalışma, Türkiye'nin Gaziantep ilinin İslahiye ilçesinden alınan verileri kullanarak pamuk ve silajlık mısırın kısıntılı sulamaya verdiği verim tepki faktörlerini karşılaştırmayı ve analiz etmeyi amaçlamaktadır. Verim tepki faktörü K_y , on günlük periyotlarda (t), potansiyel bitki su tüketimindeki azalmaya karşı bitki veriminin duyarlılığını gösterir. Ana veri kaynağı, TAGEM ve DSI tarafından FAO Penman-Monteith yöntemi kullanılarak hazırlanan 2016 Türkiye Bitki Su Tüketimi Rehberi'dir. Verim tepki faktörleri, büyüme aşamasına ve kısıntılı sulama yüzdesine bağlı olarak değişmektedir. Modelimiz su üretim fonksiyonu ve toprak nem dengesi denklemine dayalı olup, otsu bitkiler, ağaçlar ve asmalar dahil tüm bitkilere uygulanabilir. Kısıntılı sulama oranı her bir t için aynıdır. Karma Tamsayılı Programlama (MIP) Çözücü ile maksimizasyon modelinin çözümü, her iki ürünün veriminin de

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Anahtar Kelimeler

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Silajlık mısır
Toprak-nem dengesi
Verim tepki faktörü
Kısıntılı sulama

benzer duyarlılıklar göstererek kısıntılı sulamadan önemli ölçüde etkilendiğini ortaya koymuştur. Ancak, pamuk %15 kısıntılı sulama oranına kadar daha duyarlı iken, silajlık mısır bu değerin ötesinde daha duyarlı hale gelmektedir. Bulgular, su tahsisi karar alma sürecinde önemli bir etkiye sahip olacak ve bu temel kaynağın optimum şekilde kullanılmasını sağlayacaktır. Bu model ve çözümü, kısıntılı sulamayı daha iyi yönetmek ve ürün verimlerini optimize etmek için yeni bilgi ve bakış açılarıyla optimizasyon algoritmalarını kullanmaktadır.

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Nomenclature

AREA _c	The cultivated area of crop c per unit area	FC	Critical soil water depletion fraction	n	Number of time slots	SMW _c	Soil moisture wilting point for crop c, in mm per unit depth
AET ₀ ^t	Reference evapotranspiration in time slot t, in mm	GW	Groundwater resources	QWT	QWT is the known amount of water (volume) available for the entire season, m3 ha-1	SMINIT _c	The constant soil water content of deeper layers before the cultivation of crop c, in mm per unit depth
AET _c ^t	Actual evapotranspiration of crop c in time slot t, in mm	DP _c ^t	Deep percolation for crop c in time slot t in mm	PET ₀ ^t	Potential evapotranspiration of reference crop c in time slot t, in mm	SMAV _c ^t	Average soil moisture is the arithmetic mean of the sum of added irrigation water at the end and soil water content at the beginning of time slot t.
c	Type of crop	IRR _c ^t	Irrigation water in time slot t for crop c, in mm	PET _c ^t	Potential evapotranspiration of crop c in time slot t, in mm	TAGEM	General Directorate of Agricultural Research and Policies
CE	Conveyance efficiency of irrigation water, dimensionless	IAE _c	Irrigation water application efficiency for crop c, dimensionless	RAIN ^t	Rainfall in time slot t, in mm	t	Time period or time slot (10 days)
CWR	Crop Water Requirement	Ky _{ass}	Crop yield response factor to water deficit, dimensionless, assumed in the beginning	Root _c ^t	Root depth of crop c at the beginning of time slot t, in cm	Ya _c	Actual yield of crop c in kg per unit area
DSI	General Directorate State Hydraulic Works	Ky _c	Crop yield response factor to water deficit, dimensionless	SMA _c ^t	Soil moisture at the beginning of time slot t for crop c, in mm per unit depth	Ym _c	Maximum yield of crop c in kg per unit area
FAO	Food and Agricultural Organization of the United Nations	m	Number of crops	SMF	Soil moisture field capacity, in mm per unit depth	Yr	Yield Ratio of crop c (Ya _c /Ym _c)

INTRODUCTION

Approximately 70 % of freshwater resources are used for agriculture globally (UN-Water, 2023). While the supply of freshwater resources has been diminishing over time because of global warming and environmental pollution, the demand for freshwater has been increasing depending on population growth and living standards. Countries may be considered water-stressed if they withdraw more than 25 percent of their renewable freshwater resources. They approach physical water scarcity when more than 60 percent is withdrawn, and face severe physical water scarcity when more than 75 percent is withdrawn (FAO, 2017) (IPCC, 2021).

In the next few decades, water sharing among countries that use the same resources may become a global problem, especially in the Eastern Mediterranean region. Additionally, water deficits are linked to 10% of the rise in global migration, and climate change is accelerating the global water crisis (Worldbank, 2021). For this reason, agricultural water should be used effectively and safely to increase the production and yield of crops. Since

agriculture uses the most available fresh water, conservation or optimization of water usage in this sector will be more impactful than managing water consumption in other areas like domestic and industrial use. Water-saving irrigation systems should be followed to save water and maximize yield. To ensure the sustainable development of water resources, it is essential to consider effective utilization and management methods, and to develop scientific and reasonable systems for crop irrigation (Jia, et al. 2024).

The most important guide is the guides published by FAO, the Food and Agricultural Organization of the United Nations. FAO Irrigation and Drainage paper, No. 33, Yield response to water (Doorenbos and Kassam 1979) published in Rome, is a milestone. FAO Irrigation and Drainage Paper No. 56 (Allen, et al. 1998) and FAO Irrigation and Drainage Paper No. 66, Crop yield response to water (Steduto, et al. 2012) contribute to agricultural irrigation. The books Water Sources System Planning and Management (Loucks and Van 2017), and Water Resources Systems Modelling Techniques and Analysis (Vedula and Mujumdar 2016) are other valuable references. The linear or nonlinear models for optimizing irrigation water and crop pattern determination problems for a limited amount of water (reservoir), especially in arid or semi-arid regions, have been studied. The research studies conducted by Ghahraman and Sepaskhah in (2002) and (2004) serve as notable examples.

Biotic and abiotic stresses negatively affect the growth and development of plants, and water stress is a prominent abiotic stress factor (Akçay and Dagdelen 2016). The impact of water deficit on crop yield is considerable, and understanding its effects is crucial for growers and researchers. The yield response factor K_y quantifies the reduction in yield due to inadequate water availability. The specific impact can vary based on factors such as the severity and duration of the water deficit, crop variety, and growth stage. This study compares yield response factors of cotton and summer-planted silage maize to water deficit. This comparison determines which and how to prioritize in case of drought and water deficit when both plants consume the most water in July-September. To optimize water consumption and allocation, it is necessary to analyze the water sensitivity of plants if a limited amount of water is shared by different crops competing for water. K_y is not the only factor determining the allocation of a limited water source. However, other criteria, such as cost-benefit relationships, food security, and social-economic subventions, also contribute to this decision-making.

The study used the data from İslahiye town of Gaziantep Province in Türkiye. Nearby this town, Tahtaköprü Dam, which irrigates İslahiye and the Amik Plain, is available. The source of almost all parameters of İslahiye is the Türkiye Water Consumption Guide of Crops (TAGEM, DSİ, 2016). This guide is prepared using the FAO Penman-Monteith method, making it a unique and valuable source for irrigation studies related to the Eastern Mediterranean region affected by global warming and migration.

The paper is organized as follows. The second section of this study is about material and methods. Firstly, linear models and formulas are introduced. Then summarized definitions of evapotranspiration and crop yield factor K_y were given. Model sets and parameters are detailed in cotton and silage maize potential water demand, evapotranspiration, PET for İslahiye were given. Cotton and silage maize (second product) are spring and summer crops, respectively. The result section discusses the solution of a linear model focusing on crop yield sensitivities and water deficits at different ratios for cotton and silage maize. Comparison of water deficit ratio $[1 - AET(t)/PET(t)]$ in per cent concerning K_y crop yield factor is finalized the discussion of the results. The findings of the study are discussed in the conclusion section.

To the best of the authors' knowledge, this research addresses a spatial gap in the existing literature by focusing on the Eastern Mediterranean region. The limitations of linear solutions, which fail to adequately capture the dynamic nature of climatic data, highlight the urgent need for our proposed advanced forecasting models and AI algorithms. These innovative approaches, with their stimulating capabilities, are crucial in addressing the water stress in this region.

MATERIAL and METHODS

Models and Formulas

Evapotranspiration And The Penman-Monteith Method

Evapotranspiration is the process that combines water loss from the soil through evaporation and from plants through transpiration, playing a crucial role in the water cycle, especially in regions with arid and semiarid climates where irrigation is needed for plant growth and yield due to a lack of enough precipitation (Irmak, 2008). The amount of evapotranspiration depends on climatic variables, crop characteristics, management, and environmental aspects (Allen, et al. 1998). Potential (PET) or maximum evapotranspiration refers to the amount of water that would evaporate and transpire from a specific area if there were always enough water available for plants and soil. Reference evapotranspiration (ET_0) is a measure of how much water evaporates and transpires from a standardized surface, not from the actual crops but from an ideal grass field under specific conditions (Irmak, 2008). AET represents the actual evapotranspiration-water consumption in given conditions. The Blaney-

Criddle method has found wide use to calculate the evapotranspiration in Türkiye instead of the Penman-Monteith method because it requires fewer meteorological elements and the calculation steps are easier. However, the literature states that methods based on more climate parameters, such as the Penman-Monteith method, give more realistic results (Allen, et al. 1998), (Droogers and Allen 2002) and (Xing, et al. 2023). Türkiye Water Consumption Guide of Crops (TAGEM and DSI 2016) uses the FAO Penman-Monteith method.

Crop yield response factor (Ky)

It is important to understand crop yield response factor (Ky) when dealing with irrigation, especially when limited water resources are available. Ky is an indicator of a crop's sensitivity to water deficits. Plants require water for various functions, including nutrient uptake, photosynthesis, and cell growth. Water shortages can negatively impact these processes, causing crop loss. As a result of water shortage, actual crop yield (Ya) decreases relative to actual evapotranspiration (AET). The Ky values are crop specific and vary over the growing season according to growth stages (Steduto, et al. 2012). Ky bigger than 1 indicates high crop sensitivity. These crops need a steady and sufficient water supply throughout the entire growing season. Ky values less than one indicates that the crop is more tolerant to water deficit. These crops can withstand some level of water stress and might recover partially. Finally, when Ky equals 1, it indicates that the reduction in yield is proportional to the water deficit. The following linear relationship (Eq.1) (Doorenbos and Kassam 1979) between relative yield and relative evapotranspiration is presented as follows:

$$\left(1 - \frac{Y_a}{Y_m}\right) = 1 - K_y \left(1 - \frac{AET}{PET}\right) \quad (1)$$

Where Ya and Ym are actual and maximum crop yields, correspond to AET and PET, actual and maximum evapotranspiration, respectively, and Ky is the crop yield response factor. In this equation, referred to as the FAO 33 method, Ky varies depending on species, variety, irrigation method and management, and growth stage when deficit evapotranspiration is imposed. Rao et al. (1988) proposed a multiplicative form of equation (Eq.2) that covers all growth stages simultaneously and is an extended form of one growth stage of water production function developed by the FAO 33 method (Doorenbos and Kassam 1979). The FAO 33 method proposed a multiplicative equation that covers all growth stages simultaneously, and is an extension of one growth stage of water production function for single crop:

$$\frac{Y_a}{Y_m} = 1 - \prod_i^n K_{y_i} \left(1 - \frac{AET_i}{PET_i}\right) \quad (2)$$

where i is an index for the growth stages and it corresponds to time slot t in our notation, and n is the total number of crop growth stages. In addition to the multiplicative form of the above-given equation, it also has been addressed in an additive form as follows:

$$\frac{Y_a}{Y_m} = 1 - \sum_i^n K_{y_i} \left(1 - \frac{AET_i}{PET_i}\right) \quad (3)$$

It was reported that both equations, multiplicative (Eq.2) and additive (Eq.3) forms, bring similar results. In our model, the additive form gives more feasible results.

Modelling of irrigation water allocation

Crop yield optimization for multiple crops

The goal of irrigation water management is to maximize crop production while considering the crop's response to irrigation. This is typically achieved through mathematical programming techniques using crop production functions. A simple additive form of the production function given in Eq.4 is used to discuss the formulation of the optimization problems (Vedula and Mujumdar 2016). This is :

$$\left[\frac{Y_{a_c}}{Y_{m_c}}\right] = 1 - \sum_t^n \sum_c^m \left\{ K_{y_c}^t \left(1 - \frac{AET_c^t}{PET_c^t}\right) \right\} \quad (4)$$

Where, n is the number of time slot, m is the number of crops, $K_{y_c}^t$ is the yield response factor for the growth stages t (10 days time slot) of crop c, y_{a_c} is the actual yield of the crop c, AET_c^t is the actual evapotranspiration at time slot t and PET_c^t is the potential evapotranspiration at time slot t for the crop c.

Soil moisture balance

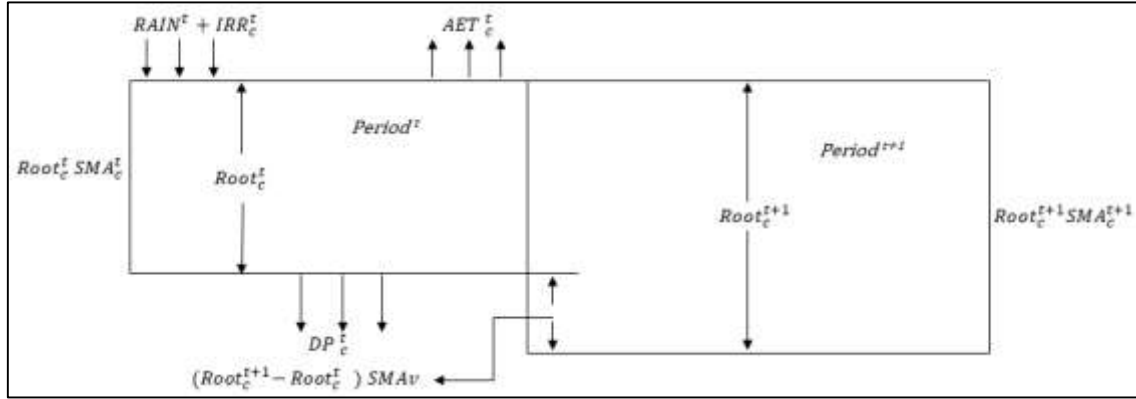


Figure 1 Schematic Diagram for Soil Moisture Balance (Vedula and Mujumdar 2016)
Şekil 1 Toprak Nem Dengesi Şematik Diyagram (Vedula and Mujumdar 2016)

In Eq.5, SMA_c^t is the soil moisture of crop c at the beginning of the period t , $Root_c^t$ is the root depth of crop c during period t , $RAIN^t$ is the effective rainfall (contribution of rainfall to soil moisture) in the command area in period t , IRR_c^t is the irrigation application to crop c in period t , AET_c^t is the actual evapotranspiration of crop c in period t , SMI_c is the initial soil moisture in the soil zone into which the crop root extends at the beginning of period $t + 1$, GW^t groundwater in time slot t and DP_c^t is the deep percolation. The soil moisture values SMA_c^t , SMI_c and SMA_v^t are in units of depth per unit root depth (mm/cm), and all other terms are in-depth units, namely mm. Schematic Diagram for Soil Moisture Balance (Vedula and Mujumdar 2016) figured out in Figure1.

Actual Evapotranspiration AET_c^t is equal or less than Potential Evapotranspiration (Eq.6). The field capacity of soil, SMF , represents the maximum amount of water that can be held in soil after excess water has percolated out through gravity. Actual Soil Moisture SMA_c^{t+1} is equal or less than soil field capacity SMF (Eq.7 and Eq.13). The permanent wilting point of soil, SMW , represents the point at which roots cannot absorb water remaining in the soil. If the soil's moisture reaches this point, plants die. Eq.8 and Eq.14 are to meet this constraint. For use in linear models, the AET constraint is written as:

$$AET_c^t \leq (SMA_c^t + IRR_c^t + RAIN^t - SMW) PET_c^t / (SMF - SMW) \quad \forall t, c \quad (5)$$

$$AET_c^t \leq PET_c^t \quad \forall t, c \quad (6)$$

$$SMA_c^{t+1} \leq SMF \quad \forall t, c \quad (7)$$

$$SMW \leq SMA_c^t \leq SMF \quad \forall t, c \quad (8)$$

The optimization model for single crop

The optimization problem for single crop (c) may be formulated as follows:

$$\text{Max} \quad \left[\frac{Y_a}{Y_m} \right] = 1 - \sum_t^n \left\{ K_y^t \left(1 - \frac{AET^t}{PET^t} \right) \right\} \quad \forall t, \text{ where } t = 1, 2, \dots, n \quad (9)$$

Subject to:

$$(SMA^{t+1} Root^{t+1}) = (SMA^t Root^t) + RAIN^t + IRR^t - AET^t - DP^t SMA_v (Root^{t+1} - Root^t) \quad \forall t \quad (10)$$

$$AET^t \leq \frac{(SMA^t + RAIN^t + IRR^t - SMW)(PET^t)}{SMF - SMW} \quad \forall t \quad (11)$$

$$\frac{AET^t}{PET^t} \geq 0.5 \quad \forall t \quad (12)$$

$$SMA^{t+1} \leq SMF \quad \forall t \quad (13)$$

$$SMW \leq SMA^t \leq SMF \quad \forall t \quad (14)$$

$$DP^t \geq IRR^t (1 - IAE) \quad \forall t \quad (15)$$

$$\sum \text{IRR}^t \text{ AREA} \leq \text{QWT} \quad \forall t \quad (16)$$

Eq.9 is the objective function of optimization problem to maximize the yield ratio Y_a/Y_m . QWT is the known amount of water (volume) available for the entire season, IRR^t is the amount of water (depth) allocated in period t , and AREA is the crop area. Eq.16 constraints that total irrigation water should be equal or less than total available water, QWT. The problem is formulated for a constant root depth so that the soil moistures, SMF and SMW in the above formulation are in-depth units.

The amount of irrigation water was found for a predetermined cultivation area. The amount of water supply was accepted as constant, although it was not. However, this model can be considered a core model running correctly. It should be enhanced, revised, and some additional constraints or subtractions of some assumptions to adapt it to real-world conditions, overcoming the handicaps of uncertainties of this problem. The number of crops is limited to a single. The time slots are 10 days. If one crop is modelled, it starts from the sowing or planting time to the final harvesting. For example, while the number of time slots is 18 for cotton, it is 10 for silage maize. Irrigation application efficiency (IAE) of less than 100% causes some water percolation below the root zone. Therefore, the model structure needs to include a constraint (Eq.15). If water application efficiency is 100 %, then DP is taken as zero. If the soil water content is less than or equal to FC, then DP becomes zero. For simplicity, 100% irrigation uniformity is assumed. In the soil water balance equation (Eq.10), the initial soil water content is considered constant through the deeper layers of the soil. As the roots deepen, this extra soil water contributes to the equation. Surface runoff water was ignored under the water deficit. The amount of groundwater (GW) is considered negligible. The variation of soil water content between time slot t is considered linear. Eq.1 is taken as a sum of addition. The water deficit ratio is restricted to 50 per cent (Eq.12) since the model is not reliable below this value. Average root length is 100 cm for all crops at all vegetation periods. This study does not consider the other factors which improve the yield including weather conditions, soil type, and fertilizer application. Considering the amount and intensity of rainfall during the summer in the region, this study does not require calculating the effective rainfall.

Model Sets and Parameters

Islahiye town is located in Gaziantep Türkiye, with Latitude 37.0253 (N), Longitude: 36.6311 (E), and Elevation in 518 m (TAGEM and DSI 2016). A study (Asil, 2018) examined the changes in plant patterns in irrigation areas during dry years. Droughts occurred in Islahiye in 2007, 2008, 2011, 2014, and 2016. The plant patterns of the irrigation areas of these years were examined, and it was observed that the planting rates of cotton, wheat, corn, vineyards, and carrots in the irrigation areas varied from year to year due to drought. The selection of cotton and silage maize is not a coincidence; they were selected on purpose.

Türkiye has 29 different climate regions, and Islahiye is located in the region numbered 22 and named the Eastern Mediterranean and Southeastern Anatolia corridor (TAGEM and DSI 2016). It is primarily influenced by the Mediterranean climate. The total annual average rainfall of Islahiye town is 797 mm. When Figure 2 is examined, it is seen that the global maximum points of rainfall are 62 mm in mid-December and 54 mm in mid-February. The driest period starts in mid-June and lasts until mid-September. There is no precipitation at all during this period.

Cotton and water demand

Cotton is primarily cultivated as a raw material for the global textile industry, including in Türkiye. A research study conducted by the Kahramanmaraş Eastern Mediterranean Transition Zone Agricultural Research Institute found that the average yield of cotton was 3,450 kg per hectare during the 2012-2013 period (Gonen and Tanriverdi 2021). As the world cotton production in 2022/23 is 24.62 million tons, Turkey is the 6th largest producer with a production of 0.87 million tons (Ozudogru and TEPGE 2024).

The growth stages of cotton for Islahiye are given in Table 1. Cotton needs 700 to 1300 mm of water to meet its PET requirements depending on climate and the length of the total growing period. In the early vegetative period, crop water requirements are low, about 10 per cent of the total. They are high during the flowering and boll formation period when the leaf area is at its maximum or about 50 to 60 per cent of the total. In the growing period, the requirements decline.

Water consumption of cotton, the potential evapotranspiration $\text{PET}(t)$ mm of cotton, 882 mm per year for Islahiye is given in Figure 3 (TAGEM and DSI 2016). Figure 3 shows the maximum amount of water consumed by cotton from planting in the first ten days of May ($t=1$) to harvesting in the second 10 days of October ($t=18$). This vegetation period lasts approximately 180 days. Based on the data presented in Figure 3, there appears to be a

significant lack of rainfall from mid-June to mid-September, a crucial period for cotton crop growth and maintenance. During this timeframe, approximately 80% of irrigation water is utilized. While there is a small amount of rainfall in May and October, totaling approximately 110 mm, it is dispersed and does not contribute greatly to irrigation efficacy, particularly for cotton crops. The maximum water consumptions of cotton is from July-11-20 to August 21-31. The study does not require calculating effective rainfall, considering rainfall amount and intensity in summer season of İslahiye.

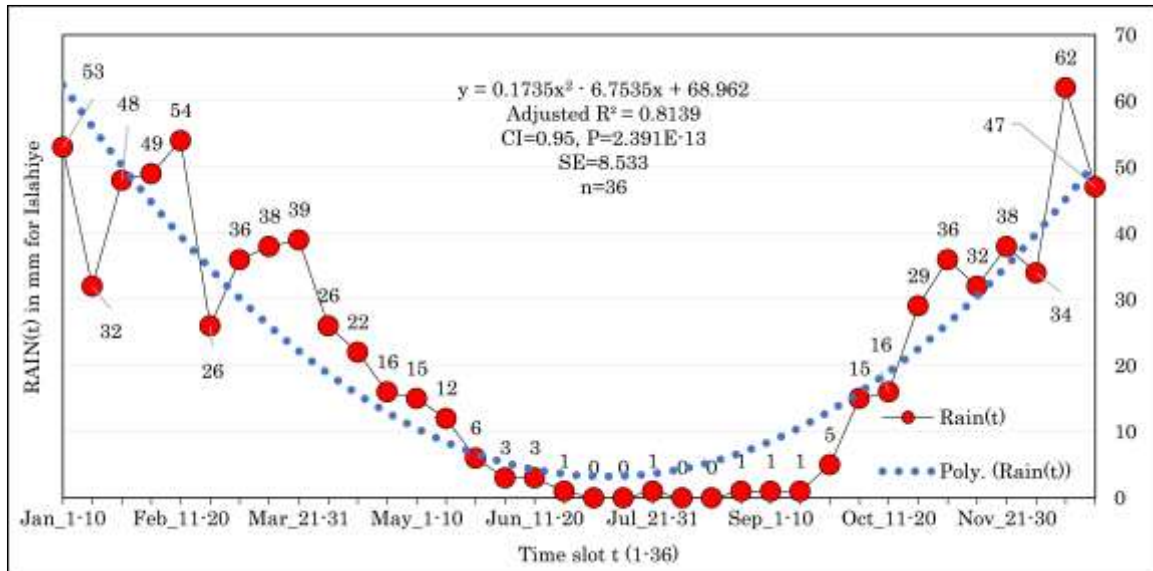


Figure 2 Region 22: Eastern Mediterranean and Southeastern Anatolia corridor region, Rainfall in İslahiye, Annually 797 mm (TAGEM and DSI 2016)

Şekil 2 Bölge 22: Doğu Akdeniz ve Güneydoğu Anadolu geçit bölgesi, İslahiye'de yıllık yağış miktarı 797 mm (TAGEM and DSI 2016)

The values in Figure 2 were used directly without additional calculations to find the net amount of rainfall. Almost half of the total irrigation need is consumed in the period from July II to August III. Crop water requirement, IRR(t), is 82, 94, 83, 82, and 83 mm on July 10-21, July 21-31, August 1-10, August 11-20, and August 21-31, respectively. The maximum amount of water is needed in the third ten days period of July.

The adjusted R^2 of the quadratic trendline in Figure 2 is 0.8025. This indicates that the relationship between time slot t and RAIN(t) is relatively strong, as the adjusted R^2 value is above 70%. The p-value for x^2 is 2.391E-13. Since the p-value for x^2 is less than 0.05 ($p < 0.05$), the null hypothesis H_0 is rejected. The high adjusted R^2 (0.8025) and low p-value for x^2 together suggest that the model is statistically significant and practically meaningful. In other words, a statistically significant quadratic relationship between time slot t and RAIN(t) exists. Since the range of RAIN(t) is 0 to 54 mm, SE = 8.53 is moderate relative to the range of RAIN(t), as it accounts for 15.8% of the total range. In many fields, an SE that is between 10-20 % of the range is considered moderate.

Table 1 Region 22: Eastern Mediterranean and Southeastern Anatolia corridor region crop growing periods for cotton and silage maize (TAGEM and DSI 2016)

Çizelge 1 Bölge 22: Doğu Akdeniz ve Güneydoğu Anadolu geçit bölgesi, pamuk ve silajlık mısır için ürün yetiştirme dönemleri

Crop	Sowing (Planting time)	First period (Germination)	Second period (Vegetation)	Third period (Flowering and fruit growing)	Fourth period (Maturity and harvesting)	Total Vegetation period
Cotton	Apr_11-20	30 days (t=1-3)	46 days(t=4-8)	60 days(t=9-14)	43 days (t=15-18)	179 days
Silage maize (summer)	Jul_I-10	20 days (t=1-2)	30 days(t=3-5)	40 days(t=6-9)	10 days(t=10)	100 days

Maize and water demand

Maize is the crop with the largest cultivation area after wheat and barley in Türkiye (Koc, ve diğerleri 2022). It is an essential plant that considerably contributes to human and animal nutrition and is cultivated over vast areas. It is highly valued as a green crop and for silage production. Maize can be cultivated as either a primary or secondary crop. In Türkiye, approximately 70% of the maize produced is utilized for livestock feed, including cattle, sheep, and poultry (Ozturk ve Orak 2020). Maize variety is widely grown as silage feed worldwide and in Türkiye due to its high dry matter production per unit area. According to a research study carried out by Kahramanmaraş Eastern Mediterranean Transition Zone Agricultural Research Institute, it was determined that the average forage yield of maize was 63,630 kg ha⁻¹ in the years 2019-2020 (Kizilsimsek, Gunaydin and Akbay 2024). In the 2022/23 season, world maize production is 1.16 billion tons, while it is 8.5 million tons for Turkey (Tasdan and TEPGE 2024).

The growth stages of silage maize for Islahiye are given in Table 1. Silage maize needs 400 to 700 mm of water to meet its PET requirements depending on climate and the length of the total growing period. In the early vegetative period, crop water requirements are low, or about 25 percent of the total. They are high during the flowering and grain formation period. Approximately ten days before harvesting the requirements decline. Water consumption of silage maize, the potential evapotranspiration PET(t) mm of cotton, 487 mm per year for Islahiye is given in Figure 4 (TAGEM and DSI 2016). According to Figure 4, there seems to be a notable lack of precipitation from July to October. This timeframe is essential for cultivating and maintaining silage maize crops, with almost all irrigation water being utilized during this period. Although a meagre quantity of rainfall, around 20 mm, is observed in the last ten days of September and the first ten days of October. It is scattered and does not contribute to irrigation efficacy for silage maize. The maximum water consumption of silage maize is from Aug_1-1 to Sep_11-20, which is almost 70% of the total consumption of 487 mm or 4,870 m³ ha⁻¹. In this study, effective rainfall was not calculated. Crop Water Requirement CWR, is 55, 74, 83, 64, and 57 mm for the time slots of Aug_1-10, Aug_11-20, Aug_21-31, Sep_1-10, and Sep_11-20, respectively. The highest water requirement is during the third ten days of August (Aug_21-31).

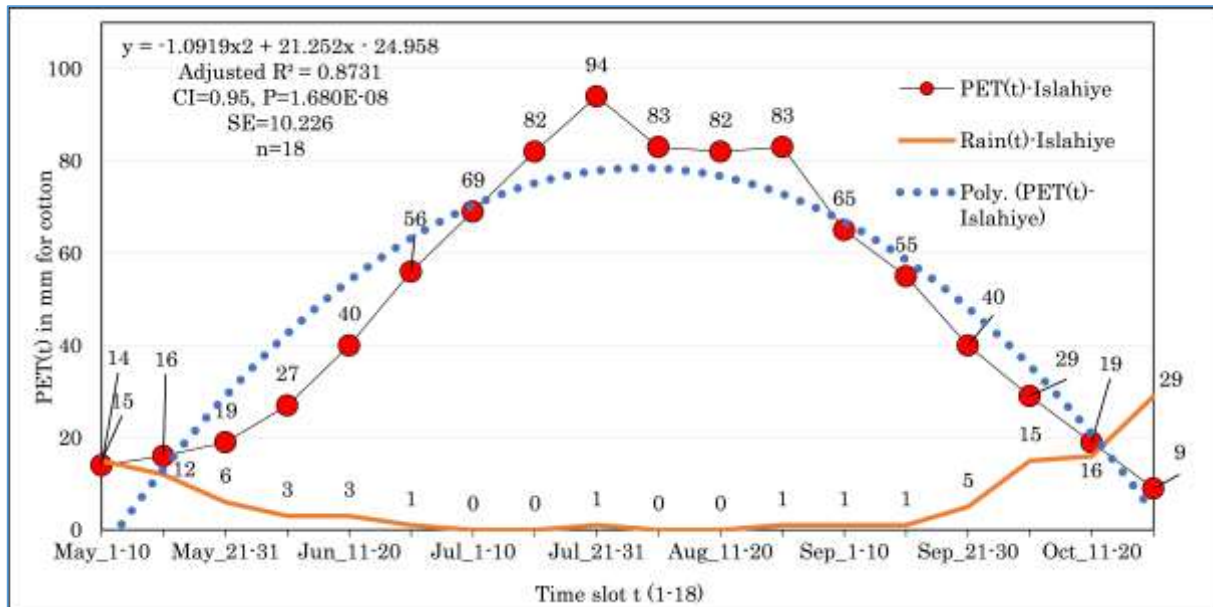


Figure 3 Potential Evapotranspiration PET(t) mm of cotton, 882 mm per year for Islahiye and RAIN(t) (TAGEM and DSI 2016)

Şekil 3 Pamuğun Potansiyel Evapotranspirasyon PET(t) mm değeri, İslahiye için yıllık 882 mm ve yıllık yağış miktarı RAIN(t) (TAGEM and DSI 2016)

The adjusted R² of the quadratic trendline in Figure 3 is 0.8731. This indicates that the relationship between time slot t and PET(t) is relatively strong, as the adjusted R² value is above 70%. The p-value for x² is 1.680E-08. Since the p-value for x² is less than 0.05 (p < 0.05), the null hypothesis H₀ is rejected. The high adjusted R² (0.8731) and low p-value for x² together suggest that the model is statistically significant and practically meaningful. In other words, a statistically significant quadratic relationship between time slot t and PET(t) exists. Since the range of PET(t) is 9 to 94 mm, SE = 10.22 is moderate relative to the range of RAIN(t), as it accounts for 12.0% of the total range. In many fields, an SE that is between 10-20 % of the range is considered moderate.

Statistical Analysis

MS Excel 2016 TOOLPAK for the Regression analysis tool which performs linear regression analysis (Output: Analysis of Variance, ANOVA) by using the "least squares" method to fit a line/curve through a set of observations by a single dependent one or more independent variables was used to analyze the data of Figures 2, 3, 4, 5, 7 and 9 (CI=0.95, p<.05).

Since we have more than one (five) dependent variables or predictors, STATGRAPHICS Centurion v16, multiple sample comparison analysis (Output: Analysis of Variance, ANOVA) was used to analyze the data of Figures 6 and 8.

RESULTS and DISCUSSION

Crop Yield Calculation of Cotton

The optimization model in equations 11-16 was solved to maximize the objective function yield ratio (Ya/Ym) by a Mixed-Integer Programming (MIP) Solver. Variable IRR(t), AET(t), SMA(t) and DP(t) were calculated. The time slot set t is valued 1-18 and corresponds to May_1-10 to Oct_21-31. Scalar values: SMF, Soil field capacity maximum soil moisture is 3.2 mm per cm, SMW, Soil wilting point is 1.6 mm per cm, AE, Irrigation application efficiency is 0.85, DF, Critical soil water depletion fraction is 0.5, SMAINIT, Initial soil moisture at time t=1 is 2.8mm per cm, and AREA, Total command area is 10,000 m² or one hectare.

Parameters: RAIN(t), Rainfall in time slot t for Islahiye in mm is given in Figure 2, PET(t), Standard potential evapotranspiration for cotton in time slot t for Islahiye is given in Figure 3 in mm, Ky_{ass}(t), Yield factor values for cotton in time slot t from 1 to 18 are assumed as 0.2, 0.2, 0.25, 0.25, 0.25, 0.25, 0.35, 0.45, 0.5, 0.5, 0.45, 0.4, 0.3, 0.25, 0.25, 0.25, 0.2, and 0.20, respectively. While the Actual yield over maximum yield, Ya/Ym is free variable;

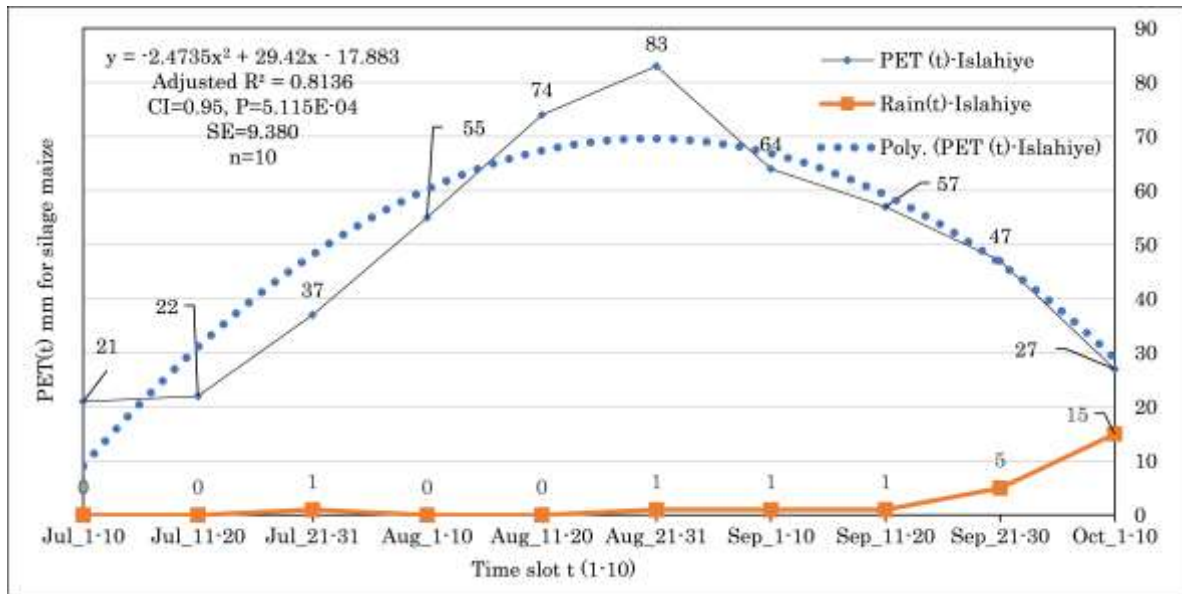


Figure 4 Potential Evapotranspiration PET(t) mm of silage maize, 487 mm per year for Islahiye and RAIN(t) (TAGEM and DSI 2016)

Şekil 4 Silajlık mısırın Potansiyel Evapotranspirasyon PET(t) mm değeri, Islahiye için yıllık 487 mm ve yıllık yağış miktarı RAIN(t) (TAGEM and DSI 2016)

The adjusted R² of the quadratic trendline in Figure 4 is 0.8136. This indicates that the relationship between time slot t and PET(t) is relatively strong, as the adjusted R² value is above 70%. The p-value for x² is 5.115E-04. Since the p-value for x² is less than 0.05 (p < .05), the null hypothesis H₀ is rejected. The high adjusted R² (0.8136) and low p-value for x² together suggest that the model is statistically significant and practically meaningful. In other words, a statistically significant quadratic relationship between time slot t and PET(t) exists. Since the range of PET(t) is 21 to 83 mm, SE = 9.380 is moderate relative to the range of RAIN(t), as it accounts for 15.1% of the total range. In many fields, an SE that is between 10-20 % of the range is considered moderate.

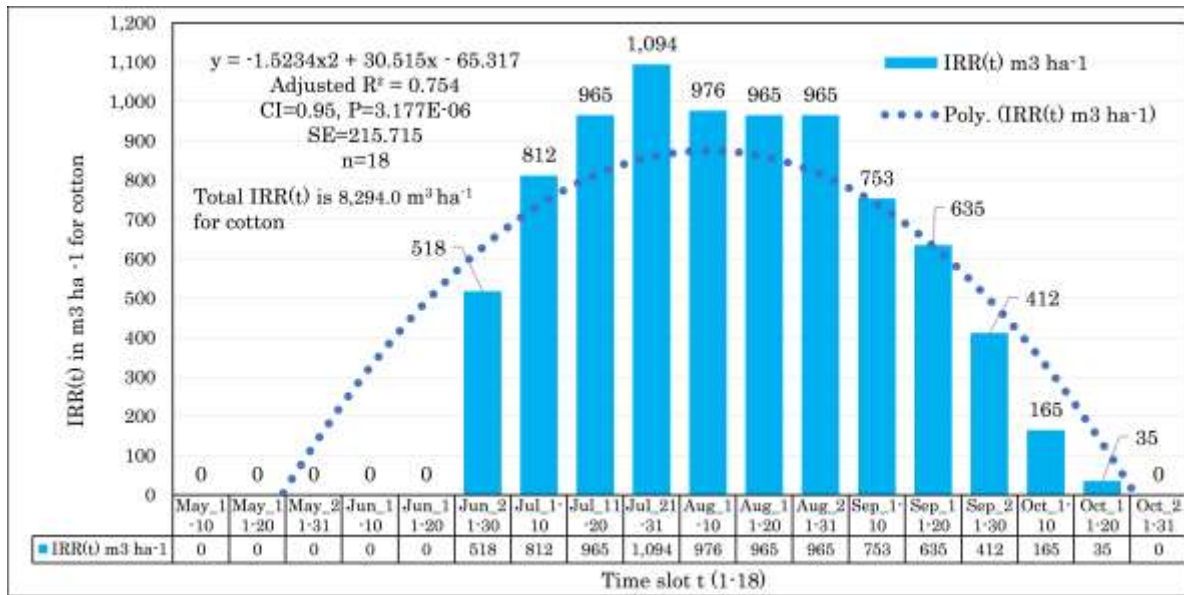


Figure 5 IRR(t) Irrigation amount of water in time slot t of cotton in m³ ha⁻¹ for Islahiye
Şekil 5 IRR(t) Islahiye'de t zaman diliminde pamuk için m³ ha⁻¹ olarak sulama suyu miktarı

Actual Soil moisture SMA(t), Actual evapotranspiration AET(t), Deep Percolation DP(t) and Allocated amount of water IRR(t) are positive variables. According to Figure 5, the crop water requirement of cotton starts at time t equals 6 (The third ten-day of June) with 517.7 m³ ha⁻¹ and ends at t equals 17 (The second ten-day of October) with 35 m³ ha⁻¹. The irrigation water requirement of cotton for the maximum yield is 8,294 m³ ha⁻¹ for Islahiye for the whole vegetation season, 180 days. Maximum yield means actual yield Ya equals Ym, and then Ya/Ym= 1.

The adjusted R² of the quadratic trendline in Figure 5 is 0.754. This indicates that the relationship between time slot t and IRR(t) is relatively strong, as the adjusted R² value is above 70%. The p-value for x² is 3.177E-06. Since the p-value for x² is less than 0.05 (p < .05), the null hypothesis H₀ is rejected. The high adjusted R² (0.754) and low p-value for x² together suggest that the model is statistically significant and practically meaningful. In other words, a statistically significant quadratic relationship between time slot t and IRR(t) exists. Since the range of IRR(t) is 0 to 1.094 mm, SE = 215.71 is moderate relative to the range of IRR(t), as it accounts for 19.7 % of the total range. In many fields, an SE that is between 10-20 % of the range is considered moderate.

Table 2 gives the amount of IRR(t) for different percentages of water deficit, starting from 0% up to 20%, incrementing 5% per step, applied to the whole vegetation period of cotton. The content of Table 2 is graphically represented in Figure 6. In this study, the efficiency of the ideal irrigation amount was compared with the efficiency at various water shortage rates. Water scarcity rates were 95%, 90%, 85% and 80%. At higher rates of water scarcity, the model does not work properly. Yield ratio Ya/Ym and water deficit per cent relationship of cotton for Islahiye were given in Table 2. Comparison of water deficit ratio [1-AET(t)/PET(t)] in per cent with respect to water consumption of cotton.

Table 2 Cotton yield response factor (water deficit sensitivity) Ky for Islahiye for water deficit 5 %, 10%, 15% and 20%

Çizelge 2 Pamuğun verim tepki faktörünün (Kısıntılı Sulama hassasiyeti), %5, %10, %15 ve %20 kısıntılı sulamaya bağlı olarak değişen değerleri

Cotton water sensitivity				Total IRR(t) in m³ per hectare (Islahiye)
AET(t) PET(t) ⁻¹	Water Deficit %	Ya Ym ⁻¹	Ky	
1.00	0%	1.00	1.00	8,300
0.95	5%	0.83	3.36	7,890
0.90	10%	0.66	3.40	7,470
0.85	15%	0.48	3.47	7,060
0.80	20%	0.30	3.50	6,640

Crop Yield Calculation of Silage Maize

The optimization model in equations 11-16 was solved to maximize the objective function yield ratio Y_r (Y_a/Y_m) by a Mixed-Integer Programming (MIP) Solver. Variable $IRR(t)$, $AET(t)$, $SMA(t)$ and $DP(t)$ were calculated. The time period set t is valued 1-10 and corresponds to Jul_1-10 to Oct_1-10. Scalar values: SMF, Soil field capacity maximum soil moisture is 3.2 mm per cm, SMW, Soil wilting point is 1.6mm per cm, IAE, Irrigation application efficiency is 0.85, DF, Critical soil water depletion fraction is 0.5, SMAINIT, Initial soil moisture at time $t=1$ is 2.8 mm per cm, and AREA, Total command area is 10,000 m² or one hectare.

Parameters: RAIN(t), Rainfall in time slot t for Islahiye in mm is given in Figure 2, PET(t), Standard potential evapotranspiration for silage maize in time slot t for Islahiye are given in Figure 4. Kyass(t), Yield response factor values for silage maize in time slot t from 1 to 10 are assumed as 0.4, 0.4, 0.9, 1.5, 1.5, 1.5, 0.9, 0.5, 0.20 and 0.2, respectively. While Actual yield over maximum yield, Y_a/Y_m is free variable; $SMA(t)$, Actual Soil moisture, $AET(t)$,

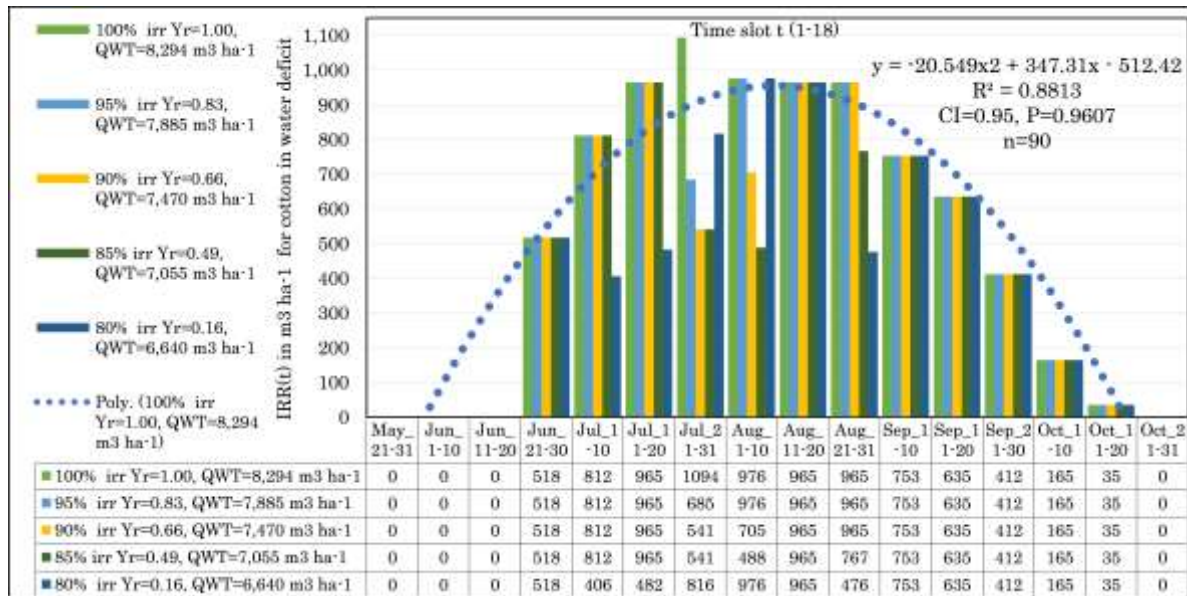


Figure 6 Yield ratio Y_a/Y_m and water deficit per cent relationship of cotton for Islahiye for water deficit 5 %, 10%, 15%, and 20%

Figure 6 Islahiye'de pamuk için Y_a/Y_m verim oranıyla, %5, %10, %15 ve %20 kısıntılı sulama arasındaki ilişki

For the data of Figure 6, one dependent (time slot t) and 5 independent variables (irrigation amount in 5 different deficit ratios), the ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. The F ratio, which in this case equals 0,154065, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is greater than or equal to 0,05, there is not a statistically significant difference between the means of the 5 variables at the 95,0% confidence level.

Actual evapotranspiration, $DP(t)$, Deep Percolation and $IRR(t)$, Allocated amount of water in cubic meter are positive variables. According to Figure 7, the crop water requirement of silage maize starts at time slot t number 1(The first ten-day of July) with 271 m3 ha-1 and ends at t number 10 (The first ten-day of October) with 142 m3 ha-1. The irrigation water requirement of silage maize for one hectare for Islahiye is 5,470 m3 for the whole vegetation season, 100 days, for the maximum yield. Maximum yield means actual yield Y_a equals Y_m , and then $Y_a/Y_m = 1$.

The adjusted R^2 of the quadratic trendline in Figure 7 is 0.797. This indicates that the relationship between time slot t and $IRR(t)$ is relatively strong, as the adjusted R^2 value is above 70%. The p-value for x^2 is 5.124E-04. Since the p-value for x^2 is less than 0.05 ($p < .05$), the null hypothesis H_0 is rejected. The high adjusted R^2 (0.797) and low p-value for x^2 together suggest that the model is statistically significant and practically meaningful. In other words, a statistically significant quadratic relationship between time slot t and $IRR(t)$ exists. The range of $IRR(t)$ is 141.18 to 964.71mm and $SE = 12.375$ is regarded as a good fit to the range of $IRR(t)$, as it accounts for 1.50 % of the total range. In many fields, an SE that is less than 10% of the range is considered a good fit.

Yield ratio Y_a/Y_m and water deficit per cent relationship of silage maize for Islahiye were given in Table 3. Comparison of water deficit ratio [$1-AET(t)/PET(t)$] in per cent with respect to water consumption of silage maize. Table 3 gives the amount of $IRR(t)$ for different percentages of water deficit, starting from 0% up to 20%,

incrementing 5% per step, applied to the whole vegetation period of silage maize. The content of Table 3 are graphically represented in Figure 8. In this study, the efficiency of the ideal irrigation amount was compared with the efficiency at various water shortage rates. Water scarcity rates were 95%, 90%, 85% and 80%. At higher rates of water scarcity, the model does not work properly.

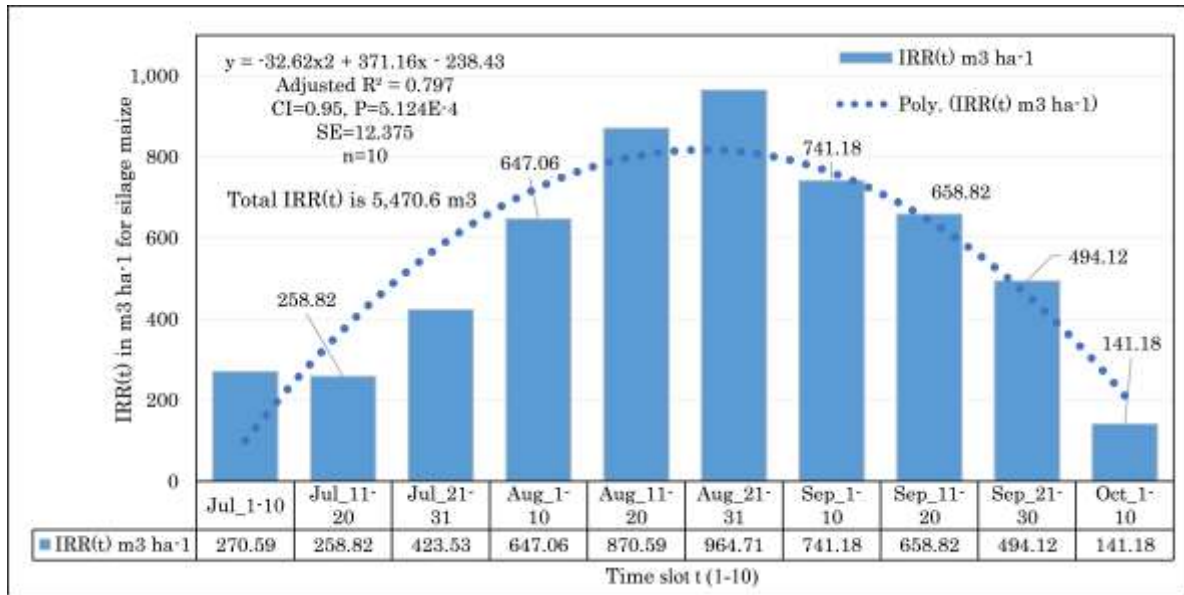


Figure 7 IRR(t) Irrigation amount of water in time slot t in m³ ha⁻¹ of silage maize for Islahiye
Şekil 7 IRR(t) Islahiye’de t zaman diliminde silajlık mısır için m³ ha⁻¹ olarak sulama suyu miktarı

Table 3 Silage maize yield response factor (water deficit sensitivity) K_y for Islahiye for water deficit 5 %, 10%, 15% and 20%

Çizelge 3 Silajlık mısırın verim tepki faktörünün (su kıtlığı hassasiyeti) %5, %10, %15 ve %20 kısıntılı sulama oranlarına bağlı olarak değişen değerleri

Silage-maize yield response factor to water deficit				Total IRR(t) in m³ ha⁻¹ (Islahiye)
AET(t) PET(t)⁻¹	Water Deficit	Y _a Y _m ⁻¹	K _y	
1.00	0%	1.00	0	5,470
0.95	5%	0.90	2	5,210
0.90	10%	0.72	2.8	4,930
0.85	15%	0.50	3.34	4,660
0.80	20%	0.17	4.14	4,380

Water deficit concerning K_y

Analyzing the yield water sensitivities of cotton and silage maize at different water deficit rates needs to be clarified. The Average K_y of cotton and silage maize given in Table 2 and Table 3 are 3.43 and 3.07, respectively. If the water deficit sensitivities of these two crops are compared, the average K_y of cotton is higher than that of silage maize's. According to Figure 9 the silage maize with a regression (trendline) line $y = 0.696x + 1.33$, $R^2 = 0.9944$ comparing with regression line of cotton $y = 0.0487x + 3.31$, $R^2 = 0.9841$ are considered, the silage maize is more sensitive to water deficit than cotton beyond water deficit per cent more than 15%.

For the data of Figure 8, one dependent (time slot t) and 5 independent variables (irrigation amount in 5 different deficit ratios), the ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. The F ratio, which in this case equals 0.208587, is a ratio of the between-group estimate to the within-group estimate. Since the P-value (0.9321) of the F-test is greater than 0.05, there is not a statistically significant difference between the means of the 5 variables at the 95,0% confidence level.

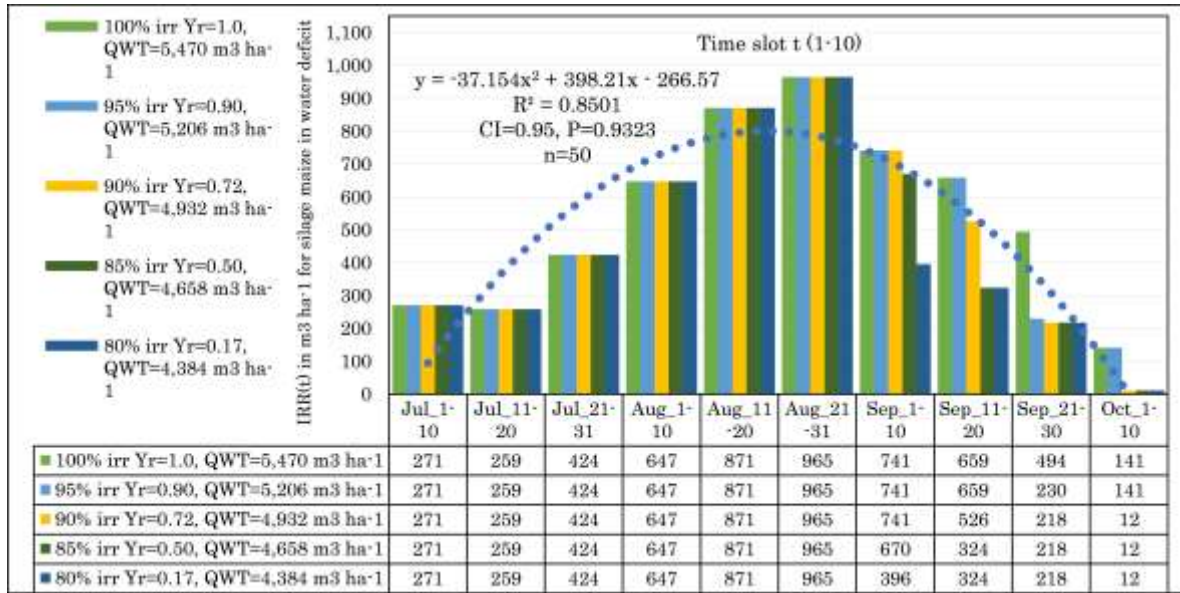


Figure 8 Yield ratio $Y_r = Y_a/Y_m$ and water deficit percent relationship of silage maize for İslahiye for water deficit 5 %, 10%, 15% and 20%

Şekil 8 İslahiye'de silajlık mısır için Y_a/Y_m verim oranıyla, %5, %10, %15 ve %20 kısıntılı sulama oranları arasındaki ilişki

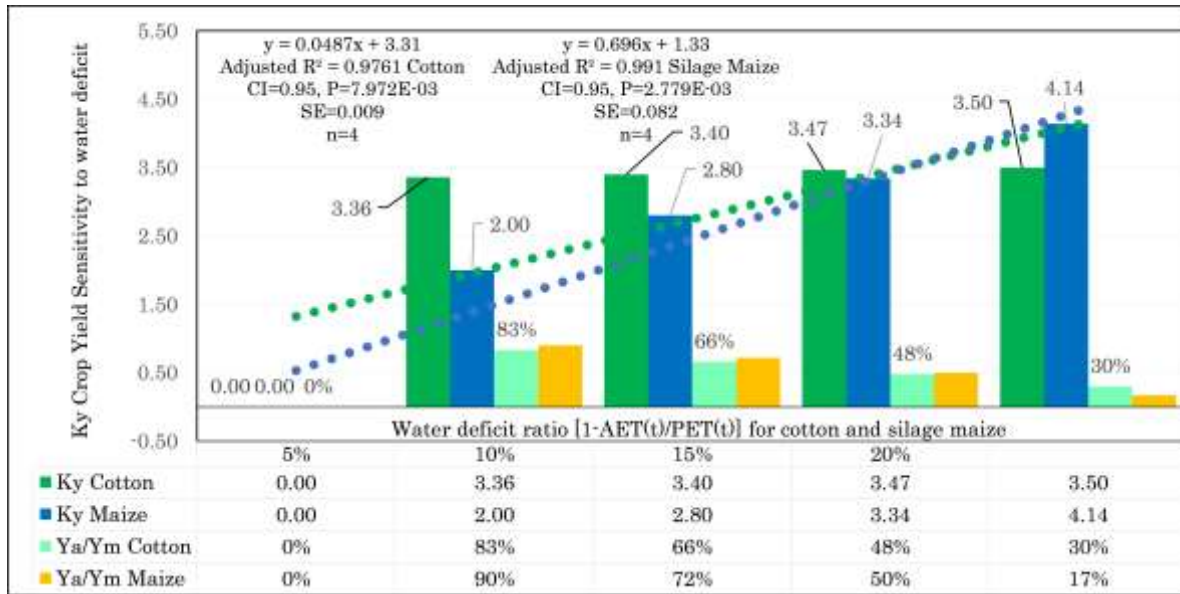


Figure 9 The relationship with water deficit $[1-AET(t)/PET(t)]$ in per cent 5 %, 10%, 15% and 20%. and crop yield ratio Y_a/Y_m with respect to Ky crop yield factor

Şekil 9 Verim tepki faktörü K_y ve bitki verim katsayısı Y_a/Y_m ile %5, %10, %15 ve kısıntılı sulama oranları $[1-AET(t)/PET(t)]$ arasındaki ilişki

The x-axis of Figure 9 represents the water deficit ratio ranging from 0% to 20%, indicating increasing water stress. The y-axis represents two independent variables K_y for cotton and silage maize. Their trendlines are $y = 0.0487x + 3.31$ with an Adjusted $R^2 = 0.9761$ for cotton and $y = 0.696x + 1.33$ with an Adjusted $R^2 = 0.991$ for silage maize. It can be deduced that there is a strong positive linear relationship between the water deficit ratio and K_y for both. Confidence Interval (CI) in the results is 0.95 for both crops. The P value is $= 7.972 \text{ E-}03$ for cotton and $2.779 \text{ E-}03$ for silage maize. Statistically, both values are significant since they are highly bigger than 0.05. Then the null hypothesis H_0 are rejected for both cotton and silage maize. The Standard Error (SE) is 0.009, which indicates high precision for cotton, while 0.082 for silage maize designates a slight increase over 0.05 but still represents a high level of precision. The two crops have a total of 4 data points, which has a small sample size ($n=4$), but is sufficient for the analysis of the trends.

Silage Maize has a steeper slope (0.696)) compared to cotton (0.0487)), meaning silage maize is more sensitive to water deficit. Its performance declines more rapidly as water stress increases. At 0% water deficit (no stress), both crops perform well, but as the deficit increases, their performance declines. Silage Maize shows a sharper decline, which makes it more vulnerable to water stress compared to cotton. Maize is significantly affected by water stress, which impacts various yield components such as plant height, dry weight, and grain yield. The correlation between these traits and yield is notably altered under stress conditions, emphasizing the crop's sensitivity to water deficit. (Khatibi, et al. 2023). Drought stress affects maize forage yield and quality, with significant reductions in wet and dry forage yields under reduced irrigation (Lobell, Deines and Tommaso 2020).

While cotton yield drops from 100% to 30% as water deficit increases, silage maize yield drops from 100% to 17%, showing greater yield sensitivity. The larger slope for silage maize shows it suffers a greater yield loss per unit of water deficit than cotton. Cotton is more resistant to water deficit, but both crops experience severe yield loss beyond a 15-20% deficit.

If they compete for the same water resources which are limited or unreliable and the only factor is the yield to take a decision, choose cotton. Cotton's yield remains stable even under water stress, making it a more resilient choice. Choose silage maize only if water resources are abundant and consistent. Its yield may be higher under optimal conditions since it is more vulnerable to water stress.

While silage maize is more sensitive to water stress, it may offer higher yields under optimal conditions. The choice between silage maize and cotton should consider not only water availability but also economic and agronomic factors such as market value, soil suitability, and pest resistance (Wei, et al. 2019). These considerations are crucial for making informed decisions. There is no doubt that not only economic but social factors such as agricultural practices also affect the decision. We should consider all the factors have an impact on the decision.

CONCLUSION

The main data source used in this study is the Türkiye Plant Water Consumption Guide, published by TAGEM and DSI in 2016. This guide was prepared using the FAO Penman-Monteith method and this makes it a unique and valuable source for irrigation studies of Türkiye. This study takes related data, including PET_c of crops, growing periods, vegetation time, and rainfall values from this source. Studies related to crop yield sensitivity factors are mainly supported by practical field studies, considerably contribute to the country's economy and the sustainable use of natural resources, usually conducted by agricultural scientists in Türkiye. Our model and solution of the problem using the optimization algorithms make our study one step ahead.

In this article, we present the findings of our unbiased investigation into the impact of water scarcity on the yield of cotton and silage maize. Our research, conducted using the data of Gaziantep's İslahiye district, reveals an interesting observation. Despite both crops competing for water during the same period, the difference in their water-yield sensitivity relationship is moderate.

This study reveals that the yields of both crops are highly affected by water deficit. Their sensitivities change depending on the percentage of water deficit. Until a 15 per cent deficit, cotton is more sensitive, and beyond this value, silage maize has higher sensitivity.

Our model was developed under the assumption that water deficit remains consistent across all time periods (t). The crop response factor to water deficit, K_y^t , varies for each plant depending on its growth stage. Typically, the flowering and fruiting stages are the most vulnerable to drought. For instance, the highest K_y^t for cotton occurs during the flowering and boll formation period, which corresponds to time periods t₉ to t₁₄. In contrast, for maize, the peak K_y^t is observed during the grain development period, which spans from t₆ to t₉. Incorporating these considerations into future model studies will yield more meaningful results for practical applications.

To make a decision, choose cotton over silage maize if there are limited or unreliable water resources and only the yield matters. The yield of cotton remains stable under water stress, which makes it more resilient. If water resources are abundant and consistent, silage maize is a good choice. As it is more sensitive to water stress, its yield may be higher under ideal conditions.

This article points out a critical knowledge gap regarding water stress forecasting in the Eastern Mediterranean region. Since linear models are inherently limited in capturing non-linear dynamics of climate data, advanced forecast models and artificial intelligence (AI) algorithms are recommended. These innovative approaches, with their continually evolving capabilities, are crucial in addressing the water stress in the region and improving agricultural water management.

Summary of Researchers' Contribution Declaration

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

Conflict of Interest Statement

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

REFERENCES

- Akçay, S., and N. Dagdelen. 2016. "Water Use Efficiency, Yield and Yield Components of Second Crop Sunflower Under Deficit Irrigation." *Turkish Journal of Field Crops* 21(2), 190-199. doi:10.17557/tjfc.05690.
- Allen, R. G., L. S. Pereira, D. Raes, and M. Smith. 1998. "FAO Irrigation and Drainage Paper No. 56 Crop Evapotranspiration." (Food and Agriculture Organization of the United Nations).
- Asil, H. 2018. "Irrigation from Tahtakopru Dam Change of Plant Pattern in the Dry Years of Irrigation." *International Eurasian Conference on Science, Engineering and Technology November 22-23, 2018*. Ankara: (EurasianSciEnTech 2018). Accessed October 30, 2024. <https://www.researchgate.net/publication/329972930-Tahtakopru-barajindan-sulama-yapan-sulama-birliklerinin-kurak-yillarda-bitki-deseni-degisi-kligi>.
- Doorenbos, J., and A. H. Kassam. 1979. "Yield Response to Water FAO-33". Rome, The Food and Agriculture Organization of the United Nations.
- Droogers, P., and R. G. Allen. 2002. "Estimating Reference Evapotranspiration Under Inaccurate Data Conditions." *Irrigation and Drainage Systems (Food and Agriculture Organization of the United Nations)* 16, 33-45. doi:doi.org/10.1023/A:1015508322413.
- FAO. 2017. "The Future of Food and Agriculture Trends and Challenges". Rome: FAO.
- Ghahraman, B., and A. R. Sepaskhah. 2004. "Linear and non-linear optimization models for allocation of a limited water supply." *Irrigation and Drainage* 1, 39-54. doi:doi.org/10.1002/ird.108.
- Ghahraman, B., and A. R. Sepaskhah. 2002. "Optimal allocation of water from a single purpose reservoir to an irrigation project with pre-determined multiple cropping patterns." *Irrig Sci* 21, 127-137. doi:doi.org/10.1007/s002710100040.
- Gonen, E., and C. Tanriverdi. 2021. "The Effects of Different Nitrogen Levels and Irrigation Intervals on Yield and Water Productivity of Cotton Grown in the Eastern Mediterranean Region." *Turkish Journal of Agricultural and Natural Sciences (TURKJANS)* 1, 898 - 904. doi:doi.org/10.30910/turkjans.789963.
- IPCC. 2021. *Climate Change (IPCC)*. Cambridge University Press, 2023. Accessed February 5, 2025. doi:https://doi.org/10.1017/9781009157896.
- Irmak, S. 2008. "Evapotranspiration." In Encyclopedia of Ecology, by Fath B. D. Jørgensen S.E., 1432-1438. Academic Press 1. doi:doi.org/10.1016/B978-008045405-4.00270-6.
- Jia, L., L. Xuecan, S. Yuanyuan, and Y. Haiye. 2024. "Different Irrigation Rates Effect on the Yield and Quality of Greenhouse Cultivated Platycodon Grandiflorum Crops Considering Transpiration." *Turkish Journal of Agriculture and Forestry (Tubitak)* 48, 1-14. doi:10.55730/1300-011X.3158.
- Khatibi, A., S. Omrani, A. Omrani, S. H. Shojaei, A. Illés, C. Bojtor, S. Mousavi, and J. Nagy. 2023. "Study of drought stress correlation on yield and yield components of maize cultivars (Zea mays L.)." *Acta Agraria Debreceniensis*. doi:https://doi.org/10.34101/actaagrar/1/11495.
- Kizilsimsek, M., T. Gunaydin, and F. Akbay. 2024. "Determination of Feed Quality Characteristics of Soma Silage Maize (Zea Mays L.) Hybrids Cultivated in Eastern Mediterranean Conditions." *Turkish Journal of Field Crops* 29 (1), 46-53. doi:10.17557/tjfc.1458251.
- Koc, D. L., M. Unlu, A. Nur, and R. Kanber. 2022. "Determination of Crop Evapotranspiration and Single Crop Coefficients of Maize Using by a Weighing Lysimeter in Mediterranean Region in Turkey." *KSU J. Agric Nat* 25 (4), 890-900. doi:10.18016/ksutarimdog.vi.802192.
- Lobell, D. B., J. M. Deines, and S. Tommaso. 2020. "Changes in the drought sensitivity of US maize yields." *Nature Food*, 729-735. doi:https://doi.org/10.1038/s43016-020-00165-w.
- Loucks, D., and Beek E. Van. 2017. *Water Resource Systems Planning and Management An Introduction to Methods, Models, and Applications*.
- Ozturk, Yasin, and Adnan Orak. 2020. "Determination of the Yield and Yield Components of Some Corn Varieties as a Second Crop in Tekirdag." *KSU J. Agric Nat* 23 (6), 1634-1646. doi:10.18016/ksutarimdog.vi.727880.
- Ozudogru, T., and TEPGE. 2024. *Agricultural Products Market Cotton*. Monthly, Ankara: TEPGE-Agricultural Economics and Policy Development Institute. Accessed February 12, 2025. <https://arastirma.tarimorman.gov.tr/tepge>.
- Rao, N. H., P. B. S. Sarma, and S. Chander. 1988. "A Simple Dated Water-Production Function for Use in Irrigated Agriculture." *Agricultural Water Management* 13, 25-32. doi:doi.org/10.1016/0378-3774(88)90130-8.
- Steduto, P., T. C. Hsiao, E. Fereres, and D. Raes. 2012. "Crop yield." *FAO Irrigation and Drainage Paper (FAO)* 66. Accessed October 30, 2024. <https://www.fao.org/4/i2800e/i2800e00.pdf>.
- TAGEM, and DSI. 2016. *Plant-Water Use Guide of irrigated crops in Turkey*. Ankara.

- Tasdan, K., and TEPGE. 2024. *Agricultural Products Markets Misir*. Ankara: TEPGE. Accessed February 12, 2025. <https://arastirma.tarimorman.gov.tr/tepge>.
- UN-Water,. 2023. *Sustainable Development Goal 6 Synthesis Report on Water and Sanitation 2023*. Newyork: United Nations Publications.
- Vedula, S., and P. P. Mujumdar. 2016. "Applications of Linear Programming." Chap. 7 in *Water Resources Systems - Modelling Techniques and Analysis*, 207-214. Bangalore: Indian Institute of Science.
- Wei, Y., J. Jin, S. Jiang, S. Ning, Y. Cui, and Y. Zhou. 2019. "Simulated Assessment of Summer Maize Drought Loss Sensitivity in Huaibei Plain, China." *Agronomy* 9(2), 78 . doi:<https://doi.org/10.3390/agronomy9020078>.
- Worldbank,. 2021. Accessed October 2, 2024. <https://www.worldbank.org/en/topic/water/publication/ebb-and-flow-water-migration-and-development>.
- Xing, L., Y. Feng, N. Cui, L. Guo, T. Du, Z. Wu, Y. Zhang, S. Wen, D. Gong, and L. Zhao. 2023. "Estimating Reference Evapotranspiration Using Penman-Monteith Equation." *Journal of Hydrology Volume 620 (Part A)*, doi:doi.org/10.1016/j.jhydrol.2023.129407.

A Scanning Electron Microscope Study of Vegetative Parts of the Genus *Vincetoxicum* in Türkiye

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ABSTRACT

In Türkiye, *Vincetoxicum* is represented by 11 taxa belonging to nine species, three of which (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz, and *V. parviflorum* Decne) are endemics. In this study, the micro-morphology of the stem, petiole, and lamina of all Turkish *Vincetoxicum* taxa was examined using a scanning electron microscope (SEM) for the first time. Micro-morphological observations were carried out on herbarium samples belonging to 21 populations from various habitats. A detailed micro-morphological description of the stem, petiole, and lamina, supported by SEM micrographs, was created for each examined taxon. It was determined that the micro-morphological characteristics such as the pattern of pubescence, the distribution and density of trichomes on the stem, petiole, and lamina, and the position of stomata on both sides of the leaf contributed to the distinction of the Turkish *Vincetoxicum* taxa. On the other hand, no difference was observed among the examined taxa in terms of the appearance of epicuticular wax, and shape and concavity/convexity properties of epidermal cells on stem, petiole, and lamina.

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Türkiye *Vincetoxicum* Cinsinin Vejetatif Kısımlarının Taramalı Elektron Mikroskobu İle İncelenmesi

ÖZET

Vincetoxicum, Türkiye’de üçü endemik (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz ve *V. parviflorum* Decne) olmak üzere dokuz türe ait 11 takson ile temsil edilmektedir. Bu çalışmada, tüm Türkiye *Vincetoxicum* taksonlarının gövde, petiyol ve lamina mikro-morfolojisi taramalı elektron mikroskobu (SEM) kullanılarak ilk kez taksonomik yönden değerlendirilmiştir. Mikro-morfolojik incelemeler, farklı habitatlardan toplanan 21 popülasyona ait herbaryum örnekleri üzerinde gerçekleştirilmiştir. İncelenen her takson için SEM fotoğraflarıyla desteklenen ayrıntılı birer gövde, petiyol ve lamina mikro-morfolojik betimi oluşturulmuştur. Türkiye *Vincetoxicum* taksonlarının ayırımında; gövde, petiyol ve lamina yüzeylerindeki tüylenme çeşidi, tüy dağılımı ve yoğunluğu ile yaprağın her iki yüzeyindeki stoma durumu gibi mikromorfolojik karakterlerin önemli rol oynadığı belirlenmiştir. Diğer taraftan, incelenen taksonlar arasında gövde, yaprak sapı ve lamina üzerindeki epikütikular mumun görünümü, epidermis hücrelerinin şekli ve içbükeylik/dışbükeylik özellikleri bakımından bir fark görülmemiştir.

Botanik

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 23.11.2024

Kabul Tarihi : 28.01.2025

Anahtar Kelimeler

Apocynaceae

Yaprak

SEM

Gövde

Vincetoxicum

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INTRODUCTION

Vincetoxicum Wolf s.str., one of the taxonomically complex genera of the subfamily Asclepiadoideae (Apocynaceae), has attracted the attention of many taxonomists for a long time. Recently, based on extensive morphological data supported by molecular evidence, Liede-Schumann et al. (2012) expanded *Vincetoxicum* by transferring the members of the seven genera in the subtribe Tylophorinae (*Biondia* Schltr., *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Pleurostelma* Baill., *Rhyncharrhena* F. Muell., *Tylophora* R. Br.) into the genus. More recent taxonomic revisions contributing to the systematics of the genus were carried out on a large group of taxa including many new regional endemic species, new combinations, new names, and typifications (Liede-Schumann & Meve, 2018; Shah et al., 2021). The current *Vincetoxicum* s.l. comprises approximately 300 natural species distributed throughout the Far East, Africa, the Mediterranean, Caucasus, southern Russia, and Europe (Liede-Schumann et al., 2016; Liede-Schumann & Meve, 2018), and some introduced species in North America (Sheeley & Raynal, 1996).

Vincetoxicum is a difficult genus due to genetic and morphological variations observed among closely related taxa (Browicz, 1978). These variations lead to difficulties in the identification of *Vincetoxicum* species based solely on traditional (phenetic) characters. Therefore, more reliable characteristics need to be revised by using different methods and techniques in the distinction of *Vincetoxicum* taxa. In order to contribute to the systematics of the genus, to date, numerous taxonomical studies based on anatomical (Metcalf & Chalk, 1950), palynological (Fishbein, 2001; Yamashiro et al., 2008; Verhoeven & Venter, 2001; Sinha & Mondal, 2011; Sreenath et al., 2012; Feng et al., 2012; Chang et al., 2012; Shah & Ahmad, 2014; Yaseen & Perveen, 2014), cytological (Yamashiro et al., 2002), and molecular phylogenetic data (Civeyrel et al., 1998; Liede & Täuber, 2002; Liede et al., 2002; Sennblad & Bremer, 2002; Yamashiro et al., 2004; Liede-Schumann et al., 2012; Liede-Schumann et al., 2016) have been performed on the members of *Vincetoxicum*, excluding Türkiye.

In Türkiye, the genus *Vincetoxicum* is represented by nine species and 11 taxa, three of which (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz, and *V. parviflorum* Decne) are endemics (Güner, 2012; Güven et al., 2021a,b; Makbul & Güven, 2022). The Turkish *Vicetoxicum* is composed of perennial, herbaceous plants growing in dry river valleys, open rocky slopes, steppes, and mountain slopes to shrublands and *Quercus*-dominated forests (Browicz, 1978; Güven, 2017). The detailed morphological properties including color, shape, and indumentum of corolla, size, and shape of corona and gynostegium, and indumentum of stem and leaf were examined using a stereomicroscope for the genus *Vincetoxicum* in Türkiye (Güven, 2017; Güven et al., 2021a). Additionally, the seed micro-morphological (İlçim et al., 2010; Güven et al., 2021a), palynological (Güven et al., 2015, 2021a), cytological and molecular characteristics (Güven et al., 2019) of the Turkish *Vincetoxicum* taxa were also examined comparatively. Although palynological characters such as corpusculum shape (ovate or oblong), pollinium shape (elliptic, clavate, ovate or obovate) and surface ornamentation (rugulate or gemmate) supported the separation of the taxa at the species level (Güven et al., 2015, 2021a), cytological and molecular data (Güven et al., 2019), and seed micro-morphological features (İlçim et al., 2010; Güven et al., 2021a) provided only limited information for the genus.

Micro-morphological properties of vegetative organs including size and shape of epidermal cells, cell wall undulations, epicuticular wax structure, stomatal morphology, patterning, and trichome morphology were very valuable in angiosperm taxonomy (Behnke & Barthlott, 1983; Stace et al., 1984; Sivarajan, 1991; Barthlott, 1994; Theisen & Barthlott, 1994; Barthlott et al., 1998; Carpenter, 2005; Tomaszewski & Zieliński, 2014). However, there is a very limited number of studies on the leaf and stem micro-morphology of Apocynaceae taxa (Joubert, 2007; Xin-sheng, 2010; Carvalho et al., 2017; Bashir et al., 2020; El-Taher, et al, 2020; Medina et al., 2021; Abeysinghe & Scharaschkin, 2022; Ridzuan & Kalu, 2023; Malaspina et al., 2024) including a few numbers of *Vincetoxicum* (Shah et al., 2018). Although stem and leaf indumentum have been considered diagnostic characters within the *Vincetoxicum*, information regarding the vegetative micro-morphological features of the related species is still lacking for the members of Turkish *Vincetoxium*. Therefore, the present study aimed: (1) to document and illustrate a detailed description of the micro-morphology of stem, petiole, and lamina for each Turkish *Vincetoxicum* species using scanning electron microscopy (SEM), (2) to evaluate the taxonomic or diagnostic importance of stem, petiole, and lamina micro-morphological characteristics among the members of *Vincetoxicum* in Türkiye.

MATERIAL and METHOD

Specimens

Micro-morphological observations were carried out on plant samples deposited at the Herbarium of Biology (RUB) at Recep Tayyip Erdogan University. The present study included individuals from 21 different populations,

representing 11 taxa of *Vincetoxicum*, and detailed locality information and distribution in Türkiye of the investigated taxa is listed in Table 1 and Figure 1, respectively.

Scanning electron microscopy

Micro-morphological examinations were carried out on five individuals per population for each examined *Vincetoxicum* taxon, excluding *V. funebre* (10 individuals were used from one population). Only healthy individuals without visible damage or contamination, were selected, and stem parts between the third and fourth nodes of the plant from the base, and fully expanded leaves from the third or fourth nodes were randomly sampled. For each of 10 individuals per 11 examined taxon, small pieces (5 x 5 mm) were cut out from the stem, petiole, and lamina separately, and all pieces were passed through an ethanol series (60-80%) to remove the debris present on the surface. For SEM studies, the cleaned stem petiole and lamina samples were air-dried under ambient conditions and then were transferred on aluminum stubs, coated with gold for 4 min in a sputter-coater and observed by a JEOL-JSM 6610 microscope at the Central Research Laboratories of Recep Tayyip Erdogan University. In this study, SEM examinations were carried out following Güzel (2021) and Aybeke (2022), and we classified the stomatal type following Dilcher (1974). A detailed micro-morphological description of the stem, petiole, and lamina, supported by SEM micrographs, was created for each examined taxon. However, since no differences could be detected between individuals belonging to different populations of each *Vincetoxicum* taxon employing stem, petiole, and lamina micro-morphology, SEM micrographs obtained from only one locality were used for the description of each taxon. The micro-morphological terminology followed that of Barthlott et al. (1998), Stearn (1992), Koch et al. (2008), and Prüm et al. (2012).

Table 1 Locality informations of the investigated taxa

Çizelge 1. İncelenen taksonların lokalite bilgileri

Taxa (Taksonlar)	No	Locality (Lokalite)	Collector number (Toplayıcı numarası)
<i>Vincetoxicum canescens</i>	1	Erzincan: Erzincan to Kemah	S. Güven 36 & S. Makbul (RUB)
(Willd.) Decne subsp.	2	Adana: Pozantı	S. Güven 90 & S. Makbul (RUB)
<i>canescens</i>			
<i>Vincetoxicum canescens</i>	3	Manisa: Kula	S. Güven 51 & S. Makbul (RUB)
(Willd.) Decne subsp.	4	Muğla: Yılanlı Mountain	S. Güven 56 & S. Makbul (RUB)
<i>pedunculata</i> Browicz			
<i>Vincetoxicum coskuncelebiana</i>	5	Ardahan: Çıldır, Taşbaşı Village	S. Güven 69 & S. Makbul (RUB)
S. Makbul & S. Güven	6	Ardahan: Çıldır	S. Güven 127 & S. Makbul
<i>Vincetoxicum funebre</i> Boiss. & Kotschy	7	Ardahan: Posof	S. Güven 126 & S. Makbul (RUB)
<i>Vincetoxicum fuscatum</i>	8	Niğde: Ulukışla	S. Güven 93 & S. Makbul (RUB)
(Hornem.) subsp. <i>fuscatum</i>	9	İstanbul: Paşaköy	S. Güven 22 & S. Makbul (RUB)
<i>Vincetoxicum fuscatum</i>	10	Erzincan: Erzincan to Sakaltutan	S. Güven 35 & S. Makbul (RUB)
(Hornem.) subsp. <i>boissieri</i>	11	Bayburt: Değirmencik Village	S. Güven 73 & S. Makbul (RUB)
(Kusn.) Browicz			
<i>Vincetoxicum hirundinaria</i>	12	Kırklareli: Demirköy	S. Güven 28 & S. Makbul (RUB)
Medicus subsp. <i>hirundinaria</i>	13	Kırklareli: Vize, Kızılağaç	S. Güven 19 & S. Makbul (RUB)
<i>Vincetoxicum parviflorum</i>	14	Tunceli: Ovacık, Karagöl	S. Güven 80 & S. Makbul (RUB)
Decne	15	Kayseri: Yahyalı	S. Güven 95 & S. Makbul (RUB)
<i>Vincetoxicum scandens</i> Somm. & Lev.	16	Trabzon: Çaykara	S. Güven 169 & S. Makbul (RUB)
	17	Rize: Çamlıkköy	S. Güven 30 & S. Makbul (RUB)
<i>Vincetoxicum speciosum</i>	18	Tekirdağ: Saray	S. Güven 20 & S. Makbul (RUB)
Boiss. & Spruner	19	İstanbul: Çatalca	S. Güven 132 & S. Makbul (RUB)
<i>Vincetoxicum tmoleum</i> Boiss.	20	Ankara: Kızılcahamam	S. Güven 61 & S. Makbul (RUB)
	21	Erzurum: Oltu	S. Güven 72 & S. Makbul (RUB)

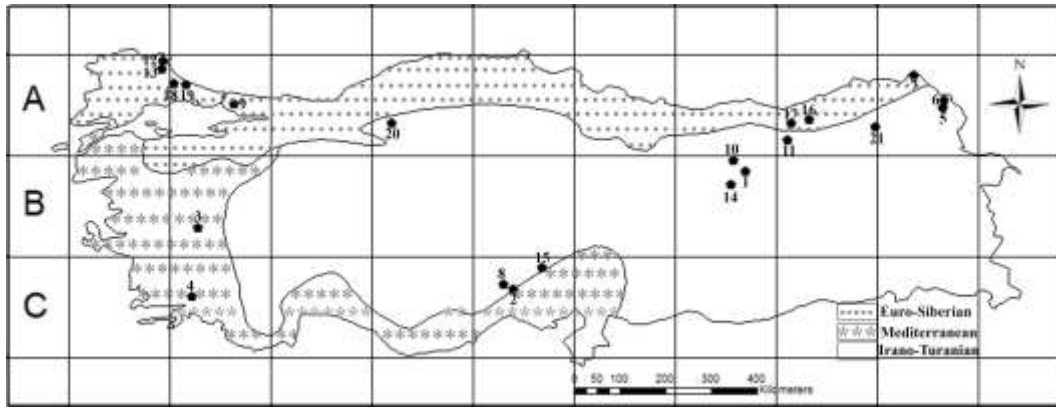


Figure 1. Distribution map of the examined taxa according to phytogeographic regions [The map of Türkiye according to the grid system of P. H. Davis (1965); refer to Table 1 for explanation of accession no].

Şekil 1. İncelenen taksonların fitocoğrafik bölgelere göre dağılım haritası [P. H. Davis (1965)'de kareleme sistemine göre Türkiye haritası; örnek numarasının açıklaması için Tablo 1'e bakınız].

RESULTS

***Vincetoxicum canescens* (Willd.) Decne subsp. *canescens*:** Stem and petiole surfaces with densely canescent-tomentose indumenta all around, consisting of non-glandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 2a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely canescent-tomentose indumenta all around, consisting of nonglandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channeled and straight, epicuticular wax striate (Figure 2g-l).

***Vincetoxicum canescens* (Willd.) Decne subsp. *pedunculata* Browicz:** Stem and petiole surfaces with densely canescent-tomentose indumenta all around, consisting of non-glandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 3a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely canescent-tomentose indumenta all around, consisting of nonglandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channeled and straight, epicuticular wax striate (Figure 3g-l).

***Vincetoxicum coskuncelebiana* S.Makbul & S.Güven:** Stem surface with a crisped indument along one side (uniseriably) (Figure 4a-c) and petiole surface with a sparsely crisped indument all around (Figure 4d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 4a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 4g-l).

***Vincetoxicum funebre* Boiss. & Kotschy:** Stem surface with a crisped indument along two sides (biseriably) (Figure 5a-c) and petiole surface with a densely crisped indument all around (Figure 5d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 5a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 5g-l).

***Vincetoxicum fuscatum* (Hornem.) subsp. *fuscatum*:** Stem surface with a crisped indument along two sides (biseriably) (Figure 6a-c) and petiole surface with a sparsely crisped indument all around (Figure 6d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 6a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 6g-l).

***Vincetoxicum fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz:** Stem surface with a crisped indument along two sides (biseriably) (Figure 7a-c) and petiole surface with a sparsely crisped indument all around (Figure 7d-f),

consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 7a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 7g-l).

***Vincetoxicum hirundinaria* Medicus subsp. *hirundinaria*:** Stem surface with a crisped indument along two sides (biserially) (Figure 8a-c) and petiole surface with a sparsely crisped indument all around (Figure 8d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 8a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 8g-l).

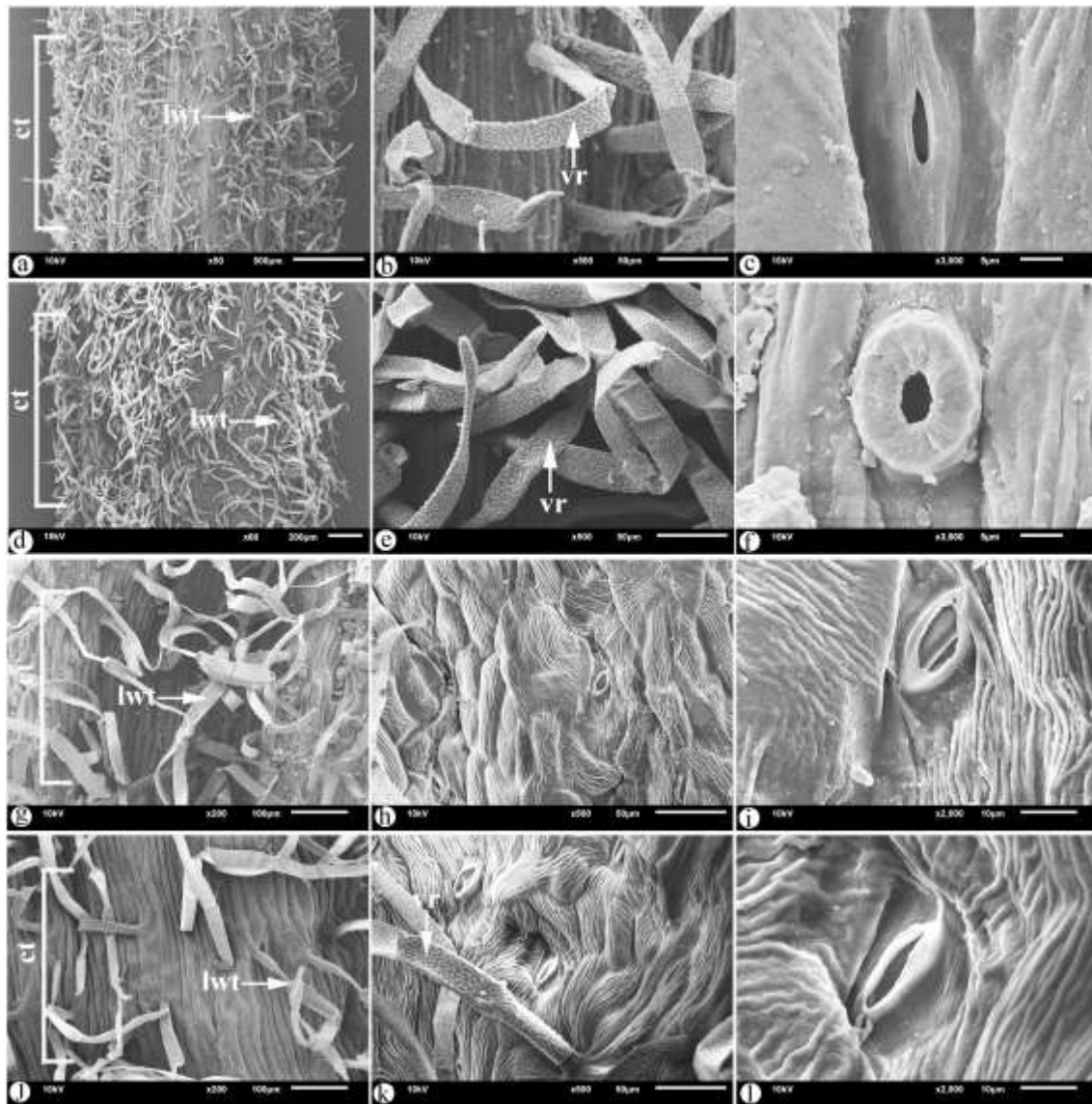


Figure 2. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum canescens* subsp. *canescens* (S.Güven 90 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, ct: canescent-tomentose indumentum (all around), lwt: long wool-like trichome, vr: verrucose trichome ornamentation.

Şekil 2. *Vincetoxicum canescens* subsp. *canescens* (S.Güven 90 & S.Makbul)'in gövde, petiyol ve yaprak yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, ct: canescent-tomentose tüy örtüsü (tüm yüzey boyunca), lwt: uzun yünsü tüy, vr: verrucose tüy ornamentasyonu.

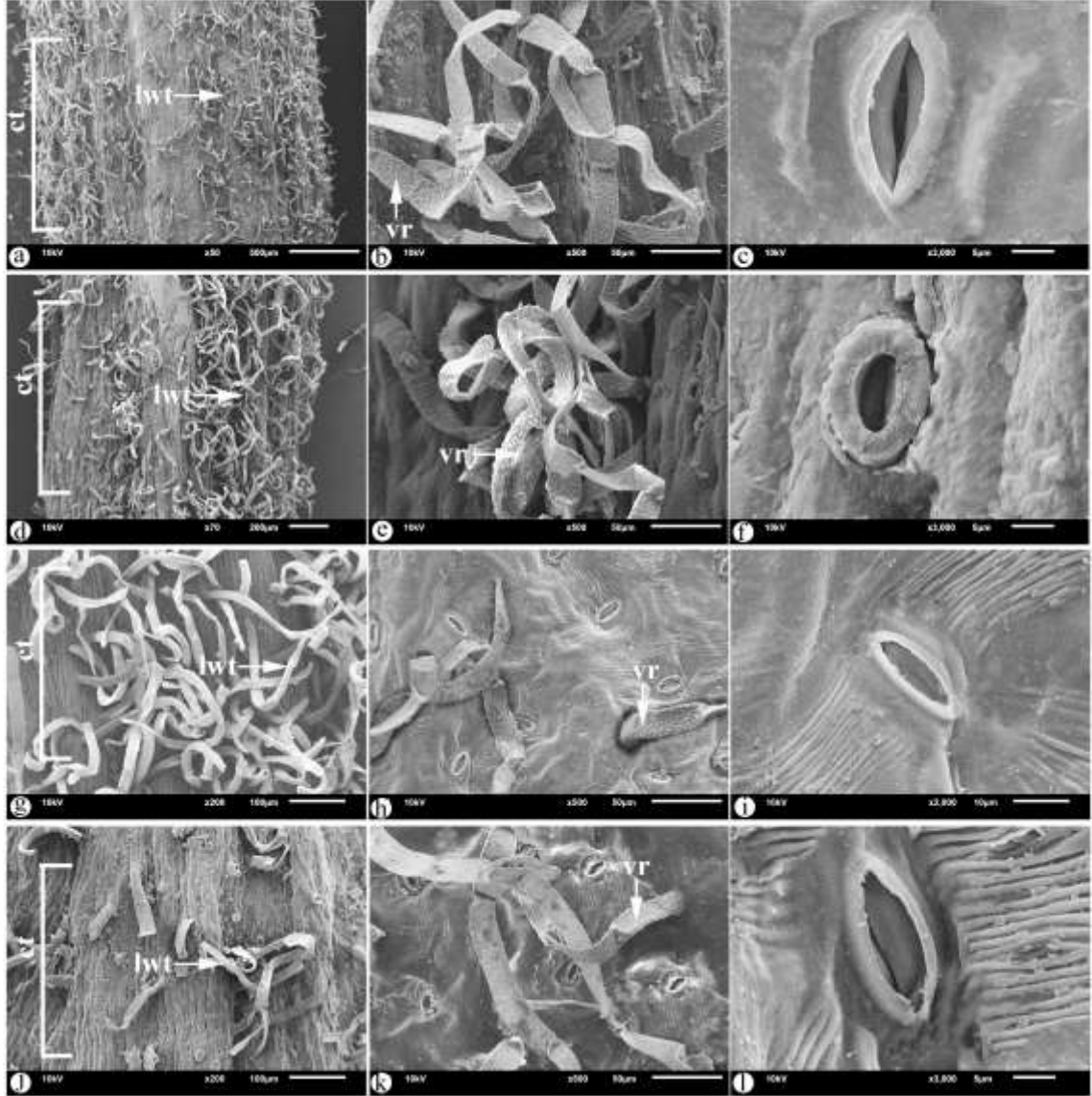


Figure 3. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum canescens* subsp. *pedunculata* (S.Güven 56 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, ct: canescent-tomentose indumentum (all around), lwt: long wool-like trichome, vr: verrucose trichome ornamentation.

Şekil 3. *Vincetoxicum canescens* subsp. *pedunculata* (S.Güven 56 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, ct: canescent-tomentose tüy örtüsü (tüm yüzey boyunca), lwt: uzun yünsü tüy, vr: verrucose tüy ornamentasyonu.

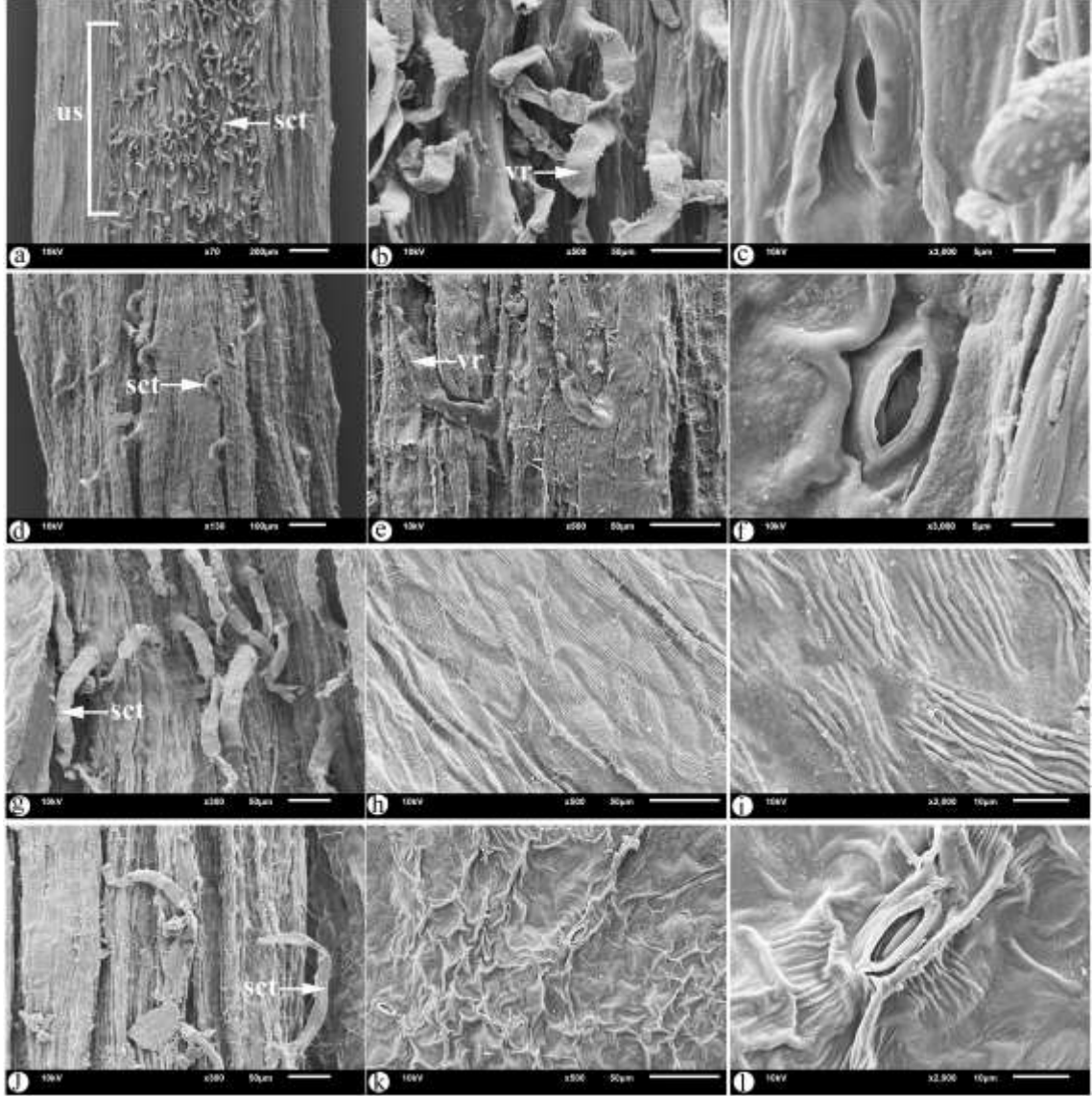


Figure 4. Scanning electron micrographs of stem, petiole, and lamina surfaces of *Vincetoxicum coskuncelebiana* (S.Güven 127 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, us: hairy along one side (uniseriell), vr: verrucose trichome ornamentation.

Şekil 4. *Vincetoxicum coskuncelebiana* (S.Güven 127 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, us: bir taraf boyunca tüylü (uniseriell), vr: verrucose tüy ornamentasyonu.

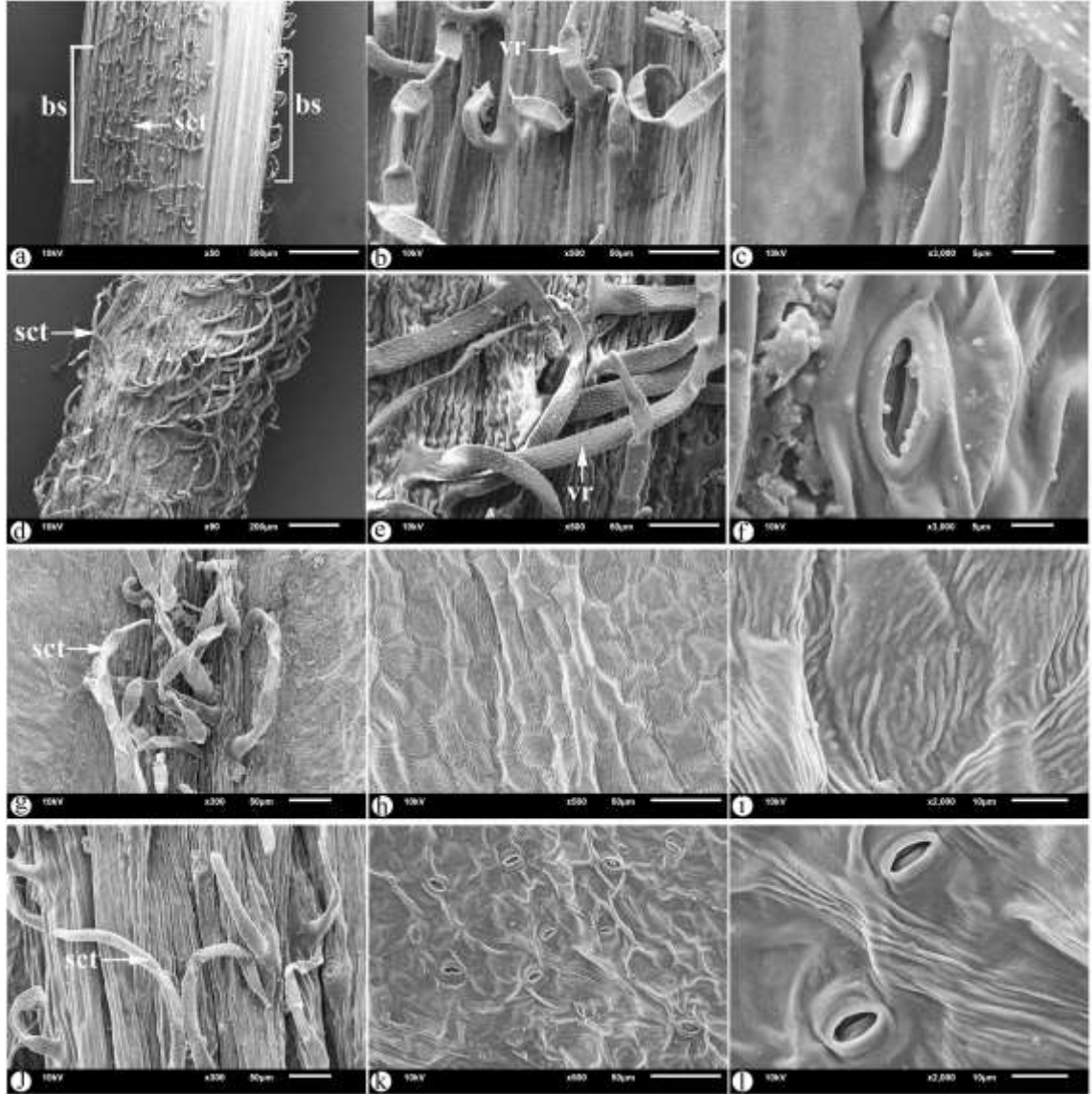


Figure 5. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum funebre* (S.Güven 126 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 5. *Vincetoxicum funebre* (S.Güven 126 & S.Makbul)'nin gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

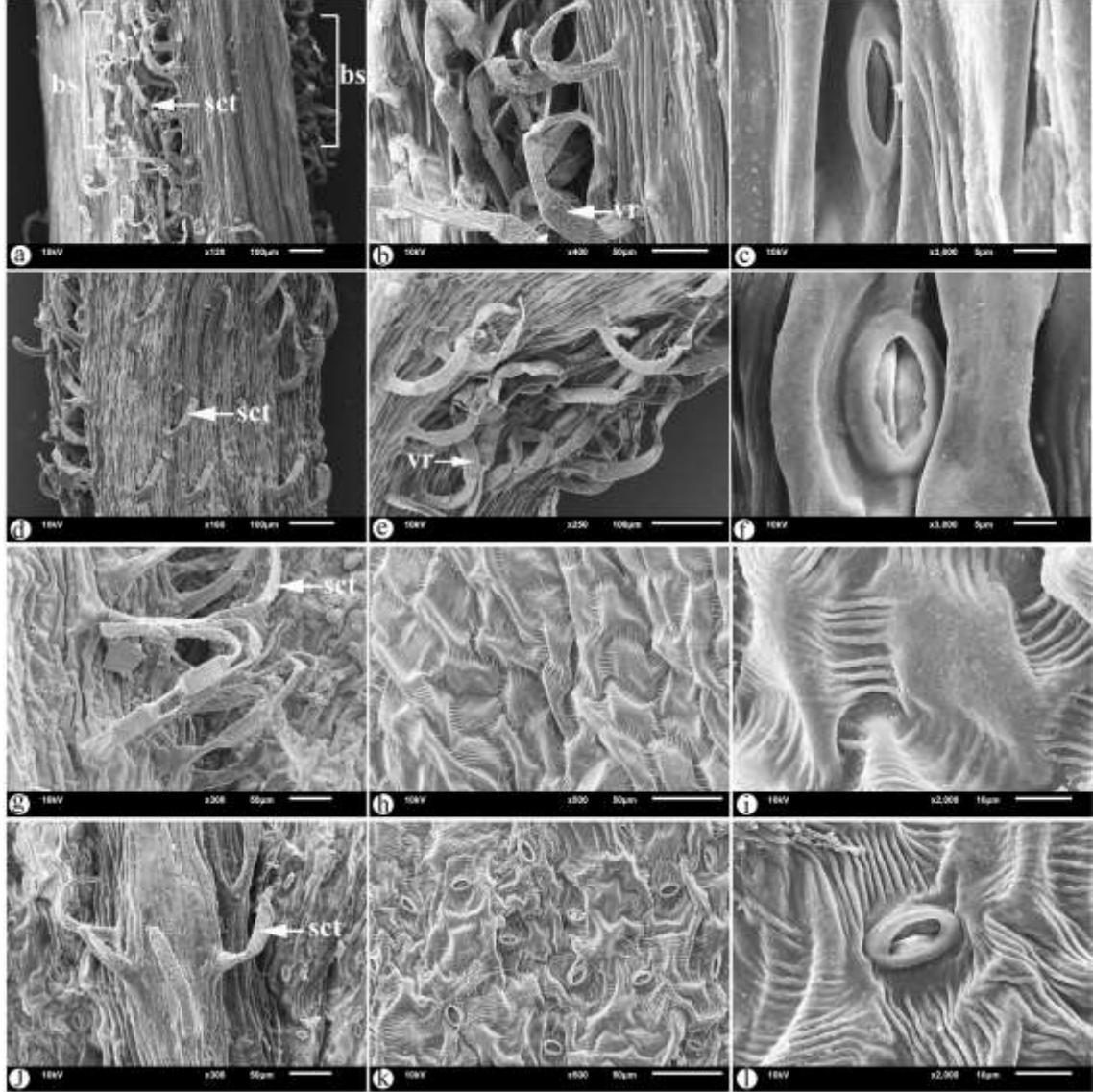


Figure 6. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum fuscatum* subsp. *fuscatum* (S.Güven 22 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biseri ally), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 6. *Vincetoxicum fuscatum* subsp. *fuscatum* (S.Güven 22 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biseri ally), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

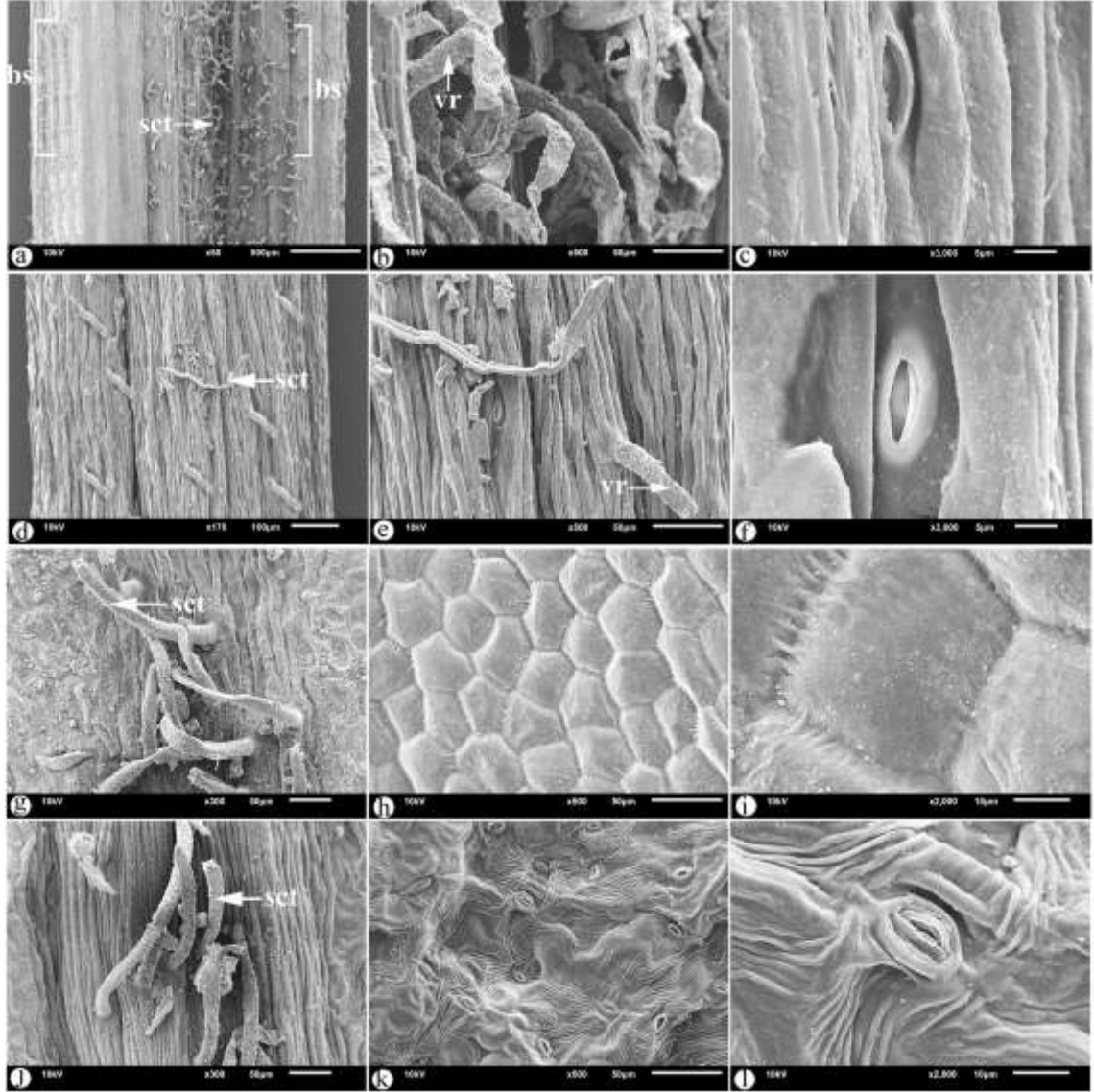


Figure 7. Scanning electron micrographs of stem, petiole and lamina surfaces *Vincetoxicum fuscatum* subsp. *boissieri* (S.Güven 73 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 7. *Vincetoxicum fuscatum* subsp. *boissieri* (S.Güven 73 & S.Makbul)'nin gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), crt: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

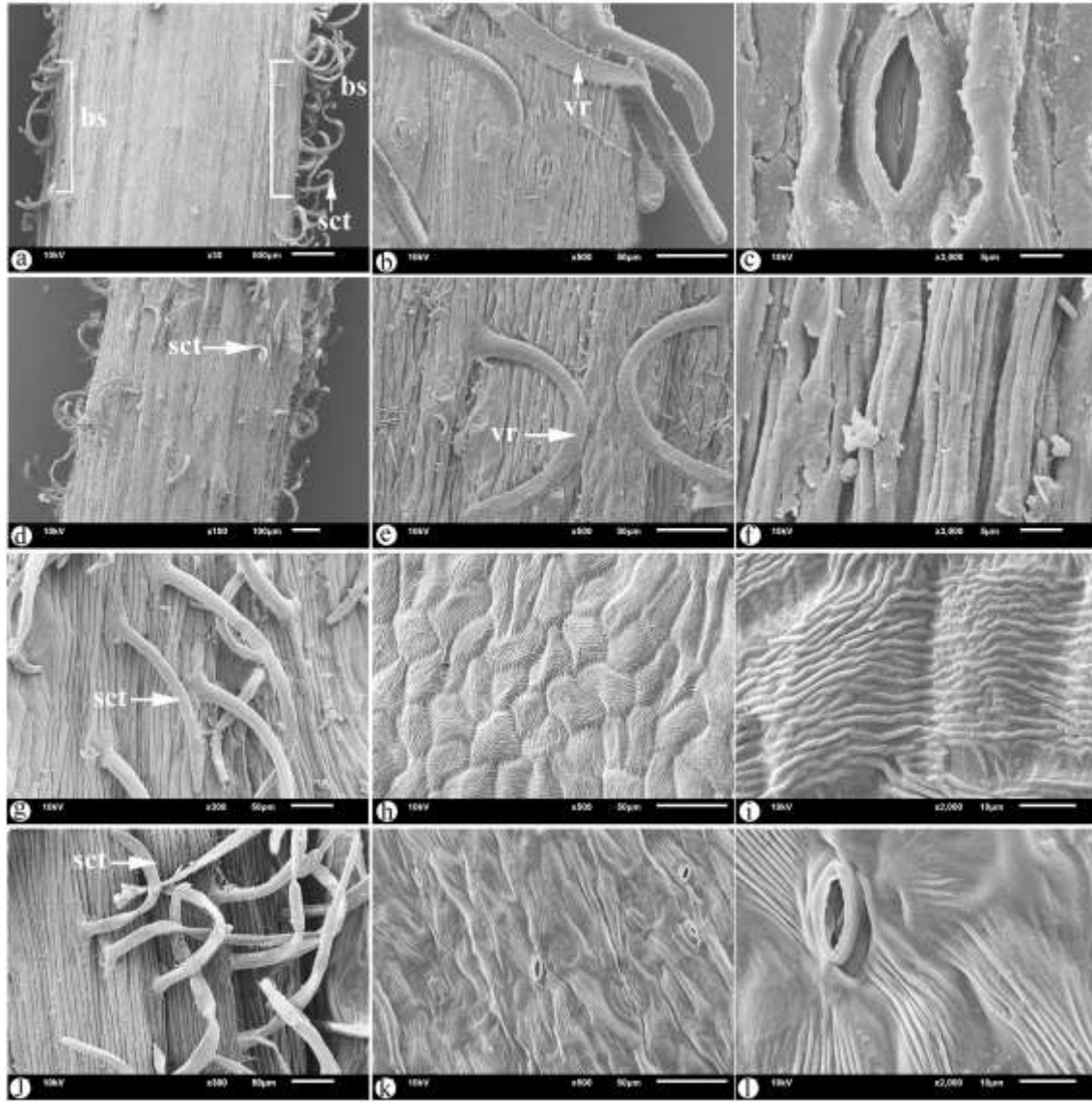


Figure 8. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum hirundinaria* subsp. *hirundinaria* (S.Güven 19 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 8. *Vincetoxicum hirundinaria* subsp. *hirundinaria* (S.Güven 19 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

***Vincetoxicum parviflorum* Decne:** Stem and petiole surfaces with sparsely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 9a-f). Leaf: amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with sparsely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channelled, straight, epicuticular wax striate (Figure 9g-l).

***Vincetoxicum scandens* Somm. & Lev.:** Stem surface with a crisped indument along one side (uniserially) (Figure 10a-c) and petiole surface with a sparsely crisped indument all around (Figure 10d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 10a-f). Leaf: hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 10g-l).

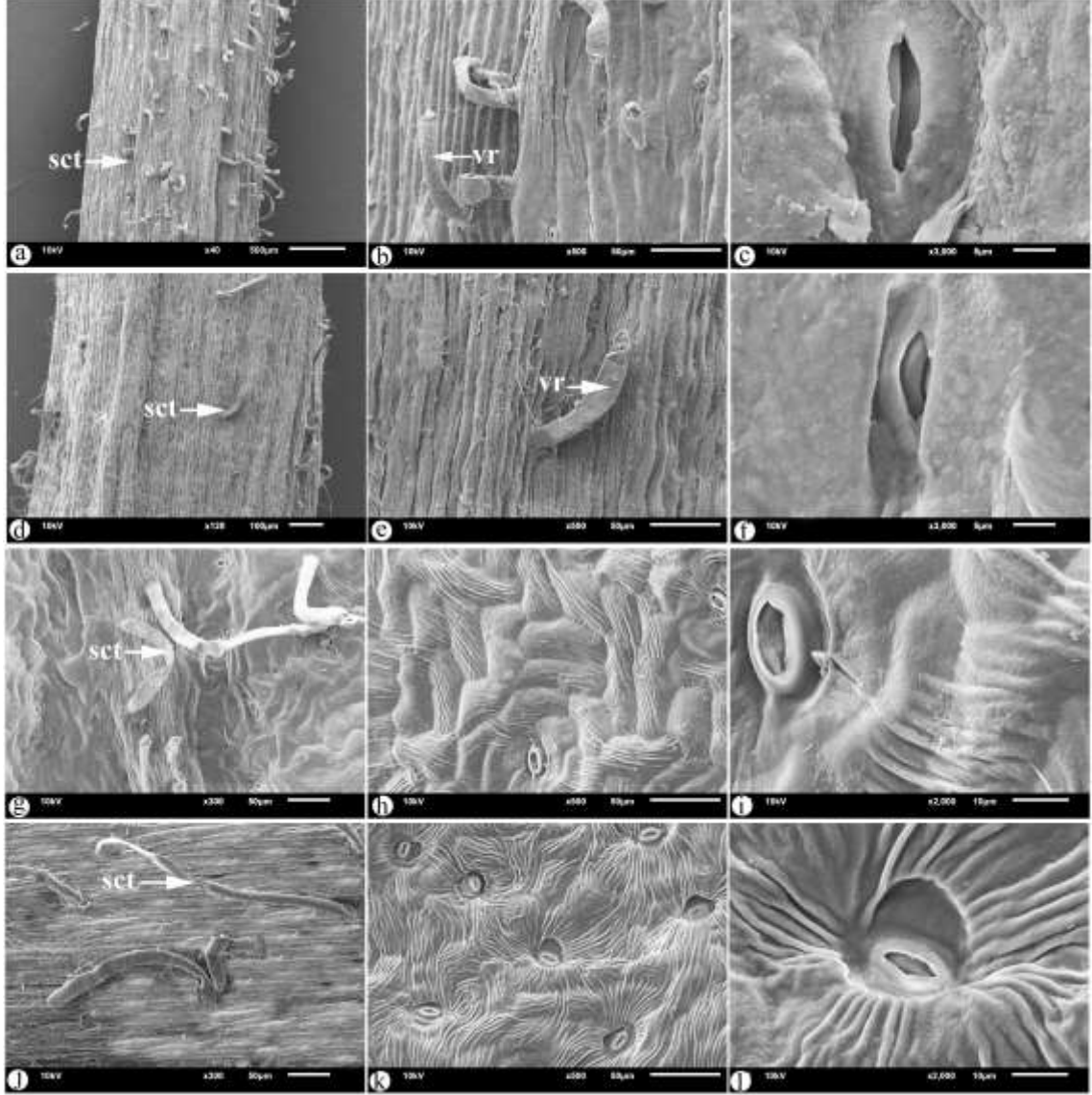


Figure 9. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum parviflorum* (S.Güven 95 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 9. *Vincetoxicum parviflorum* (S.Güven 95 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

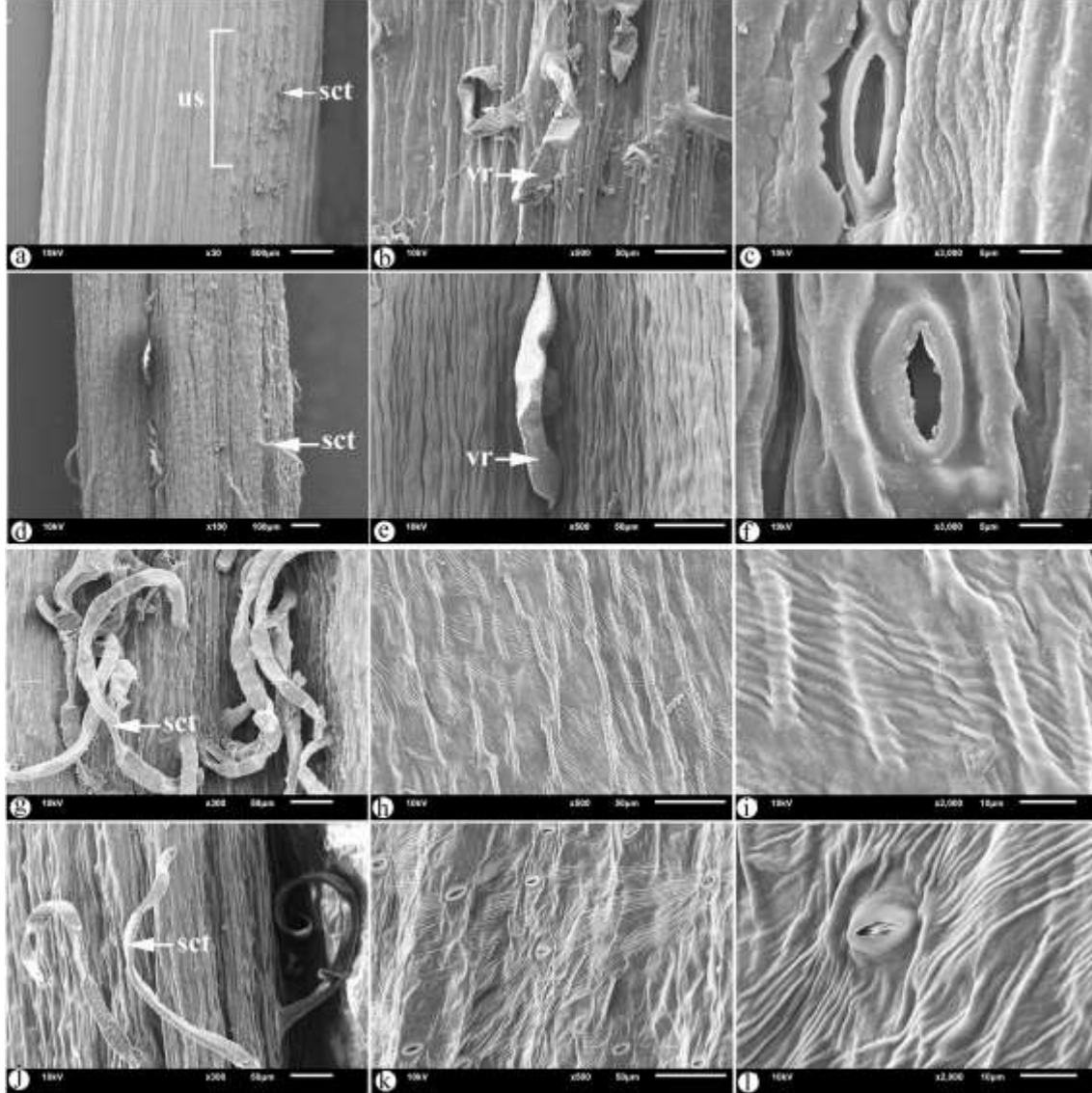


Figure 10. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum scandens* (S.Güven 169 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, us: hairy along one side (uniseriably), vr: verrucose trichome ornamentation.

Şekil 10. *Vincetoxicum scandens* (S.Güven 169 & S.Makbul)'in gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, us: bir taraf boyunca tüylü (uniseriably), vr: verrucose tüy ornamentasyonu.

***Vincetoxicum speciosum* Boiss. & Spruner:** Stem and petiole surfaces with densely velutinous-lanate indumenta all around, consisting of non-glandular, long, wooly, curled or wavy trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 11a-f). Leaf: hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with densely velutinous-lanate indumenta all around, consisting of non-glandular, long, wooly, curled or wavy trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channeled, straight to slightly undulate, epicuticular wax striate (Figure 11g-l).

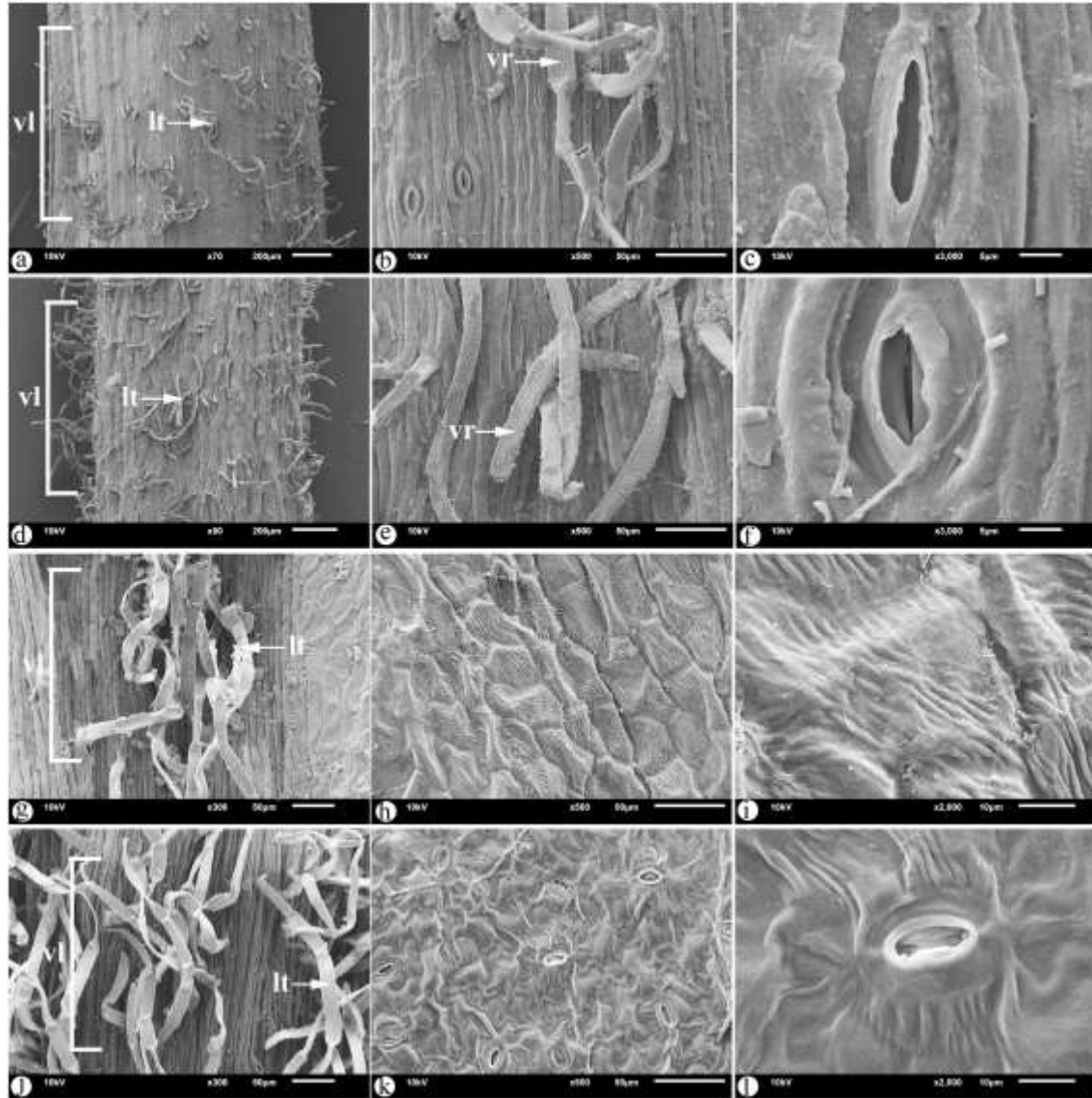


Figure 11. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum speciosum* (S.Güven 132 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, lt: lanate trichome, vl: velutinous-lanate indument (all around), vr: verrucose trichome ornamentation.

Şekil 11. *Vincetoxicum speciosum* (S.Güven 132 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, lt: lanat tüy, vl: velutinous-lanate tüy örtüsü (tüm yüzey boyunca), vr: verrucose tüy ornamentasyonu.

***Vincetoxicum tmoleum* Boiss.:** Stem and petiole surfaces with densely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 12a-f). Leaf: amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely crisped indumenta all around, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 12g-l).

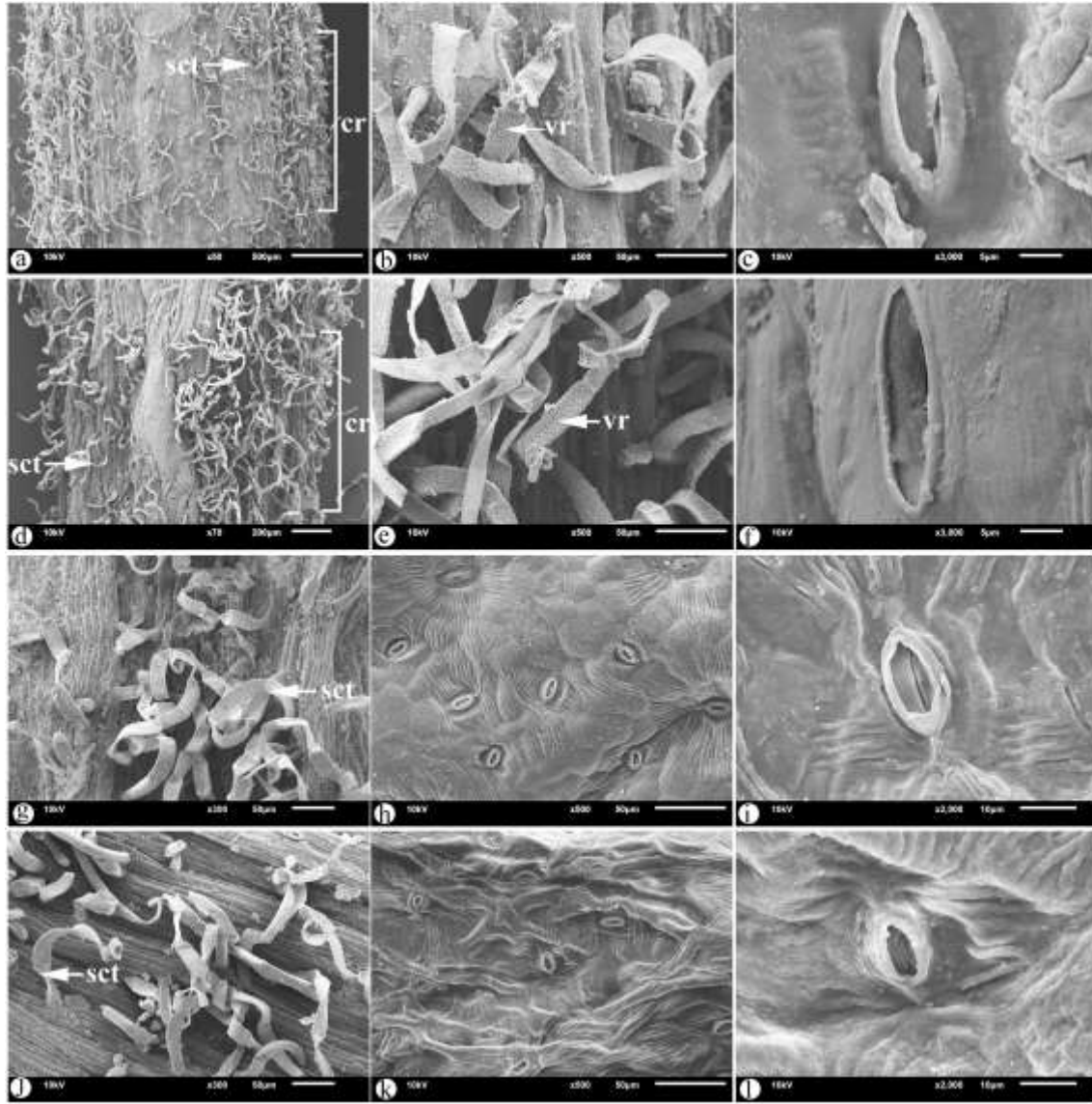


Figure 12. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum tmoaleum* (S.Güven 61 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, cr: crisped indument (all around), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 12. *Vincetoxicum tmoaleum* (S.Güven 61 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskopu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, cr: kısa kıvrık tüy örtüsü (tüm yüzey boyunca), vr: verrucose tüy ornamentasyonu.

DISCUSSION

The present study is the first comprehensive report regarding the surface micro-morphology of vegetative organs of all the genus *Vincetoxicum* in Türkiye. Detailed surface feature of stem, petiole and lamina based on SEM analyzes played an important role in identification and classification of flowering plants (Tomaszewski & Zieliński, 2014). In this study, 11 *Vincetoxicum* taxa were compared on the basis of micro-morphological characteristics of vegetative parts such as stem, petiole and lamina. The most important distinguishing micro-morphological characters for Turkish *Vincetoxicum* species were determined as the distribution of stomata on leaves, and intumentum properties of stem, petiole and lamina, such as the pattern of pubescence, and the distribution and density of trichomes. On the other hand, no difference was observed among the examined taxa in terms of appearance of epicuticular wax, and shape and concavity/convexity properties of epidermal cells on stem, petiole and lamina (Figures 2-12). All the distinguishing micro-morphological characteristics were compared in both Appendix and Figure 13.

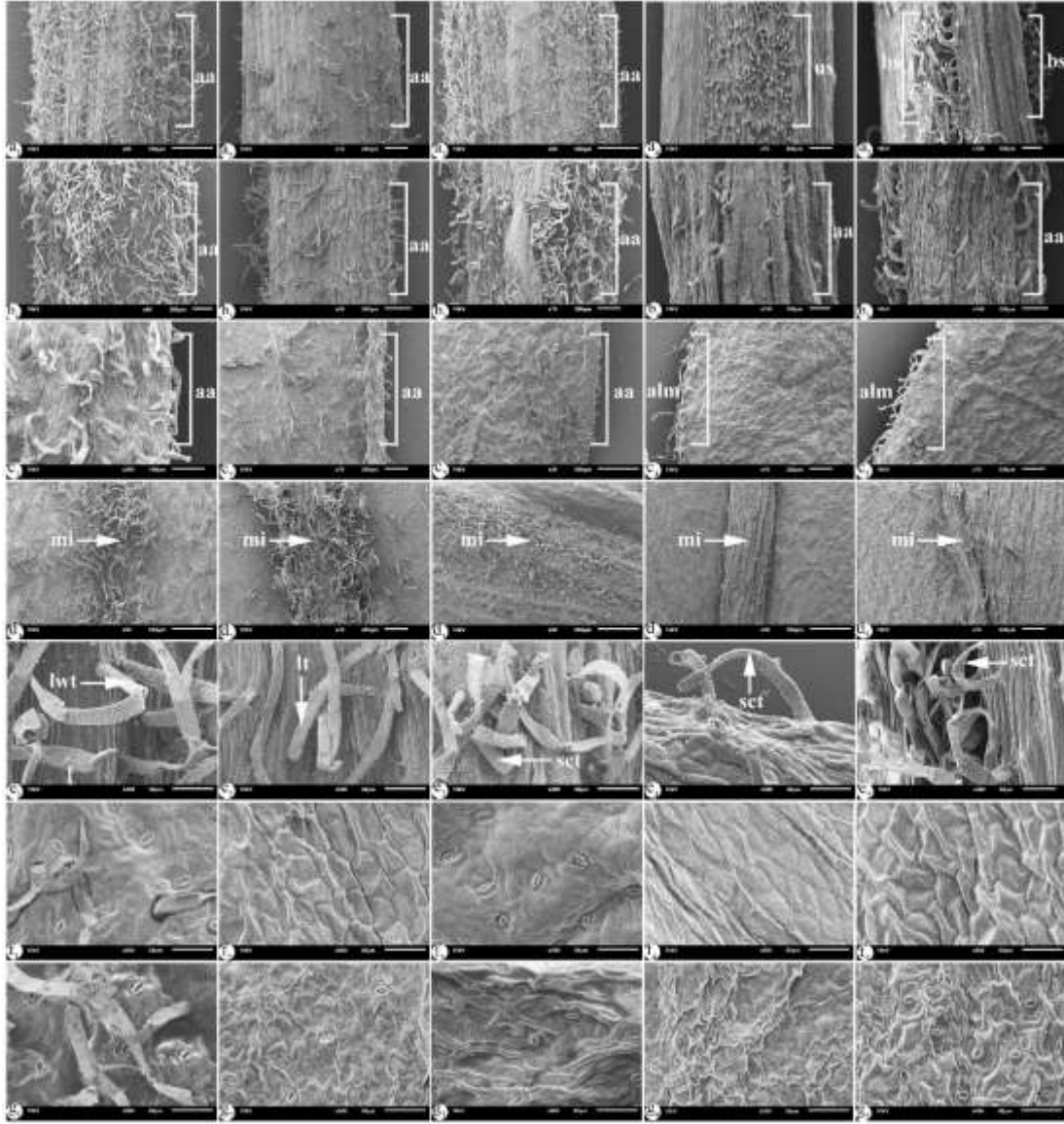


Figure 13. Distinguishing micro-morphological characteristics of stem, petiole, and lamina of Turkish *Vincetoxicum* taxa a. Distribution of indumentum on stem: a1-a3: densely hairy all around (aa), a4: sparsely hairy along one side-uniseriably (us), a5: sparsely hairy along two sides-biseriably (bs); b. Distribution and density of indumentum on petiole: b1-b3: densely hairy all around (aa), b4-b5: sparsely hairy all around (aa), c. Distribution of indumentum on lamina and leaf margin: c1-c3 densely hairy all around, c4-c5: sparsely hairy along margins and veins (alm), d. Distribution of indumentum on lamina and midrib (mi): d1-d3: densely hairy all around, d4-d5: sparsely hairy along midrib, e. The pattern of pubescence on stem, petiole, and lamina e1. grayish white, matted, long wool-like trichome (lwt), e2. lanate trichome (lt), e3-e5. short curly trichome (sct); f. Adaxial surface of lamina, f1 and f3: amohistomatic, f2, f4 and f5: hipostomatic, g. Abaxial surface of lamina. 1. *Vincetoxicum canescens* subsp. *canescens*, 2. *V. speciosum*, 3. *V. tmoleum*, 4. *V. coscuncelebiana*, 5. *V. fuscatum* subsp. *fuscatum*

Şekil 13. Türkiye *Vincetoxicum* taksonlarının gövde, yaprak sapı ve laminasına ait ayırtedici mikromorfolojik özellikleri. a. Gövde üzerindeki tüy dağılımı: a1-a3: tüm yüzey boyunca yoğun tüylü (aa), a4: bir yüzey boyunca seyrek tüylü-tek sıralı (us), a5: iki yüzey boyunca seyrek tüylü-iki sıralı (bs); b. Yaprak sapı üzerindeki tüy dağılımı ve yoğunluğu: b1-b3: tüm yüzey boyunca yoğun tüylü (aa), b4-b5: tüm yüzey boyunca seyrek tüylü (aa), c. Yaprak laminası ve kenarı üzerindeki tüy dağılımı: c1-c3 tüm yüzey boyunca yoğun tüylü, c4-c5: kenarlar ve damarlar boyunca seyrek tüylü (alm), d. Lamina ve orta damar üzerindeki tüy dağılımı (mi): d1-d3: tüm yüzey boyunca yoğun tüylü, d4-d5: orta damar boyunca seyrek tüylü, e. Gövde, yaprak sapı ve lamina üzerindeki tüylenme deseni e1. gri-beyaz, karışık, uzun yünsü tüy (lwt), e2. lanat tüy (lt), e3-e5. kısa kıvrık tüy (sct); f. Laminanın adaksiyal yüzeyi, f1 ve f3: amhistomatik, f2, f4 ve f5: hipostomatik, g. Laminanın abaksiyal yüzeyi. 1. *Vincetoxicum canescens* subsp. *canescens*, 2. *V. speciosum*, 3. *V. tmoleum*, 4. *V. coscuncelebiana*, 5. *V. fuscatum* subsp. *fuscatum*

The present investigations revealed that pubescence properties could be useful diagnostic characteristics for Turkish *Vincetoxicum*. The stem, petiole, and lamina indumentum of the examined taxa, composed of multicellular non-glandular trichomes, could clearly be divided into three groups based on the pattern of pubescence; (1) canescent-tomentose with grayish white, matted, long wool-like trichomes in *V. canescens*, (2) velutinous-lanate with long, wooly, curled or wavy trichomes in *V. speciosum*, and (3) crisped hairy with short curly trichomes in the remaining taxa (Appendix, Figure 13). It was reported that within the Apocynaceae, trichome types, ranging from unicellular to multicellular, glandular to non-glandular, showed a wide variation at both the genus and species levels. In addition, some of the Apocynaceae taxa have only simple non-glandular hairs, while some have both simple and glandular hairs (Gabr et al., 2015; El-Taher, et al, 2020; Medina et al., 2021). The trichome distribution on the stem, detected as another diagnostic character, was present along only one side (uniseriably) in *V. coskuncelebiana* (Figure 4a) and *V. scandens* (Figure 10a), along two sides (biseriably) in *V. funebre* (Figure 5a), *V. fuscatum* (Figure 6a and Figure 7a) and *V. hirundinaria* (Figure 8a), and along the entire surface in *V. canescens* (Figure 2a and Figure 3a), *V. parviflorum* (Figure 9a), *V. speciosum* (Figure 11a) and *V. tmoleum* (Figure 12a). Consistent with the stem indumentum, the trichomes were present along the entire surface on both adaxial and abaxial sides of the leaf in the taxa of *V. canescens* (Figure 2g-l and Figure 3g-l), *V. parviflorum* (Figure 9g-l), *V. speciosum* (Figure 11g-l) and *V. tmoleum* (Figure 12g-l). On the other hand, the leaves of the remaining examined taxa were hairy only along the veins and margins (Figure 13c₄-c₅ and d₄-d₅). The obtained SEM data relating to the pattern of pubescence was compatible with the previous light microscopy-based morphological studies on the genus (Pobedimova, 1952; Grossheim, 1967; Rechinger, 1970; Markgraf, 1972; Browicz, 1978; Güven et al., 2021a). The trichome ornamentation was determined as verrucose in all the investigated taxa. Consistent with our data, Liede (1996) described the trichome ornamentation as verrucose on the corolla surface of *Cynanchum* and *Vincetoxicum* taxa. Trichome surface features such as presence/absence and type of ornamentation (papillate, verrucose) were used as distinguishing characteristics for various plant groups in recent years (Martin & Juniper, 1970; Theobald et al. 1979; Freire et al., 2005). However, this character did not provide any distinction between the examined taxa.

Studies on the Apocynaceae family revealed that the distribution of stomata on the leaf contributed to the distinction of taxa at the genus and species levels (Omino, 1996; Jaleel et al., 2009; Abere & Onwukaeme, 2012). Among the studied *Vincetoxicum*, leaves were amphistomatic in *V. canescens* (Figure 2g-l and Figure 3g-l), *V. parviflorum* (Figure 9g-l), and *V. tmoleum* (Figure 12g-l) and hypostomatic in the remaining ones (Appendix). Recent micro-morphological studies on different Apocynaceae taxa also revealed the presence of amphistomatic and hypostomatic leaves in the family (Metcalf & Chalk, 1950; Joubert, 2007; Xin-sheng, 2010; Medina et al., 2021). However, this character did not sufficiently support the distinction of the examined taxa at the species level.

Leaves and stems of many plant species are covered with a whitish coating proper, composed of epicuticular waxes, external to the cuticle. Micro-morphological investigations carried out that the epicuticular wax had a crystallized or semi-crystallized structure and the outer cellular wall could have a smooth surface structure or could have crusts, platelets, granules, tubules exhibiting various ornamentations such as striate, reticulate, or micro-papillose (Barthlott et al., 1998; Tomaszewski & Zieliński, 2014). In the present study, similarities and dissimilarities between the wax structures on the stem, petiole and both sides of leaf were examined using a SEM. The epicuticular wax layer was determined as smooth on the stem and petiole and striate on the leaf surface. Additionally, a stronger similarity was observed between adaxial and abaxial leaf surfaces for all the investigated taxa. Stem, petiole, and leaf epicuticular wax structures did not provide any distinction between the examined taxa. On the other hand, the major micro-morphological difference between *Vincetoxicum stocksii* Ali & Khatoon and *V. luridum* Stocks ex S.A. Shah was determined as the presence of epicuticular wax in the form of threads on the abaxial surface of *V. stocksii* and their absence in *V. luridum* (Shah et al., 2018). In parallel with our data, it has been determined that the epicuticular wax layer of the leaf is striated in different Apocynaceae members (Joubert, 2007; Xin-sheng, 2010; Bashir et al., 2020; Medina et al., 2021).

Among the studied taxa, leaf epidermal cells were found to be polygonal or irregularly shaped with convex or concave periclinal walls, and channelled, straight to slightly undulate anticlinal walls. Similar epidermal micro-morphological features were previously reported for taxa of *Cynanchum* (Xin-sheng, 2010) and *Cryptolepis* (Joubert, 2007). However, these characters did not provide sufficient data for the delimitation of the studied *Vincetoxicum* taxa.

The taxonomic relationships of the Turkish *Vincetoxicum* were previously evaluated in the light of palynological (Güven et al., 2015), cytological, and molecular (Güven et al., 2019, 2021a) data. The studied 11 *Vincetoxicum* taxa clustered under five separate groups in the phylogenetic trees inferred from ITS sequences (Güven et al., 2021a). Of these groups, the first one included *V. parviflorum*, the second *V. fuscatum*, the third *V. tmoleum* and *V. canescens*, the fourth *V. speciosum*, and the fifth *V. coskuncelebiana* (= *V. anatolicum*), *V. funebre*, *V. scandens*, and *V. hirundinaria* members (Güven et al., 2021a). The present micro-morphological properties of vegetative parts of the investigated taxa appeared to be partially compatible with their previous phylogenetic analysis. *V. canescens*,

V. tmoleum, *V. parviflorum*, and *V. speciosum* have amphistomatic leaves (excluding *V. speciosum* with hypostomatic leaves) and a dense pubescence all around on stem, petiole and lamina. The remaining examined taxa of *Vincetoxicum* are characterized by hypostomatic leaves hairy along veins and margins, and stems uniseriably (*V. coskuncelebiana* and *V. scandens*) or biserially (*V. funebre*, *V. fuscatum* and *V. hirundinaria*) hairy (Appendix).

Browicz (1978) stated that, since *V. parviflorum* was morphologically close to *V. fuscatum* taxa, it could be a subspecies or variety of *V. fuscatum*. However, detailed taxonomic studies on the genus determined that these two taxa showed significant differences. While *V. parviflorum* was characterized with free and apically erect corona segments, sparsely crisped hairy follicles, elliptical pollinia (Güven et al., 2021a), and a somatic chromosome number of $2n=22$ (Güven et al., 2019), *V. fuscatum* taxa had partly fused and apically curved corona segments, glabrous follicle, clavate pollinia (Güven et al., 2021a), and $2n=44$ somatic chromosome number (Güven et al., 2019). These two species also clustered under different branches in the previous palynological (Güven et al., 2015) and molecular phylogenetic analyses (Güven et al., 2019, 2021a). The present micro-morphological data also supported the separation of these two species. In the examined *V. fuscatum* taxa, the thichomes are distributed biserially on the stem, and along the veins and margins on the leaves, and the leaves are hypostomatic. *V. parviflorum* is easily separated from *V. fuscatum* taxa with its leaves including stomata on both sides and stems and leaves covered with hairs along the entire surface. *V. fuscatum* subsp. *boissieri* and *V. fuscatum* subsp. *fuscatum*, two morphologically similar subspecies distinguished only by corolla indumentum (Browicz, 1978; Güven 2017), were also reported to be similar in terms of palynological (Güven et al., 2015), cytological and molecular (Güven et al., 2019, 2021a) data. Similarly, there is no difference between these two subspecies in terms of stem, petiole and lamina micro-morphology.

Molecular analyses were carried out that *V. tmoleum* and *V. canescens* taxa were closely related (Liede-Schumann et al., 2016; Güven et al., 2019, 2021a). These two species are characterized by similar yellow or greenish yellow, campanulate corolla with dense indument on the adaxial surface and free corona segments (Güven et al., 2021a), ovate pollinia, rugulate pollen surface, and ovate corpusculum (Güven et al., 2015), and $2n=22$ somatic chromosome number (Güven et al., 2019). On the other hand, *V. tmoleum* and *V. canescens* taxa exhibit quite different features in terms of stem and fruit morphology. *V. canescens* taxa, characterized by their decumbent stems and ovoid fruits, can be easily distinguished from *V. tmoleum* which has an erect stem and lanceolate fruits (Browicz, 1978; Güven et al., 2021a). The present micro-morphological studies also supported this distinction, and it was observed that the indumentum of the stems and leaves is densely canescent-tomentose in *V. canescens* while it is in densely crisped hairy in *V. tmoleum*. *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata*, which could be distinguished from each other only by the peduncle length, were also found to be similar in terms of palynological (Güven et al., 2015), cytological and molecular data (Güven et al., 2019). Similarly, the present micro-morphological characters did not contribute to the distinction of these two subspecies.

V. coskuncelebiana (= *V. anatolicum*), which was recently published as a new species from Türkiye, was clustered under the same sub-branch with *V. funebre* and *V. scandens* species in the molecular analyses carried out by Güven et al. (2021a). Although these three *Vincetoxicum* species show similarities in terms of some characteristics such as general corona structure (cup-shaped), pollinium shape (ovate), and corpusculum shape (oblong), they can be easily distinguished from each other morphologically. *V. scandens* is characterized by its twining stem up to 2 m and dark purplish-black flowers densely pilose on the upper surface, whereas *V. funebre* has an erect and 40-135 cm high stem, and dark purplish-black flowers sparsely crisped hairy on the upper surface. Compared to these two highly structured plant species, *V. coskuncelebiana* is distinguished by its shorter stem structure (25–68 cm), and yellow to light brown flowers crisped hairy on the adaxial surface (Güven et al., 2021a). In addition, the present data revealed that the stem pubescence is biserially in *V. funebre*, while it is uniseriably in *V. coskuncelebiana* and *V. scandens*.

CONCLUSION

In conclusion, the present work provided the first detailed micro-morphological descriptions of the stem, petiole, and lamina of all the *Vincetoxicum* taxa naturally distributed in Türkiye. The superficial appearance and shape of epidermal cells, ornamentation of epicuticular wax, and pattern of pubescence on stem, petiole, and lamina, and the type and distribution of stomata on both adaxial and abaxial leaf surfaces were examined in detail using SEM, and the obtained data was evaluated in a taxonomic perspective. The micro-morphological characters such as (1) indumentum pattern on stem, petiole, and lamina (grayish white, matted, long wool-like trichome; long, wooly, curled or wavy trichome; short curly trichome), (2) trichome distribution on stem (all around; uniseriably; biserially), (3) trichome distribution on lamina (all around; along margins and veins), and (4) stomata distribution on leaves (hypostomatic; amphistomatic) were found to be useful in distinction of the Turkish *Vincetoxicum* taxa. Micro-morphological observations were carried out on plant samples taken from at least two different populations

for each taxon, except for *V. funebre*, which is known from a single locality in our country. The presence or absence of both trichomes and stomata and their density were considered to be affected by various ecological factors (Song et al., 2020). However, the pattern of pubescence and their surface ornamentation, and stomata type and distribution exhibited a stable structure among the taxa examined taxa and were found to be useful for the delimitation and identification of the present taxa collected from different habitats. Although this research included a limited number of individuals, the obtained data provided additional insights into comprehensive SEM-based taxonomical studies of the genus *Vincetoxicum*.

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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 have declared no conflict of interest.

REFERENCES

- Abere, T. A., & Onwukaeme, D. N. (2012). Pharmacognostic evaluation of the leaves of *Secamone afzelii* (Schult) K.Schum (Asclepiadaceae). *Tropical Journal of Pharmaceutical Research February*, 11(1), 125–131. <http://dx.doi.org/10.4314/tjpr.v11i1.16>
- Abeyasinghe, P. D., & Scharaschkin, T. (2022). Comparative anatomy of two forms of Sri Lankan *Calotropis gigantea* (L.) R. Br. (Family Apocynaceae s.l.–Subfamily Asclepiadoideae) -Taxonomic implications. *Ceylon Journal of Science*, 51(3), 307–318. <http://doi.org/10.4038/cjs.v51i3.8038>
- Aybeke, M. (2022). Bazı Orkide taksonlarında labellum mikromorfolojisi. *KSÜ Tarım ve Doğa Derg*, 25(1), 42–48. <https://doi.org/10.18016/ksutarimdog.vi.882554>
- Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I., & Wilhelmi, H. (1998). Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society*, 126(3), 237–260. <https://doi.org/10.1111/j.1095-8339.1998.tb02529.x>
- Bashir, K., Sohail, A., Ali, U., Ullah, A., Ul Haq, Z., Gul, B., Ullah, I., Sunera, & Asghar, M. (2020). Foliar micromorphology and its role in identification of the Apocynaceae taxa. *Microscopy Research and Technique*, 83, 755–766.
- Behnke, H. D., & Barthlott, W. (1983). New evidence from the ultrastructural and micromorphological fields in angiosperm classification. *Nordic Journal of Botany*, 3, 43–66. <https://doi.org/10.1111/j.1756-1051.1983.tb01444.x>
- Browicz, K. (1978). Asclepiadaceae. In Davis, P.H. (ed) *Flora of Turkey and the East Aegean Islands, Vol. 6*. Edinburgh University Press, Edinburgh, pp 163–174.
- Carpenter, K. J. (2005). Stomatal architecture and evolution in basal angiosperms. *American Journal of Botany*, 92, 1595–1615. <https://doi.org/10.3732/ajb.92.10.1595>
- Carvalho, R., Pellissari, L. C. O., Pace, M. R., Scremin-Dias, E., Arruda, R. D. O., & Farinaccio, M. A. (2017). Leaf morphoanatomy of *Araujia* and *Morrenia* (Asclepiadoideae, Apocynaceae): phylogenetic implications and species key. *Botanical Journal of the Linnean Society*, 20, 1–14. <https://doi.org/10.1093/botlinnean/bow004>
- Chang, Y. H., Tzeng, H. Y., & Tseng, Y. H. (2012). Pollinaria morphology of *Cynanchum* L. (Asclepiadaceae) in Taiwan. *Quarterly Journal of Forestry Research*, 34(4), 251–262.
- Civeyrel, L., Thomas, A. L., Ferguson, K., & Chase, M. W. (1998). Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *matK* sequences. *Molecular Phylogenetics and Evolution*, 9(3), 517–527. <https://doi.org/10.1006/mpev.1997.0510>
- Davis, P. H. (1965). *Flora of Türkiye and the East Aegean Islands, Vol. 1*. Edinburgh: Edinburgh University Press.
- Dilcher, D. L. (1974). Approaches to the identification of angiosperm leaf remains. *Botanical Review*, 40(1), 1–160. <https://doi.org/10.1007/BF02860067>
- El-Taher, A. M., Gendy, A. E., -N. G. E., Alkahtani, J., Elshamy, A. I., & Abd-ElGawad, A. M. (2020). Taxonomic implication of integrated chemical, morphological, and anatomical attributes of leaves of eight Apocynaceae taxa. *Diversity*, 12(9), 334. <https://doi.org/10.3390/d12090334>

- Feng, Y., Qin, X., Huang, J., & Hong, W. (2012). Pollinarium morphology of 17 species and 1 subspecies of *Cynanchum* (Asclepiadaceae). *Acta Botanica Boreali-Occidentalia Sinica*, 32(9), 1758–1762.
- Freire, S. E., Arambarri, A. M., Bayón, N.D., Sancho, G., Urtubey, E., Monti, C., Novoa, M.C., & Colares, M. N. (2005). Epidermal characteristics of toxic plants for cattle from the Salado river basin (Buenos Aires, Argentina). *Boletín de la Sociedad Argentina de Botánica*, 40, 241–281.
- Fishbein, M. (2001). Evolutionary innovation and diversification in the flowers of Asclepiadaceae. *Annals of the Missouri Botanical Garden*, 88, 603–623. <http://dx.doi.org/10.2307/3298636>
- Gabr, D. G., Khafagi, A. A. F., Mohamed, A. H., & Mohamed, F. S. (2015). The significance of leaf morphological characters in the identification of some species of Apocynaceae and Asclepiadaceae. *Journal of American Science*, 11, 61–70.
- Grossheim, A. A. (1967). *Flora of the Caucasus*, Vol. 7. Academy of Science of the USSR, pp 221–225.
- Güner, A. (2012). Apocynaceae. In Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M. T. (eds.) *Türkiye bitkileri listesi (Damarlı bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, pp 83–86.
- Güven, S. (2017). Türkiye *Vincetoxicum* (Apocynaceae Asclepiadoideae) cinsinin taksonomik revizyonu. PhD Thesis, Recep Tayyip Erdoğan Üniversitesi, Rize.
- Güven, S., Makbul, S., Coskuncelebi, K., & Pınar, N. M. (2015). Pollinarium morphology of the genus *Vincetoxicum* Wolf (Apocynaceae: Asclepiadoideae) in Turkey. *Phytotaxa*, 230(1), 22–38. <https://doi.org/10.11646/phytotaxa.230.1.2>
- Güven, S., Makbul, S., & Coşkuncelebi, K. (2019). Contribution to chromosome numbers and phylogeny of Turkish *Vincetoxicum* wolf (Apocynaceae, Asclepiadoideae). *European Journal of Taxonomy*, 590, 1–19. <https://doi.org/10.5852/ejt.2019.590>.
- Güven, S., Coşkuncelebi, K., & Makbul, M. (2021a). A new *Vincetoxicum* (Apocynaceae) species from Eastern Anatolia and its relationship based on biosystematic properties. *Nordic Journal of Botany*, 39(12), e03405. <https://doi.org/10.1111/njb.03405>.
- Güven, S., Makbul, S., & Coşkuncelebi, K. (2021b). *Vincetoxicum tmoleum* Boiss. taksonunun genişletilmiş morfolojik özellikleri ve Türkiye'deki yayılışı. *Recep Tayyip Erdogan University Journal of Science and Engineering*, 2(2), 1–12. <https://doi.org/10.53501/rteufemud.945076>
- Güzel, M. E. (2021). Kültür Marulu (*Lactuca sativa* L.) ile yabancı akrabalarının yaprak mikromorfolojileri. *Bağbahçe Bilim Dergisi*, 8(1), 121–133. <https://doi.org/10.35163/bagbahce.866561>
- İlçim, A., Özay, S. G., & Kökdil, G. (2010). Exomorphic seed characters and anatomy of leaf and stem of some *Vincetoxicum* (Asclepiadaceae/Apocynaceae) species from Turkey. *Journal of Faculty of Pharmacy of Ankara University*, 39(1), 1–16. https://doi.org/10.1501/Eczfak_00000000550
- Jaleel, C. A., Gopi, R., Azooz, M. M., & Panneerselvam, R. (2009). Leaf anatomical modifications in *Catharanthus roseus* as affected by plant growth promoters and retardants. *Global Journal of Molecular Sciences*, 4(1), 01–05.
- Joubert, L. (2007). A taxonomic study of *Cryptolepis* (Apocynaceae) in Southern Africa. [Master's Thesis, Faculty of Natural and Agricultural Sciences, Department of Plant Sciences (Botany), University of the Free State Bloemfontein]. South Africa.
- Koch, K., Bhushan, B., & Barthlott, W. (2008). Diversity of structure, morphology and wetting of plant surfaces. *Soft Matter*, 4, 1943–63.
- Liede, S. (1996). *Cynanchum* – *Rhodostegiella* – *Vincetoxicum* – *Tylophora* (Asclepiadaceae): new considerations on an old problem. *Taxon*, 45, 193–211.
- Liede, S., & Täuber, A. (2002). Circumscription of the genus *Cynanchum* (Apocynaceae–Asclepiadoideae). *Systematic Botany*, 27(4), 789–800.
- Liede, S., Täuber, A., & Schneidt, J. (2002). Molecular considerations in the Tylophorinae K.Schum. (Apocynaceae–Asclepiadoideae). *Edinburgh Journal of Botany*, 59(3), 377–403. <https://doi.org/10.1017/S0960428602000239>
- Liede-Schumann, S., Kong, H., Meve, U., & Thiv, M. (2012). *Vincetoxicum* and *Tylophora* (Apocynaceae: Asclepiadoideae: Asclepiadeae) – two sides of the same medal: independent shifts from tropical to temperate habitats. *Taxon*, 61, 803–825. <https://doi.org/10.1002/tax.614007>
- Liede-Schumann, S., Khanum, R., Mumtaz, A. S., Gherghel, I., & Pahlevani, A. (2016). Going west – a subtropical lineage (*Vincetoxicum*, Apocynaceae: Asclepiadoideae) expanding into Europe. *Molecular Phylogenetics and Evolution*, 94, 436–446. <https://doi.org/10.1016/j.ympev.2015.09.021>
- Liede-Schumann, S., & Meve, U. (2018). *Vincetoxicum* (Apocynaceae–Asclepiadoideae) expanded to include *Tylophora* and allies. *Phytotaxa*, 369, 129–184. <https://doi.org/10.11646/phytotaxa.369.3.1>

- Makbul, S., & Güven, S. (2022). A new replacement name for the Turkish *Vincetoxicum anatolicum* S. Güven, S. Makbul & Coskunç. (Apocynaceae: Asclepiadoideae). *Phytotaxa*, 547(2), 217–218. <https://doi.org/10.5281/zenodo.6575118>
- Malaspina, P., Ingegneri, M., Betuzzi, F., Di Gristina, E., Cornara, L., Trombetta, D., & Smeriglio, A. (2024). Beyond the scent: new evidence about micromorphological, phytochemical and biological features of *Plumeria rubra* 'Tonda Palermitana' (Apocynaceae). *Plants*, 13, 2479. <https://doi.org/10.3390/plants13172479>
- Mansoor, A., Ibrahim, M. A., Zaidi, M. A., & Ahmed, M. (2011). Antiprotozoal activities of *Vincetoxicum stocksii* and *Carum copticum*. *Bangladesh Journal of Pharmacology*, 6(1), 51–54. <https://doi.org/10.3329/bjp.v6i1.8442>
- Markgraf, F. (1972). *Flora Europaea, Vol. 3*, Cambridge University Press, ISBN: 978-0521084895, pp 71–73.
- Martin, J. T., & Juniper, B. E. (1970). *The cuticles of plants*. New York: St. Martins.
- Medina, M. C., Sousa-Baena, M. S., Capelli, N. d. V., Koch, R., & Demarco, D. (2021). Stinging trichomes in Apocynaceae and their evolution in angiosperms. *Plants*, 10(11), 2324. <https://doi.org/10.3390/plants10112324>
- Metcalf, C. R., & Chalk, L. (1950). *Anatomy of dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses, Vol. II*. Oxford Clarendon Press.
- Omino, E. (1996). *A contribution to the leaf anatomy and taxonomy of Apocynaceae in Africa*. Backhuys Publishers, ISBN: 9073348544, 178 s.
- Pobedimova, G. (1952). *Antitoxicum*. In Shishkin, B. K. & Bobrov, E. G. (eds.) *Flora of USSR Vol. 18*. Academy of Science of the USSR Press, Leningrad-Moscow, pp 674–691.
- Prüm, B., Seidel, R., Bohn, H. F., & Speck, T. (2012). Plant surfaces with cuticular folds are slippery for beetles. *Journal of the Royal Society Interface*, 9, 127–35.
- Rechinger, K. H. (1970). *Flora Iranica, vol. 73:10*. Akademische Druck und Verlagsanstalt, p 21.
- Ridzuan, K., & Kalu, M. (2023). Comparative micromorphology leaf surface of selected *Hoya* spp. (Apocynaceae) from Sarawak, *Reinwardtia*, 22(2), 69–77.
- Sennblad, B., & Bremer, B. (2002). Classification of Apocynaceae s.l. according to a new approach combining linnaean and phylogenetic taxonomy. *Systematic Biology*, 51(3), 389–409. <https://doi.org/10.1080/10635150290069869>.
- Shah, S. A., & Ahmad, M. (2014). Reassessing palynological characters in the subfamilies Periplocoideae and Asclepiadoideae (Apocynaceae): taxonomic and evolutionary implications. *Palynology*, 38(2), 224–234. <http://dx.doi.org/10.1080/01916122.2014.893264>
- Shah, S. A., Sultan, A., Wen, J., Nisa, S.U., Su, X., Iqbal, J., & Mumtaz, A.S. (2018). *Vincetoxicum luridum* (Asclepiadeae, Asclepiadoideae, Apocynaceae), a new and long misunderstood endemic species from Balochistan, Pakistan. *Phytotaxa*, 357(2), 117–125. <https://doi.org/10.11646/phytotaxa.357.2.4>
- Shah, S. A., Sultan, A., Wen, J., Ullah, Z., Nisa, S. U., Ren, Z., Alam, M. M., Iqbal, J., & Mumtaz, A. S. (2021). Taxonomy of *Vincetoxicum* s.str. (Asclepiadoideae, Apocynaceae) from southern Asia including three new species and resurrected names. *PhytoKeys*, 179, 35–73. <https://doi.org/10.3897/phytokeys.179.62514>
- Sheeley, S. E., & Raynal, D. J. (1996). The distribution and status of species of *Vincetoxicum* in eastern North America. *Bulletin of the Torrey Botanical Club*, 123(2), 148–156. <https://doi.org/10.2307/2996072>
- Sinha, S., & Mondal, A. K. (2011). The morphological diversification of pollinia of some members of Asclepiadaceae. *African Journal of Biotechnology*, 10(41), 7981–7986.
- Sivarajan, V. V. (1991). *Introduction to the principles of plant taxonomy*. Cambridge University Press, Cambridge.
- Song, J. H., Yang, S., & Choi, G. (2020). Taxonomic implications of leaf micromorphology using microscopic analysis: A tool for identification and authentication of korean piperale. *Plants*, 9(5), 566. <https://doi.org/10.3390/plants9050566>
- Sreenath, K. P., Ramakrishna, T. M., & Babu, T. P. (2012). Perspective on polinial apparatus and carriers of Asclepiadaceae sensu lato. *Global Journal of Bio-Science and Biotechnology*, 1(1), 45–53.
- Stearn, W. T. (1992). *Botanical Latin*. London, David and Charles Publication.
- Stace, C. A., Heywood, V. H., & Moore, D.M. (1984). *The taxonomic importance of the leaf surface. Current Concepts in Plant Taxonomy*. Academic Press, London, pp 67–94.
- Theisen, I., & Barthlott, W. (1994). Mikromorphologie der epicuticularwachse und die systematik der Gentianales, Rubiales, Dipsacales und Calycerales. *Tropische und Subtropische Pflanzenwelt*, 89, 1–62.
- Theobald, W. L., Krahulik, J. L., & Rollins, R. (1979). Trichome description and classification. In Metcalfe, C. R., & Chalk, L., (eds). *Anatomy of the dicotyledons*. Oxford, Clarendon Press.
- Tomaszewski, D., & Zieliński, J. (2014). Epicuticular wax structures on stems and comparison between stems and leaves—A survey. *Flora*, 209(5–6), 215–232. <https://doi.org/10.1016/j.flora.2014.03.001>
- Verhoeven, R. L., & Venter, H. J. T. (2001). Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). *Annals of the Missouri Botanical Garden*, 88, 569–582. <http://dx.doi.org/10.2307/3298634>

- Xin-sheng, Q. (2010). Studies on the micromorphology of leaf epidermis in *Cynanchum* (Asclepiadaceae) from China under environmental scanning electron microscope (ESEM). *Journal of South China Agricultural University*, 31(3), 47–51.
- Yamashiro, T., Fukuda, T., Yokoyama, J., & Maki, M. (2004). Molecular phylogeny of *Vincetoxicum* (Apocynaceae-Asclepiadoideae) based on the nucleotide sequences of cpDNA and nrDNA. *Molecular Phylogenetics and Evolution*, 31, 689–700. <http://dx.doi.org/10.1016/j.ympev.2003.08.016>
- Yamashiro, T., Yamashiro, A., Yokoyama, J., & Maki, M. (2008). Morphological aspects and phylogenetic analyses of pollination systems in the *Tylophora–Vincetoxicum* complex (Apocynaceae-Asclepiadoideae) in Japan. *Biological Journal of the Linnean Society* 93, 325–341. <http://dx.doi.org/10.1111/j.1095-8312.2007.00896.x>
- Yaseen, S., & Perveen, A. (2014). Pollinia morphology to some members of Asclepiadaceae of Pakistan. *Bangladesh Journal of Botany*, 43(3), 249–253.
- Zaidi, M. A., & Crow Jr., S. A. (2005). Biologically active traditional medicinal herbs from Balochistan, Pakistan. *Journal of Ethnopharmacology*, 96(1-2), 331–334. <https://doi.org/10.1016/j.jep.2004.07.023>.

Appendix. Comparison of distinguishing stem, petiole and lamina characteristics of Turkish *Vincetoxicum* taxa.

Ek. Türkiye *Vincetoxicum* taksonlarının ayırt edici gövde, yaprak sapı ve lamina özelliklerinin karşılaştırılması.

	Stem indumentum			Petiole indumentum			Adaxial and abaxial lamina indumentum			Leaf
	distribution	density	pubescence pattern	distribution	density	pubescence pattern	distribution	density	pubescence pattern	
<i>V. canescens</i> subsp. <i>canescens</i>	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	amphistomatic
<i>V. canescens</i> subsp. <i>pedunculata</i>	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	amphistomatic
<i>V. coskuncelebiana</i>	along one side (uniseri ally)	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. funebre</i>	along two sides (biseri ally)	sparse	crisp ed hairy	all around	dense	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. fuscatum</i> subsp. <i>fuscatum</i>	along two sides (biseri ally)	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. fuscatum</i> subsp. <i>boissieri</i>	along two sides (biseri ally)	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. hirundinaria</i> subsp. <i>hirundinaria</i>	along two sides (biseri ally)	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. parviflorum</i>	all around	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	amphistomatic
<i>V. scandens</i>	along one side (uniseri ally)	sparse	crisp ed hairy	all around	sparse	crisp ed hairy	along margins and veins	sparse	crisp ed hairy	hypostomatic
<i>V. speciosum</i>	all around	dense	velutinous-lanate hairy	all around	dense	velutinous-lanate hairy	all around	dense	velutinous-lanate hairy	hypostomatic
<i>V. tmoleum</i>	all around	dense	crisp ed hairy	all around	dense	crisp ed hairy	all around	dense	crisp ed hairy	amphistomatic

Akdeniz, Tarsus ve Erdemli (Mersin-Türkiye) ilçelerindeki bazı doğal sit alanlarının flora, genel vejetasyon yapısı ve EUNIS habitat tiplerinin incelenmesi

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ÖZET

Bu çalışmanın amacı Mersin'in Akdeniz, Tarsus ve Erdemli ilçelerinde bulunan Adanalıoğlu Ormanı ve Sahili, Kazanlı, Tarsus Dalyanı, Alata Sahili ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanlarının floristik özelliklerini, EUNIS habitat tiplerini ve genel vejetasyon yapısını belirlemektir. Araştırmada, arazi çalışmaları ile 38 familya ve 69 cinse ait 78 takson (58 tür) tespit edilmiştir. Elde edilen bulgulara göre Adanalıoğlu Ormanı ve Sahili'nde 35, Kazanlı'da 31, Tarsus Dalyanı'nda 30, Alata Sahili'nde 13 ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı'nda 24 takson tespit edildi. Bölgede IUCN tehlike kategorisi "En Az Endişe Verici (LC)" olan nadir bir *Panocratium maritimum* L. türü tespit edilmiştir. Fitocoğrafik bölgelere göre 21 takson Akdeniz (%26,92), 1 takson Avrupa-Sibiry'a (%1,28), 1 takson İran-Turan (%1,28), 17 takson geniş yayıllı (%21,79) ve 37 taksonun fitocoğrafik bölgesi bilinmemektedir (%47,43). Sonuç olarak, EUNIS habitat sınıflandırmasına göre, 5 seviye 1 habitata bağlı 8 seviye 2 habitat tipi bulunmaktadır. Genel vejetasyon yapısı itibarıyla çalışma alanlarından Adanalıoğlu Ormanı ve Sahili, Kazanlı, Tarsus Dalyanı ve Alata Sahilinde *Juncetea maritimi* sınıfı, Adanalıoğlu Ormanı ve Sahili, Kazanlı, Tarsus Dalyanı ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı'nda *Cakiletea maritimae* ve *Astragalo-Brometea* sınıfları, Alata sahilinde *Cisto-micromerietea* ve *Salici purpureae-Populetea nigrea* sınıfları, Adanalıoğlu Ormanı ve Sahili, Kazanlı, Tarsus Dalyanı *Salicornietea* sınıfı, Alata Sahili ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı'nda *Quercetea ilicis* sintaksonomik sınıflarının karakter türlerine rastlanmıştır.

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Investigation of the flora, general vegetation structure and EUNIS habitat types of some natural protected areas in Akdeniz, Tarsus and Erdemli (Mersin-Turkey)

ABSTRACT

The aim of this study was to determine the floristic characteristics, EUNIS habitat types and general vegetation structure of Adanalıoğlu Forest and Coast, Kazanlı, Tarsus Dalyanı, Alata Coast and Alata Horticultural Research Institute Natural Sites located in Akdeniz, Tarsus and Erdemli districts of Mersin (Turkey). In the research, 78 taxa (58 species) belonging to 38 families and 69 genera were identified through field studies. According to the findings, 35 taxa were identified in Adanalıoğlu Forest and Coast, 31 in Kazanlı, 30 in Tarsus Dalyanı, 13 in Alata Coast and 24 in Alata Horticultural Research Institute Natural Sites. It was determined that there is a rare *Panocratium maritimum* L. with IUCN threatened category "Least Concern (LC)" in the area. According to the phytogeographic regions, 21 taxa are Mediterranean (26.92%), 1 taxon is European-Siberian (1.28%), 1 taxon is Irano-Turanian (1.28%), 17 taxa are widely distributed (21.79%) and 37 taxa have unknown phytogeographic region (47.43%). As a result, according to EUNIS habitat classification, there are 8 level 2 habitat types connected

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to 5 level 1 habitats. In terms of general vegetation structure, *Juncetea maritimi* class in Adanalıoğlu Forest and Coast, Kazanlı, Tarsus Dalyanı and Alata Coast, *Cakiletea maritimae* and *Astragalo-Brometea* classes in Adanalıoğlu Forest and Coast, Kazanlı, Tarsus Dalyanı and Alata Horticultural Research Institute Natural Protected Area, Character species of *Cisto-micromerietea* and *Salici purpureae-Populetea nigrea* classes were found in Alata coast, *Salicornietea* class in Adanalıoğlu Forest and Coast, Kazanlı, Tarsus Dalyanı, *Quercetea ilicis* syntaxonomic classes in Alata Coast and Alata Horticultural Research Institute Natural Sites.

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GİRİŞ

Türkiye, bitki biyoçeşitliliği ve endemik takson sayısı bakımından dünyanın önemli bölgelerinden biridir (Avcı, 1993). Türkiye'nin coğrafi bölgeleri arasında iklim, toprak ve topografya farklılıkları bulunmasından dolayı bitki çeşitliliği oldukça zengindir (Davis, 1965-1985; Davis & Hedge, 1975; Davis ve ark., 1988; Güner ve ark., 2000). Türkiye florasında 11707 takson bulunmakta olup bunların 3649 (%31.82)'u endemiktir (Güner ve ark., 2012). Bu rakam Avrupa kıtasının toplam bitki taksonu sayısı olan yaklaşık 20.000 (Lončarević ve ark., 2024) ile karşılaştırıldığında Türkiye Florası'nın zenginliği ve önemi daha iyi anlaşılmaktadır. Türkiye bitkileri üzerine ilk çalışmalar 1700'lü yılların başında Tournefort ve arkasından Boissier tarafından yapılmıştır (Boissier 1867-1888; Baytop, 2010). En kapsamlı araştırma ise Davis ve ark. (1965-1985; 1988) ve Güner ve ark. (2000) tarafından gerçekleştirilmiştir. Günümüzde ise Resimli Türkiye Florası yayımlama çalışmaları devam etmektedir (Güner ve ark., 2014; 2018; 2022; 2023; 2024).

Bu çalışmadaki araştırma alanları Mersin ili Akdemiz, Tarsus ve Erdemli ilçelerinde bulunan beş doğal sit alanıdır. Çalışma alanları coğrafi olarak Türkiye'nin Akdeniz fitocoğrafik bölgesinde yer almaktadır. Türkiye'de İran-Turan fitocoğrafik bölgesi daha geniş bir alan kaplamasına rağmen coğrafi bölgeler arasında endemik taksonların dağılışının en yüksek olduğu bölge Akdeniz Bölgesidir (Avcı, 1993; Güner ve ark., 2012; Şenkul & Kaya, 2017). Akdeniz bölgesinin bitki biyoçeşitliliği yüksek olmasına rağmen bu bölgede flora ve genel vejetasyon yapısının çalışması yapılmayan birçok alan bulunmaktadır (Tel ve ark., 2022a). Bu bakımdan alanın floristik ve genel vejetasyon yapısının tespit edilmesi, Türkiye ve Akdeniz bölgesi flora ve vejetasyonunun daha iyi tanınmasına katkı sağlayacaktır. Çalışma alanının yakın çevresinde Düzenli ve ark. (1996), Zeren & İspirgil (2001), Uçar (2002), Everest & Rauss (2004), Orcan ve ark. (2004), Aksay (2006), Karaömerlioğlu (2007), Karaömerlioğlu & Düzenli (2008), Dinç (2008), Yıldıztuğay & Küçüköyük (2010), Şirin (2012), Şirin & Ertuğrul (2015), Şen (2019), Üzgör-Ün ve ark. (2021) ve Topal ve ark. (2022)'nin flora ve vejetasyon araştırmaları bulunmaktadır. Ayrıca, kültürel ve doğal sit alanlarında Tel (2009), Tel ve ark. (2010), Tel & Tak (2012), Tel & Eğilmez (2015), Tel & İlçim (2016), Anonim (2016), Ortaç (2017), Tel & Tak (2018), Tel ve ark. (2018; 2019), Ortaç & Tel (2021), Tel ve ark. (2021; 2022a; 2022b; 2023) tarafından gerçekleştirilmiş flora ve vejetasyon çalışmaları bulunmaktadır.

Araştırma alanında habitat sınıflandırmasına yönelik daha önce yapılmış herhangi bir çalışma bulunmamaktadır. Son yıllarda habitatlar insan faaliyetleri nedeniyle tahrip ve yok olma tehlikesiyle karşı karşıyadır (Arslan ve ark., 2012). Bu nedenle, habitat sınıflandırmasının yapılması doğal kaynakların sürdürülebilirliği bakımından önem arz etmektedir (Moss & Roy, 1998; Davies ve ark., 2004; Anonim, 2024a). Ancak, bu konuda Türkiye'de yapılan çalışmalar yetersiz olup Arslan & Arslantürk (2009), Arslan ve ark. (2012); Ulu Ağır ve ark. (2014), Mergen & Karacaoğlu (2015), Çiftçi (2015), Erdoğan (2016), Geven ve ark., (2016), Şahin & Karavelioğlu (2018a), Şahin & Karavelioğlu (2018b), Tug ve ark. (2018), Seyfe (2019), Özen & Ürker (2020), Çakmak & Aytaç (2020), Çakmak & Aytaç (2021) ve Demir ve ark. (2022)'nin bölgesel çalışmaları bulunmaktadır.

Anonim (2024a) EUNIS habitat tipleri belirli bir düzen içinde sıralanmış ve 10 ana kategori ile bunlara ait alt başlıklarından oluşmaktadır. Bunlar; A: Denizel habitatlar, B: Kıyı habitatları, C: İç su habitatları, D: Bataklıklar, E: Otlaklar, yosunlar veya likenlerin hakim olduğu otlaklar ve araziler, F: Fundalık, çalılık ve tundralar, G: Ağaçlık alanlar, orman ve diğer ormanlık alanlar, H: İç kesimlerdeki bitki örtüsü olmayan veya seyrek bitki örtüsüne sahip habitatlar, I: Düzenli veya yakın zamanda işlenen tarım alanları, bahçe ve evsel habitatlar, J: Yapay, endüstriyel ve diğer yapay habitatlar olarak sınıflandırılmıştır (Anonim, 2024a). EUNIS habitat

sınıflandırmasında koruma statüsü bulunan alanların kesin olarak belirtilmesi gerekmektedir. Türkiye’de korunan alanlar bazı türler veya özel bitki topluluklarının bulunup bulunmasına göre değişik isimlerde koruma statüsüne sahip sahalara olarak korunmaktadır. Böylece bu alanlar da bir veya birden fazla habitat tipi korunabilmektedir (Arslan ve ark., 2012). Bu çalışmanın gerçekleştirildiği alanlar da koruma statüsüne sahip doğal sit alanları kapsamında bulunmaktadır.

Kültür ve Tabiat Varlıklarını Koruma Kanununa göre doğal sit alanı “Jeolojik devirlere ait, ender bulunmaları nedeniyle olağanüstü özelliklere sahip olan korunması gerekli alanlar” olarak tanımlanmaktadır (Anonim, 1983). Doğal sitler araştırmanın gerçekleştirildiği tarihlerde I., II. ve III. derece doğal sit olarak tanımlanmaktayken 2022 tarihinde yayımlanan yönetmelikle Kesin korunması gereken hassas bölgeler, Nitelikli koruma sahaları ve Sürdürülebilir koruma ve kullanım sahaları olarak üç kategoride yeniden tanımlanmıştır (Anonim, 2022).

Bu araştırma Mersin ili Akdeniz, Tarsus ve Erdemli ilçelerinde bulunan Adanalıoğlu ormanı ve sahili, Kazanlı, Tarsus dalyanı, Alata sahili ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal sit alanı olmak üzere beş doğal sit alanının floristik özelliklerini, EUNIS habitat tiplerini ve genel vejetasyon yapısını incelemek amacıyla gerçekleştirilmiştir. Bu çalışma ile ülkemizde EUNIS habitat tiplerinin belirlenmesine katkı sağlanmıştır. Ayrıca, çalışma alanları olan doğal sitlerin floristik özellikleri ve genel vejetasyon yapılarını anlaşılmasına katkı sağlanmıştır.

Araştırma Sahası ve Özellikleri

Bu çalışmadaki araştırma sahaları, Mersin’in Akdeniz, Tarsus ve Erdemli ilçelerinde yer alan Adanalıoğlu ormanı ve sahili, Kazanlı, Tarsus dalyanı, Alata sahili ve Alata Bahçe Kültürleri Araştırma Enstitüsü doğal sit alanlarını kapsamaktadır (Çizelge 1; Şekil 1). Bu alanların hepsi doğal sit statüsündeki alanlardır.

Çizelge 1. Araştırma alanlarının genel özellikleri, koordinatları ve koruma statüleri (Anonim, 2016)

Table 1. General characteristics, coordinates and protection status of the research areas (Anonymous, 2016)

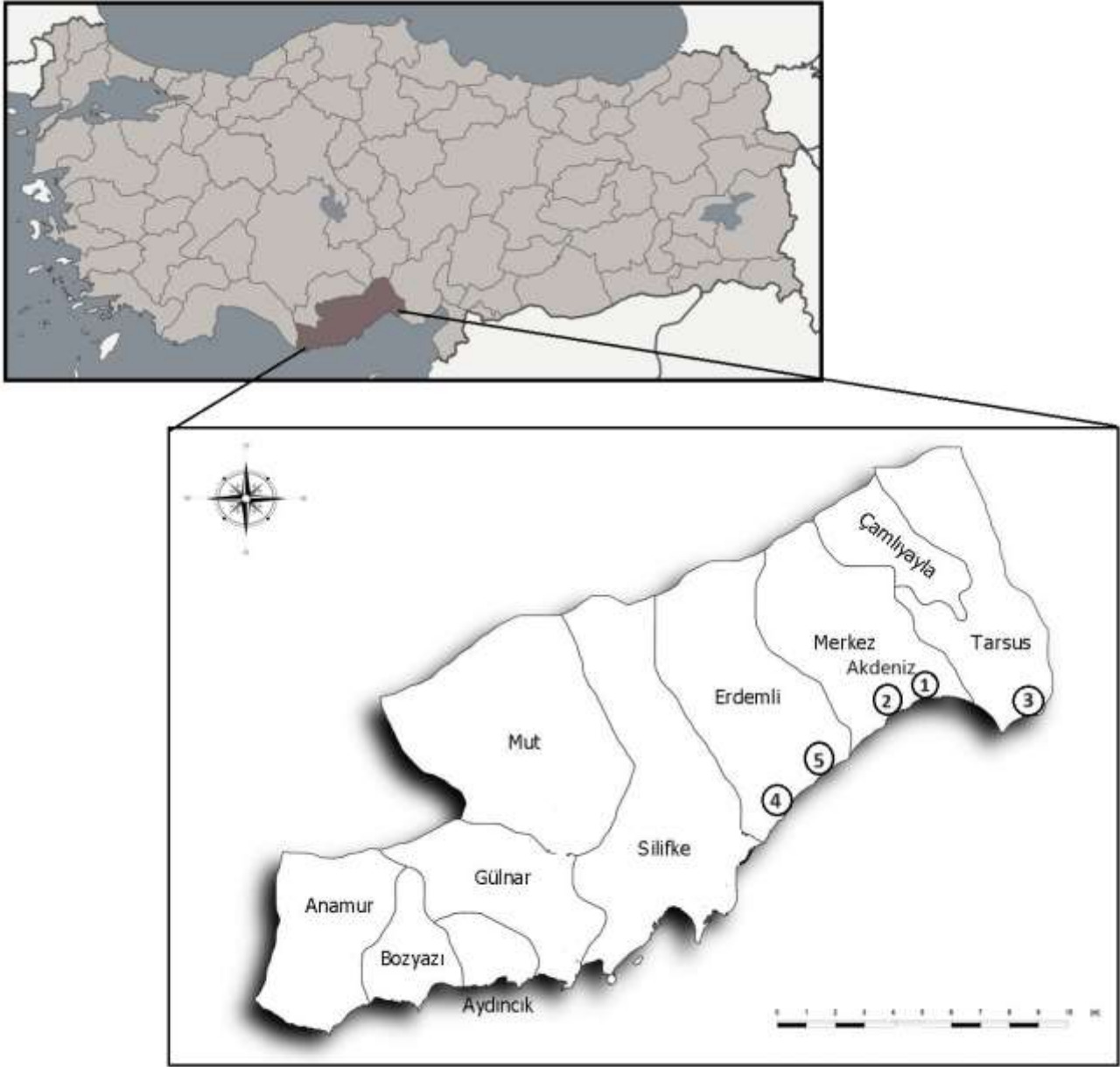
No	Çalışma Alanı	Alan Büyüklüğü(ha.)	Koordinatlar	Koruma Statüsü	İlçe
1	Adanalıoğlu Ormanı ve Sahili	135	36° 47' 39. 59" Kuzey 34° 37' 36.19" Doğu	I. ve II. Derece Doğal Sit	Akdeniz
2	Kazanlı	87.83	36° 49' 10. 79" Kuzey 34° 45' 04.75" Doğu	I.Derece Doğal Sit	Akdeniz
3	Tarsus Dalyanı	1122	36° 48' 51. 45" Kuzey 34° 52' 47.26" Doğu	I.Derece Doğal Sit	Tarsus
4	Alata Sahili	9.68	36° 36' 36. 57" Kuzey 34° 19' 01.01" Doğu	I.Derece Doğal Sit	Erdemli
5	Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı	105.95	36° 36' 58. 49" Kuzey 34° 19' 43.09" Doğu	I.Derece Doğal Sit	Erdemli

1- Adanalıoğlu orman alanı ve sahili, Mersin ili Akdeniz ilçesinde yer almaktadır. Kıyı şeridi *Caretta caretta* L. ve *Chelonia mydas* L. yuvalama alanı olması nedeniyle I. Derece, kıyı arkası ise II. Derece doğal sit olarak belirlenmiştir. Alan 135 ha. büyüklüğe sahiptir. Çalışma alanı deniz seviyesinden 4 metre yüksekliktedir. Kıyı kumullarında kompozisyonunu çoğunlukla kumul bitkilerinin oluşturduğu kıyı kumul vejetasyonu görülmektedir. Arka kısımlarda ise tipik maki elemanları bulunmaktadır (Anonim, 2016).

2- Kazanlı doğal sit alanı; Mersin ilinin Akdeniz ilçesinde yer almaktadır. I. Derece doğal sit alanı statüsünde olup kıyı kumullarında kompozisyonunu çoğunlukla kumul bitkilerinin oluşturduğu kıyı kumul vejetasyonu görülmektedir. Arka kısımlarda ise tipik maki elemanları ile kaplıdır. Kazanlı Doğal Sit alanı, yaklaşık 2.2 km.'lik bir kıyı şeridi, karaya doğru kıyı çizgisinin gerisinde yer alan tarım alanları, küçük ölçekli yerleşimler ve bir tatil sitesi nedeniyle doğal peyzajın değişime uğradığı bir alan haline gelmiştir. Jeomorfolojik açıdan düz bir arazi yapısına sahip olup çevresinde herhangi bir yükselti yer almamaktadır. Çalışma alanı 4 m. yükseltiye sahiptir. Yakın çevresi ile ele alındığında kırsal yerleşimler, kırsal yerleşimlere ait yollar ve tarım arazileri ile kıyı şeridine ulaşım amaçlı açılan kılcal yollar bulunmaktadır. Kazanlı doğal sit alanı toplam olarak 87.83 ha. alan kaplamaktadır (Anonim, 2016).

3- Tarsus Dalyanı; Mersin ili Tarsus ilçesinde yer almakta ve I. Derece doğal sit alanı statüsündedir. Tarsus Dalyanı, sulak alan vasfından dolayı koruma altındadır (Anonim, 2016). Yaklaşık 11.5 km.'lik kıyı şeridine sahip olan sahada tarım alanları ile küçük ölçekli yerleşimler doğal peyzajın değişimine neden olmuştur. Alanda deniz, kumsal, dalyan bölgesi ile Adana ırmağına ait menderesler ve su yüzeyleri öne çıkmaktadır. Çalışma alanı 2 metre

yükseltiye sahiptir. Tarsus Dalyanı toplam olarak 1122 ha. alan kaplamaktadır.



Şekil 1. Çalışma alanları yer gösterim haritası 1. Adanalıoğlu orman alanı ve sahili, 2. Kazanlı, 3. Tarsus Dalyanı, 4. Alata Sahili, 5. Alata Bahçe Kùltürleri Araştırma Enstitüsü Doğal Sit Alanı. (Anonim, 2024b; Anonim, 2024c)

Figure1. Location map of the study areas 1. Adanalıoğlu forest area and coast, 2. Kazanlı, 3. Tarsus Dalyan, 4. Alata Coast, 5. Alata Horticultural Research Institute Natural Protected Area (Anonymous, 2024b; Anonymous, 2024c)

4- Alata sahili; I. Derece doğal sit olup Mersin ili Erdemli ilçesinde bulunmaktadır. Saha yaklaşık 9.68 ha. Büyüklüğe sahiptir. Alan ve çevresi tarımsal amaçlı kullanılmaktadır. Kumsal *Caretta caretta* ve *Chelonia mydas*'ın üreme alanıdır. Alanın 7.36 ha. sulu tarım, 2.32 ha. ı ise kıyı kumullarından oluşmaktadır. Çalışma alanı deniz seviyesinden 11 metre yükseltiye sahip olup dalgaların kıyıyı aşındırması sonucu oluşmuş tipik bir kıyı topografyası göstermektedir.

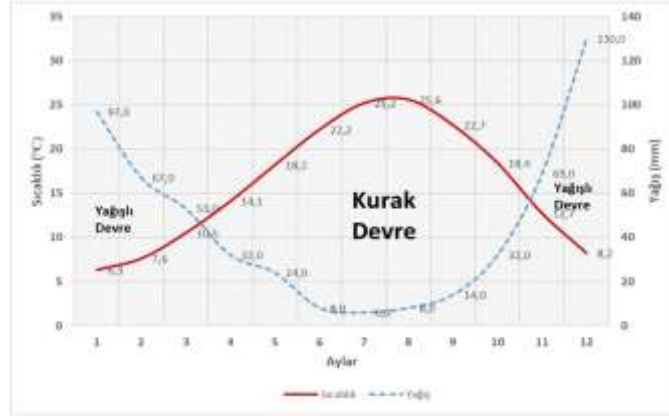
5- Alata Bahçe Kùltürleri Araştırma Enstitüsü doğal sit alanı; Mersin ili Erdemli ilçesinde yer almakta olup I. Derece doğal sit statüsündedir. Alanın ortasından karayolu geçmektedir. Bahçe Kùltürleri Araştırma Enstitüsü arazisi olduğundan narenciye ve zeytin ağaçları bulunmakta ve korunmaktadır. Tipik maki elemanları ile kaplıdır.

Alan toplam 105.95 ha. Rakımı ise 2 metredir. Dalgaların kıyıyı aşındırması sonucu oluşmuş tipik bir kıyı topografyası göstermektedir.

Çalışma Alanlarının İklim Özellikleri

Akdeniz ilçesi iklim özellikleri

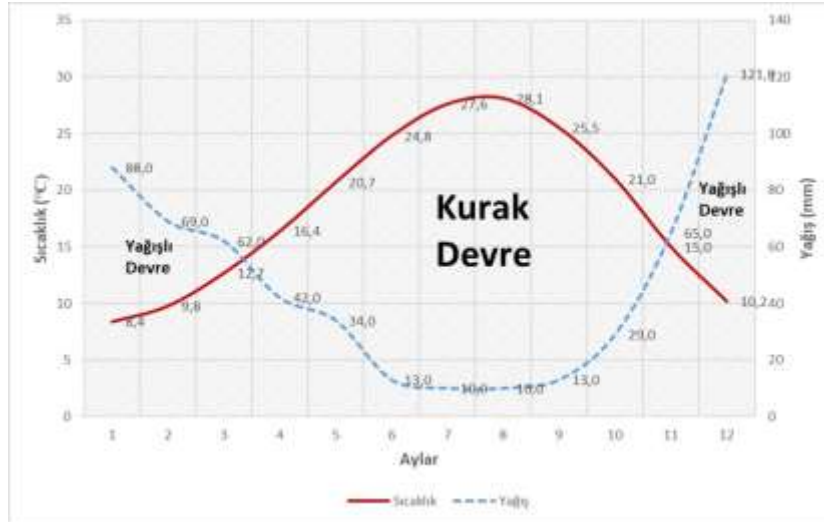
Akdeniz ilçesi Mersin'in merkez ilçesi olup tipik Akdeniz iklimi özellikleri görülür (Anonim, 2024d) Kış ayları yaz aylarına nispeten daha fazla yağışlıdır. Yıllık sıcaklık ortalaması 16 °C'dir. İlçede ortalama yıllık yağış 540 mm'dir. Temmuz ayı en kurak ay olup yağış miktarı 6 mm'dir. Aralık ayı 130 mm yağış ile en fazla yağış olan aydır. En sıcak ay 25.6 °C sıcaklıkla Ağustos'tur. Yıllık en düşük sıcaklık ortalaması 6.3 °C ile Ocak ayında görülmektedir (Anonim, 2024d). Akdeniz ilçesinin Ombro-termik iklim diyagramı Şekil 2'de verilmiştir.



Şekil 2. Akdeniz ilçesi ombro-termik iklim diyagramı
Figure 2. Akdeniz district ombro-thermal climate diagram

Tarsus ilçesi iklim özellikleri

Tarsus ilçesi sıcak ve ılıman bir iklime sahiptir. Kış mevsiminde yaza nazaran daha fazla yağış görülür. Tarsus'ta yıllık ortalama sıcaklık 18.3 °C'dir. Ortalama yağış miktarı yıllık 556 mm'dir. Temmuz yılın en kurak ayı olup 10 mm yağış düşmektedir. En fazla yağış ortalama 121 mm yağış miktarıyla Aralık ayında görülür (Anonim, 2024e). Tarsus ilçesinin Ombro-termik iklim diyagramı Şekil 3'te verilmiştir.

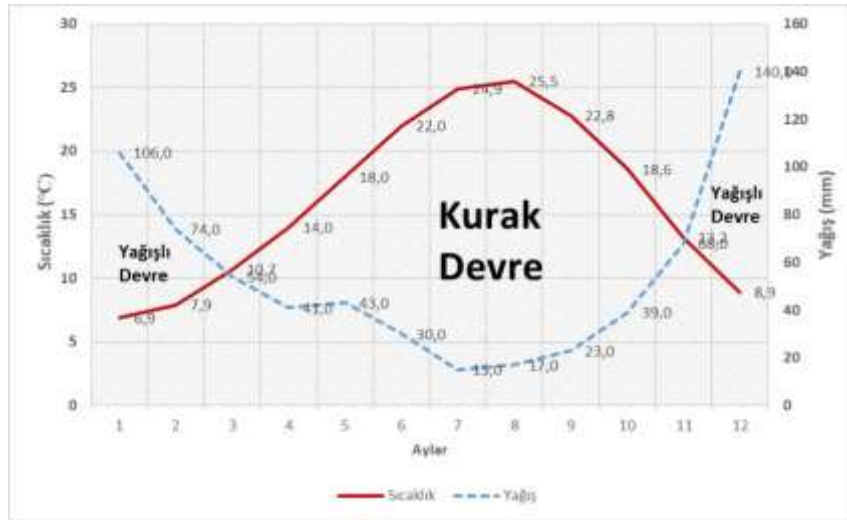


Şekil 3. Tarsus ilçesi ombro-termik iklim diyagramı
Figure 3. Tarsus district ombro-thermal climate diagram

Erdemli ilçesi iklim özellikleri

Erdemli Erdemli ilçesinin iklimi tipik Akdeniz iklimidir (Anonim, 2024f). Erdemlide kış ayları yaz aylarına nispeten daha fazla yağışlıdır. Erdemli ilçesinde yıllık ortalama sıcaklık 16.1 °C'dir. Yıl boyunca düşen yağış miktarı ortalama 650 mm'dir. Temmuz yılın en kurak ayı olup 15 mm yağış düşer. Ortalama 140 mm yağış miktarıyla en fazla yağış Aralık ayında görülür. Yılın en sıcak ayı olan Ağustos'ta sıcaklık ortalaması 25.5 °C'dir.

En düşük sıcaklık görülen ay Ocak ayı olup 6.9 °C'dir (Anonim, 2024f). Erdemli ilçesinin Ombro-termik iklim diyagramı Şekil 4'te verilmiştir.



Şekil 4. Erdemli ilçesi ombro-termik iklim diyagramı
Figure 4. Erdemli district ombro-thermal climate diagram

MATERYAL ve METOD

Bu araştırmanın materyali çalışma alanlarından 2014-2016 yılları arasında toplanan bitki örnekleridir. Bitki taksonlarının toplandığı sahalar şekil 1'de gösterilmiş ve çizelge 1'de genel özellikleri verilmiştir. Bitkilerin tehlike kategorileri Ekim ve ark. (2000), Güner ve ark. (2012) ve Anonim (2024j)'e göre belirlenmiştir.

Örnekleme Yöntemi

Çalışma alanları Mersin ilinin üç farklı ilçesinde bulunmakta olup habitat ve genel vejetasyon yapıları benzer olduğundan aynı çalışmada ele alınmıştır. Araştırma alanından toplanan bitkiler herbaryum tekniğine uygun olarak kurutulmuş, teşhisleri yapılmış ve Adıyaman Üniversitesi herbaryumuna yerleştirilmiştir (Seçmen ve ark., 2000). Çalışma alanlarındaki habitat tipleri EUNIS sınıflandırma sistemi kullanılarak (Anonim, 2024a) gözleme dayalı şekilde kayıt edilmiştir.

Laboratuvar analizleri

Örneklerin teşhis ve tayinlerinde Flora of Turkey and the East Aegean Islands adlı eserden yararlanılmıştır (Davis, 1965-1985; Davis ve ark., 1988; Güner ve ark., 2000). Herbaryum örneği haline getirilen bitkiler Adıyaman Üniversitesi Herbaryumunda muhafaza edilmektedir. Takson isimleri verilirken Uluslararası Bitki İsimleri İndeksi (Anonim, 2024g), The WFO PlantList (Anonim, 2024h) ve Bizim bitkiler (Anonim, 2024i)'a göre geçerli olanlar kullanılmıştır. Endemik bitkilerin IUCN tehlike kategorileri Anonim (2024j)'ye göre verilmiştir. Çalışma alanlarındaki habitatlar tipleri Davies ve ark. (2004) ve Anonim (2024a)'dan istifade edilerek belirlenmiştir. Çalışma alanlarının vejetasyon değerlendirmesinde Braun-Blanquet yöntemi kullanılmamıştır. Yapılan gözlemler ve toplanan bitki örneklerinin değerlendirilmesi sonucunda alanların genel vejetasyon yapısı karakter türler ve sintaksonomik üst birimlere göre gözleme dayalı olarak belirlenmiştir.

BULGULAR ve TARTIŞMA

Araştırmada toplam 38 familya, 69 cins ve bu cinslere ait 78 (58 tür, 20 tür altı takson) farklı takson tespit edilmiştir. Alanlardaki tür sayılarının az olması alanın iklim ve istasyon tiplerinin stabil olmasıyla açıklanabilir. Alanların floristik özelliklerine ayrı ayrı bakacak olursak; Adana'ya 35, Kazanlı'da 31, Tarsus Dalıyanında 30, Alata Sahilinde 13 ve Alata Bahçe Kùltürleri Araştırma Enstitüsü Doğal Sit Alanı'nda 24 takson belirlenmiştir (Çizelge 2). Bitkilerin fitocoğrafik bölgelerine göre dağılışı ve dağılışı yüzdeleri çizelge 3'te sunulmuştur.

Çizelge 2. Çalışma alanlarındaki familya, takson, endemik ve nadir takson sayıları

Table 2. Number of families, taxa, endemic and rare taxa in the study areas

Araştırma Alanı	Familya Sayısı	Takson Sayısı	Endemik Takson	Nadir Takson
1 Adanalıoğlu Ormanı ve Sahili	23	35	-	1
2 Kazanlı	20	31	-	1
3 Tarsus Dalyanı	19	30	-	1
4 Alata Sahili	11	13	-	1
5 Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı	19	24	-	1

Çizelge 3. Bitkilerin fitocoğrafik bölge dağılımları ve oranları

Table 3. Phytogeographical distributions and rates of plants

Fitocoğrafik Bölge	Takson Sayısı	Oran (%)
Akdeniz	21	26.92
Avrupa – Sibirya	1	1.28
İran – Turan	1	1.28
Geniş Yayılışlı	17	21.79
Bilinmeyen	37	47.43
Kültür Varyetesi	1	1.28

Adanalıoğlu ormanı ve sahilinde 23 familya ve 33 cinse ait toplam 35 takson (27 tür) belirlenmiştir (Çizelge 2; Ek-1). Doğal sit alanında nadir bir takson olan *Pancratium maritimum* L. yaygındır. Bu türün IUCN tehlike kategorisi En az endişe verici (LC) dir. (Juan Vicedo, 2018). Alanın büyük bir kısmı yerleşim yerine dönüşmüş ve alan büyük ölçüde doğallığını kaybetmiştir. Sahil kesimdeki kumullarda *Pancratium maritimum*, *Cakile maritima* Scop., *Salsola kali* L., *Daucus littoralis* Sibth. & Sm. *Euphorbia peplis* L., *Echium angustifolium* Mill., *Lactuca saligna* L. gibi bitki türleri bulunmaktadır. Bitkilerin fitocoğrafik bölge dağılımları 7 Akdeniz, 9 Geniş Yayılışlı, 1 Avrupa-Sibirya, 17 bilinmeyen ve 1 takson Kültür varyetesi şeklindedir. İran-Turan fitocoğrafik bölge elementi bulunmamaktadır.

Kazanlı'da 20 familya 31 cinse ait 31 (28 tür) takson belirlenmiştir (Çizelge 2; Ek-1). Kıyı kumullarında kompozisyonunu çoğunlukla kumul bitkilerinin oluşturduğu kıyı kumul vejetasyonu görülmektedir. Arka kısımlarda ise tipik maki elemanları ile kaplıdır. Denize doğru kıyı şeridinde doğal, karaya doğru kıyı çizgisinin gerisinde yer alan tarım arazileri ve seralar nedeniyle kültürel peyzaj özelliği taşımaktadır. Taksonların fitocoğrafik bölgeleri; 8 Akdeniz, 6 Geniş yayılışlı, 1 Av.-Sib. Elm. ve 16 takson bilinmeyen şeklindedir. İran-Turan fitocoğrafik bölge elementi bulunmamaktadır. Doğal Sit Alanında nadir bir takson olan *Pancratium maritimum* doğal yayılış göstermektedir (Anonim, 2016).

Tarsus Dalyanı'nda 19 familya 29 cinse ait toplam 30 takson (26 tür) belirlenmiştir (Çizelge 2; Ek-1). Dalyanda doğal bozulmamış alan olarak dar bir kıyı şeridi bulunmaktadır. Burada kumul vejetasyon bitkilerinden *Cakile maritima*, *Pancratium maritimum*, *Polygonum maritimum* L., *Daucus littoralis*, *Euphorbia peplis*, *Echium angustifolium* gibi bitkiler gözlenmiştir. Dalyan sınırları içerisinde bulunan alanın büyük bir kısmı tarım sahası olarak kullanılmaktadır. Alanda kumul vejetasyon bulunmaktadır. Taksonların fitocoğrafik bölgeleri; 6 Akdeniz, 7 Geniş yayılışlı, 1 Avrupa-Sibirya ve 16 takson bilinmeyen şeklinde sıralanmaktadır. İran-Turan fitocoğrafik bölge elementine rastlanmamıştır.

Alata Sahili'nde, 11 familya, 13 cinse ait 13 takson (9 tür) tespit edilmiştir (Çizelge 2; Ek-1). Bunların fitocoğrafik bölgeleri; 7 takson Akdeniz, 5 takson bilinmeyen 1 takson Kültür varyetesi şeklindedir. Alanda Geniş yayılışlı, İran-Turan ve Avrupa-Sibirya fitocoğrafik bölge elementi taksona rastlanmamıştır. Çalışma alanında endemik takson bulunmamakta olup nadir bir takson olan *Pancratium maritimum* L. bulunmaktadır (Çizelge 4).

Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı'nda, 19 familya 24 cinse ait 24 takson (22 tür) belirlenmiştir (Çizelge 2; Ek-1). Alan büyük ölçüde tahrip olmuş ve bahçeye dönüştürülmüştür. Doğal sayılabilecek sahilde dar bir kumsal bulunmaktadır. Kumsal gerisinde tipik kumul vejetasyon elemanları olan *Cakile maritima*, *Pancratium maritimum*, *Polygonum maritimum*, *Daucus littoralis*, *Euphorbia peplis*, *Echium angustifolium* bitki türleri bulunur. Bitkilerin fitocoğrafik bölgelere göre dağılımı 10 Akdeniz, 1 Avrupa-Sibirya, 1 İran-Turan, 10 bilinmeyen ve 2 geniş yayılışlı şeklindedir. Çalışma alanında endemik takson tespit edilmemiştir. Alanda nadir bir takson olan *Pancratium maritimum* (bulunmaktadır. Alanda kumul bir habitat ve bu habitata bağlı kumul vejetasyon teşekkül etmiştir.

Çalışma alanları Akdeniz kıyı bölgesinde yer almakta olup Sıcak Akdeniz İklim Katında yer almaktadır.

Dolayısıyla alanda ve yakın çevresinde Akdenizin tipik bitki örtüsü maki yer almaktadır. Tüm alanlar kıyı şeridinde yer aldığından alanlarda kumul vejetasyonu hâkim olup kumul vejetasyonu bitkilerine rastlanılmaktadır. Bunlara ilave olarak alanın kumul vejetasyonuna sahip olması nedeniyle belirlenen takson sayılarının da oldukça sınırlı olduğu görülmektedir.

Bu araştırmada endemik takson belirlenmemiş olup çalışma alanlarının tamamında nadir bir takson olan *Pancratium maritimum* L. bulunmaktadır.

Çalışma alanlarının genel vejetasyon yapısına bakıldığında; Adanalıoğlu ormanı ve sahilinde; *Juncetea maritimi* Br.-Bl. in Br.-Bl. et al. 1952 sınıfına ait *Juncetalia maritimi* Br.-Bl. ex Horvatić 1934 ordosunun *Juncion maritimi* Br.-Bl. ex Horvatić 1934 alyansı karakter türü olan *Halimione portulacoides* (L.) Aellen ile temsil edilmektedir. *Juncetea maritimi* sınıfının *Limonium angustifolium* (Tausch) Turill karakter türüyle temsil edildiği görülmüştür. *Cakiletea maritimae* Tx. et Preising in Tx. ex Br.-Bl. et Tx. 1952 sınıfına ait *Cakilion euxinae* Géhu et al. 1994 (syn.: *Cakilion maritimae* Morariu 1957) alyansı *Cakile maritima* karakter türü ile temsil edilmektedir. *Astragalo-Brometea* Quézel 1973 sınıfına ait *Onobrychido armenae-Thymetalia leucostomi* Akman, Ketenoğlu, Quézel 1985 ordosunun *Salvio tchihatcheffi-Hedysarion variaie* alyansı karakter türü olan *Helianthemum nummularium* (L.) Miller subsp. *nummularium* karakter türü ile temsil edilmektedir. *Astragalo-Brometea* Quézel 1973 sınıfı *Helianthemum nummularium* (L.) Miller subsp. *nummularium* karakter türü ile temsil edildiği görülmüştür. *Salicornietea* sınıfının *Prosopis farcta* (Banks & Sol.) Macbride ve *Quercus-Fagetea* Jakucs ex Passarge 1968 sınıfının *Pinus pinea* L. karakter türleri ile temsil edildiği ancak ordo ya da alyans karakter türünün bulunmadığı belirlenmiştir (Çizelge 4).

Çizelge 4. Adanalıoğlu ormanı ve sahilinde belirlenen sintaksonomik üst birimler ve karakter türleri
Table 4. Syntaxonomic upper units and character types determined in Adanalıoğlu forest and coast

Kategori	Sintakson	Karakter Tür
1.Sınıf	<i>Juncetea maritimi</i>	<i>Limonium angustifolium</i>
1.Ordo	<i>Juncetalia maritimi</i>	
1.Alyans	<i>Juncion maritimi</i>	<i>Halimione portulacoides</i>
2.Sınıf	<i>Cakiletea maritimae</i>	
1.Alyans	<i>Cakilion maritimae</i>	<i>Cakile maritima</i>
3.Sınıf	<i>Astragalo-brometea</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
1.Ordo	<i>Onobrychido armenae-thymetalia leucostomi</i>	
1.Alyans	<i>Salvio tchihatcheffi-Hedysarion variaie</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
4.Sınıf	<i>Salicornietea</i>	<i>Prosopis farcta</i>
5.Sınıf	<i>Quercus-Fagetea</i>	<i>Pinus pinea</i>

Kazanlı doğal sit alanında; *Juncetea maritimi* sınıfına ait *Juncetalia maritimi* ordosu *Juncion maritimi* alyansı karakter türleri olan *Halimione portulacoides* (L.) Aellen ve *Juncus maritimus* karakter türleriyle temsil edilmektedir. *Juncetea maritimi* sınıfı ve *Juncetalia maritimi* ordosunun da *Juncus maritimus* karakter türü ile temsil edildiği görülmüştür. *Cakiletea maritimae* sınıfına ait *Cakilion maritimae* alyansının *Cakile maritima* karakter türü ile temsil edildiği belirlenmiş, Ordo düzeyinde herhangi bir karakter türe rastlanmamıştır. *Astragalo-Brometea* sınıfına ait *Onobrychido armenae-Thymetalia leucostomi* ordosunun *Salvio tchihatcheffi-Hedysarion variaie* alyansı karakter türü olan *Helianthemum nummularium* (L.) Miller subsp. *nummularium* karakter türü ile temsil edilmektedir. *Astragalo-Brometea* sınıfının *Helianthemum nummularium* (L.) Miller subsp. *nummularium* karakter türü ile temsil edildiği görülmüştür. *Salicornietea* sınıfının *Prosopis farcta* ve *Quercus-Fagetea* Br.-Bl and Vlieger 1937 sınıfının *Pinus pinea* karakter türleri ile temsil edildiği, ordo ya da alyans karakter türünün bulunmadığı beirlenmiştir (Çizelge 5).

Tarsus Dalyanında; *Juncetea maritimi* sınıfına ait *Juncetalia maritimi* ordosunun *Juncion maritimi* alyansı karakter türleri olan *Juncus maritimus* karakter türü ile temsil edilmektedir. *Juncetea maritimi* sınıfı ve *Juncetalia maritimi* ordosunun da *Juncus maritimus* ile temsil edildiği görülmüştür. *Cakiletea maritimae* sınıfına ait *Cakilion maritimae* alyansı *Cakile maritima* türü ile temsil edilmesine rağmen ordo düzeyinde herhangi bir karakter türe rastlanmamıştır. *Phragmito-Magnocaricetea* sınıfına ait *Phragmitetalia* ordosunun *Phragmition communis* Koch 1926 alyansının karakter türü olan *Bolboschoenus maritimus* subsp. *maritimus* (L.) Palla karakter türü ile temsil edildiği görülmüştür. *Phragmitetalia* Koch 1926 ordosunun da *Iris pseudacorus* L. karakter türü ile temsil edildiği görülmüştür. *Salicornietea* sınıfının *Prosopis farcta*, *Quercus-Fagetea* sınıfının *Pinus pinea* ve *Heldreichietea* sınıfının *Rumex scutatus* L. ile temsil edildiği buna karşın, ordo ya da alyans düzeyinde karakter türünün bulunmadığı tespit edilmiştir (Çizelge 6).

Çizelge 5. Kazanlı sit alanında belirlenen sintaksonomik üst birimler ve karakter türleri

Table 5. *Syntaxonomic upper units and character types determined in the Kazanlı protected area*

Kategori	Sintakson	Karakter Tür
1.Sınıf	<i>Juncetea maritimi</i>	<i>Juncus maritimus</i>
1.Ordo	<i>Juncetalia maritimi</i>	<i>Juncus maritimus</i>
1.Alyans	<i>Juncion maritimi</i>	<i>Halimione portulacoides</i> <i>Juncus maritimus</i>
2.Sınıf	<i>Cakiletea maritimae</i>	
1.Alyans	<i>Cakilion maritimae</i>	<i>Cakile maritima</i>
3.Sınıf	<i>Astragalo-Brometea</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
1.Ordo	<i>Onobrychido armenae-Thymetalia leucostomi</i>	
1.Alyans	<i>Salvio tchihatcheffi-Hedysarion variaie</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
4.Sınıf	<i>Salicornietea</i>	<i>Prosopis farcta</i>

Çizelge 6. Tarsus Dalyanı sit alanında belirlenen sintaksonomik üst birimler ve karakter türleri

Table 6. *Syntaxonomic upper units and character types determined in the Tarsus Dalyan protected area*

Kategori	Sintakson	Karakter Tür
1.Sınıf	<i>Juncetea maritimi</i>	<i>Juncus maritimus</i>
1.Ordo	<i>Juncetalia maritimi</i>	<i>Juncus maritimus</i>
1.Alyans	<i>Juncion maritimi</i>	<i>Juncus maritimus</i>
2.Sınıf	<i>Cakiletea maritimae</i>	
1.Alyans	<i>Cakilion maritimae</i>	<i>Cakile maritima</i>
3.Sınıf	<i>Phragmito-magnocaricetea</i>	
1.Ordo	<i>Phragmitetalia</i>	<i>Iris pseudacorus</i>
1.Alyans	<i>Phragmition communis</i>	<i>Bolboschoenus maritimus</i> var. <i>maritimus</i>
4.Sınıf	<i>Salicornietea</i>	<i>Prosopis farcta</i>
5.Sınıf	<i>Querco-Fagetea</i>	<i>Pinus pinea</i>
6.Sınıf	<i>Heldreichietea</i>	<i>Rumex scutatus</i>

Alata sahili doğal sit alanınınında; *Cisto-Micromerietea* Oberd. 1954 sınıfına ait *Cisto-Micromerietalia Cisto-Micromerietalia* ordosu *Sarcopoterium spinosum* türüyle temsil edilmekte ve alyans seviyesinde karakter türü bulunmamaktadır. *Quercetea ilicis* Br.-Bl. ex A. Bolòs et O. de Bolòs in A. Bolòs y Vayreda 1950 sınıfına ait *Quercetalia ilicis* Br.-Bl. ex Molinier 1934 ordosu, *Olea-Ceratonion* Br.-Bl. ex Guinochet et Drouineau 1944 alyansı ile *Pistacio-Rhamnetalia* Rivas-Mart. 1975 ordosunun karakter türleri olan *Myrtus communis* subsp. *communis* L. ile temsil edilmektedir. *Astragalo-Brometea* sınıfına ait *Onobrychido armenae-Thymetalia leucostomi* ordosunun *Salvio tchihatcheffi-Hedysarion variaie* alyansı karakter türü olan *Helianthemum nummularium* subsp. *nummularium* karakter türü ile temsil edilmektedir. *Astragalo-Brometea* sınıfı *Helianthemum nummularium* subsp. *nummularium* karakter türü ile temsil edildiği görülmüştür. *Juncetea maritimi* sınıfının *Plantago maritima* L. ve *Salici purpureae-Populetea nigrae* sınıfı *Equisetum hyemale* L. karakter türleri ile temsil edilmekte olup ordo ya da alyans karakter türüne rastlanmamıştır (Çizelge 7).

Çizelge 7. Alata sahili sit alanında belirlenen sintaksonomik üst birimler ve karakter türleri

Table 7. *Syntaxonomic upper units and character types determined in the Alata coast protected area*

Kategori	Sintakson	Karakter Tür
1.Sınıf	<i>Cisto-micromerietea</i>	
1.Ordo	<i>Cisto-micromerietalia</i>	<i>Sarcopoterium spinosum</i>
2.Sınıf	<i>Quercetea ilicis</i>	
1.Ordo	<i>Quercetalia ilicis</i>	
1.Alyans	<i>Olea – ceratonion</i>	<i>Myrtus communis</i> subsp. <i>communis</i>
2.Ordo	<i>Pistacio-rhamnetalia</i>	<i>Myrtus communis</i> subsp. <i>communis</i>
3.Sınıf	<i>Astragalo-Brometea</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
1.Ordo	<i>Onobrychido armenae-Thymetalia leucostomi</i>	
1.Alyans	<i>Salvio tchihatcheffi-Hedysarion variaie</i>	<i>Helianthemum nummularium</i> subsp. <i>nummularium</i>
4.Sınıf	<i>Juncetea maritimi</i>	<i>Plantago maritima</i>
5.Sınıf	<i>Salici purpureae-populetea nigrae</i>	<i>Equisetum hyemale</i>

Alata Bahçe Kültürleri Araştırma Enstitüsü doğal Sit Alanında; *Cakiletea maritimae* sınıfına ait *Cakilion maritimae* alyansı *Cakile maritima* karakter türü ile temsil edildiği, Ordo düzeyinde herhangi bir karakter tür bulunmadığı görülmüştür. *Astragalo-Brometea* sınıfına ait *Onobrychido armenae-Thymetalia leucostomi* ordosunun *Salvio tchihatcheffi-Hedysarion varia* alyansı karakter türü olan *Helianthemum nummularium* subsp. *nummularium* karakter türü ile temsil edildiği, *Astragalo-Brometea* sınıfının *Helianthemum nummularium* subsp. *nummularium* karakter türü ile temsil edildiği görülmüştür. Ordo seviyesinde karakter türe rastlanmamıştır. *Quercetea ilicis* Br.-Bl., 1947 sınıfına ait *Quercetalia ilicis* Br.-Bl., 1947, Rivaz Martinez, 1974 ordosu, *Olea-Ceratonion* alyansının *Prasium majus* L., *Phillyrea latifolia* L. ve *Clematis cirrhosa* L. karakter türleriyle, *Pistacio-Rhamnetalia* ordosunun karakter türü olan *Clematis cirrhosa* ile temsil edildiği görülmüştür. *Quercetea ilicis* sınıfı ve *Quercetalia ilicis* ordosu *Phillyrea latifolia* karakter türü ile temsil edilmektedir (Çizelge 8).

Çizelge 8. Alata bahçe kültürleri araştırma enstitüsü sit alanında belirlenen sintaksonomik üst birimler ve karakter türleri

Table 8. Syntaxonomic upper units and character types determined in the Alata garden cultures research institute protected area

Kategori	Sintakson	Karakter Tür		
1.Sınıf	<i>Cakiletea maritimae</i>			
1.Alyans	<i>Cakilion maritimae</i>	<i>Cakile maritima</i>		
2.Sınıf	<i>Astragalo-Brometea</i>	<i>Helianthemum nummularium</i>	subsp.	
1.Ordo	<i>Onobrychido armenae-Thymetalia leucostomi</i>			
1.Alyans	<i>Salvio tchihatcheffi-Hedysarion varia</i>	<i>Helianthemum nummularium</i>	subsp.	
3.Sınıf	<i>Quercetea ilicis</i>	<i>Phillyrea latifolia</i>		
1.Ordo	<i>Quercetalia ilicis</i>	<i>Phillyrea latifolia</i>		
1.Alyans	<i>Olea – ceratonion</i>	<i>Prasium majus</i>		
		<i>Phillyrea latifolia</i>		
		<i>Clematis cirrhosa</i>		
2.Ordo	<i>Pistacio-Rhamnetalia</i>	<i>Clematis cirrhosa</i>		

Çalışma alanlarının genel vejetasyonu araştırılırken Braun-Blanquet metodu uygulanmadığından birlikler oluşturulmamış, bu nedenle yakın bölgelerdeki vejetasyon çalışmalarıyla mukayese edilememiştir. Araştırma alanlarında bulunan habitat tiplerinin EUNIS habitat tiplerine göre (Anonim, 2024a; Davies ve ark., 2004) sınıflandırılması sonucunda; değişik düzeylerde beş seviye 1 ve sekiz seviye 2 habitat tipi bulunduğu belirlenmiştir (Çizelge 9). Çalışma alanları ayrı ayrı değerlendirildiğinde; Adana’da 5 seviye 1, 9 seviye 2 habitat tipi, Kazanlı’da 3 seviye 1, 7 seviye 2 habitat tipi, Tarsus Dalyan’ında 4 seviye 1, 8 seviye 2 habitat tipi, Alata Sahili’nde 3 seviye 1, 7 seviye 2 habitat tipi ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal sit alanında 3 seviye 1, 6 seviye 2 habitat tipi bulunduğu tespit edilmiştir (Çizelge 9).

Türkiye’de Çakmak & Aytaç (2021) tarafından yapılan çalışmada EUNIS habitat sınıflandırmasındaki habitat tiplerinden (toplam 326) 138 tanesinin Türkiye’de de bulunduğu tespit edilmiştir. EUNIS habitat sınıflandırması halen devam etmekte olup İç sular, Sulak alanlar ve İnşa edilmiş, endüstriyel ve diğer yapay habitatlar değerlendirilmemiştir. Bu çalışmada da 5 seviye 1 habitat tipine bağlı 8 seviye 2 habitat tipi belirlenmiştir. Birçok ülke EUNIS habitat sınıflamasını tamamlamış olup Türkiye bu kapsamda yapılan çalışmalarla 30’a yakın ilin EUNIS habitat tipleri belirlenmiştir (Terzioğlu ve ark., 2015; Kanca ve ark., 2019). Bu illerin tamamında İç su habitatları, Otlaklar ve çimen dışı otsu habitatlar, Ormanlar, Tarım alanları ve İnsan yapımı habitatlar bulunmaktadır (Terzioğlu ve ark., 2015). Bu durum Türkiye’nin sahip olduğu habitat zenginliğinin bilinmesi, sağlıklı kullanımı ve EUNIS habitat tiplerinin tüm Türkiye için elde edilmesini sağlayacaktır. Bu çalışma ile Türkiye’nin habitat tipleri detaylıca çıkartılarak türlerin, habitatların ve illerin peyzaj haritalarında değişim olup olmadığının izlenmesine katkı sağlanmış olacaktır. Literatür çalışmalarına bakıldığında denizel habitatlar ile bataklıklar ve turbalık habitatların çok düşük düzeyde temsil edildiği, fundalık, çalılık ve tundraların temsil düzeyi ise yüksek çıktığı görülmektedir (Terzioğlu ve ark., 2015; Kanca ve ark., 2019; Çakmak & Aytaç, 2021). Bunun sebebinin çalışmaların büyük çoğunluğunun karasal alanlarda yapılması ve fundalık, çalılık ve tundralara ait verilerin fazla olmasından kaynaklandığı düşünülmektedir. Buna ilave olarak Türkiye’de 3. seviyede en çok bulunan EUNIS habitat tiplerinin sırasıyla; E1.2 (11 çalışmada), G3.5 (9 çalışmada) ve G1.7 (8 çalışmada) olduğu belirlenmiştir (Çakmak & Aytaç, 2021). Bu çalışmada çalışma alanının maki ve kıyı vejetasyonuna sahip olması nedeniyle yukarıda belirtilen habitat tiplerinden sadece T3A5 *Pinus brutia* ormanı habitatına rastlanmamıştır.

Bunun sebebinin de benzer habitat tiplerine sahip alanlarda yapılan çalışmaların fazlalığından kaynaklandığı düşünülmektedir (Şahin & Karavelioğlu (2018a), Şahin & Karavelioğlu (2018b), Tug ve ark. (2018), Seyfe (2019), Özen & Ürker (2020), Çakmak & Aytaç (2020), Çakmak & Aytaç (2021) ve Demir ve ark. (2022). Bu çalışmada ise 5 seviye 1 ve 8 seviye 2 habitat tipi belirlenmiştir. Çalışmada elde edilen habitat tiplerinin çeşitliliği bölgenin ikliminin sıcak Akdeniz iklimi olması ile doğrudan ilişkilidir. Aynı zamanda habitat tiplerinin farklı olması bölgenin florasının zengin olmasını da sağlamaktadır.

Çizelge 9. Araştırma alanlarındaki Eunis habitat sınıflandırma tipleri ve kodları

Table 9. Eunis habitat classification types and codes in research areas

Alan Adı	EUNIS Sınıflandırma Kodu	Habitat Adı	EUNIS alt sınıflandırma kodu ve açıklaması
Adanalıoğlu Orman Alanı ve sahili	N	Kıyı	Kumul N12: Kum plajı N14: Hareketli Kıyı Kumulları N1B: Kıyı Fundalıkları
	M	Çamurlu ve bataklık alanlar	MA651: Sulak alan, taşkın yatağı (Sazlık-bataklık-turbalık alanlar)
	S	Fundalık, çalılık ve tundra	S4: Maki, Ağaçsı ve Sıcak Akdeniz Çalılıkları S5: Maki; S51: Sıcak Akdeniz Çalılıkları
	T	Ağaçlık, orman habitatları ve ağaçlandırılmış alanlar	T3A5: Pinus brutia ormanı
	V	Tarım alanları	V12: Karışık ekinli tarlalar, bostanlar, bahçeler
Kazanlı	N	Kıyı	Kumul N14: Hareketli Kıyı Kumulları N1B: Kıyı Fundalıkları N12: Kum plajı
	S	Fundalık, çalılık ve tundra	S4: Maki, Ağaçsı ve Sıcak Akdeniz Çalılıkları S5: Maki; S51: Sıcak Akdeniz Çalılıkları
	V	Tarım alanları	V12: Karışık ekinli tarlalar, bostanlar, bahçeler
Tarsus Dalyanı	N	Kıyı	Kumul N14: Hareketli Kıyı Kumulları N1B: Kıyı Fundalıkları N12: Kum plajı
	M	Çamurlu ve bataklık alanlar	MA651: Sulak alan, taşkın yatağı (Sazlık-bataklık-turbalık alanlar)
	S	Fundalık, çalılık ve tundra	S4: Maki, Ağaçsı ve Sıcak Akdeniz Çalılıkları S5: Maki; S51: Sıcak Akdeniz Çalılıkları
	V	Tarım alanları	V12: Karışık ekinli tarlalar, bostanlar, bahçeler
Alata Sahili	N	Kıyı	Kumul N14: Hareketli Kıyı Kumulları N1B: Kıyı Fundalıkları N12: Kum plajı
	S	Fundalık, çalılık ve tundra	S4: Maki, Ağaçsı ve Sıcak Akdeniz Çalılıkları S5: Maki; S51: Sıcak Akdeniz Çalılıkları
	V	Tarım alanları	V12: Karışık ekinli tarlalar, bostanlar, bahçeler
Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal sit alanı	N	Kıyı	Kumul N14: Hareketli Kıyı Kumulları N1B: Kıyı Fundalıkları N12: Kum plajı
	S	Fundalık, çalılık ve tundra	S4: Maki, Ağaçsı ve Sıcak Akdeniz Çalılıkları; S5: Maki, S51: Sıcak Akdeniz Çalılıkları
	V	Tarım alanları	V12: Karışık ekinli tarlalar, bostanlar, bahçeler

Bu çalışmada 38 familya, 69 cinse ait toplam 78 takson (58 tür) tespit edilmiştir. Bu taksonların fitocoğrafik bölgeleri; 1 Avrupa-Sibirya (%1.28), 1 İran-Turan (%1.28), 17 Geniş Yayılışlı (%21.79), 37 bölgesi bilinmeyen (%47.43) ve 21 Akdeniz elementi (%26.92) şeklindedir. Akdeniz fitocoğrafik bölgesi elementlerinin fazla olması, çalışma alanlarının Akdeniz bölgesinde yer almasından kaynaklanmaktadır. Araştırma alanı ile diğer doğal sit alanlarında yapılan çalışmalar taksonların fitocoğrafik bölgelerine göre karşılaştırıldığında Ortaç & Tel (2021)'in çalışması hariç, diğer tüm alanlarda Akdeniz fitocoğrafik bölge elementinin daha fazla bulunduğu görülmüştür.

(Aksay, 2006; Uçar, 2002; Yıldızıtugay & Küçüködük, 2010; Tezcan, 1995; Tel ve ark., 2018; 2019; 2021; 2022a; 2022b; 2023). Bu sonuç çalışma alanlarının Akdeniz bölgesinde bulunması sebebiyle beklenen bir sonuçtur (Çizelge 10).

Çizelge 10. Araştırma alanı ve çevresindeki çalışmalardaki taksonların fitocoğrafik bölgelere göre dağılım oranları (%)
Table 10. Distribution rates of taxa in studies in and around the research area according to phytogeographic regions (%)

Çalışmalar	Akdeniz Elementi	Avrupa- Sibirya Elementi	İran- Turan Elementi	Geniş Yayılışlı ve Bilinmeyen	Endemizm Oranı
Çalışma Alanı	26.92	1.28	1.28	69.22	0
Anamur Antik Kenti ve Çevresinin Florası (Yıldızıtugay & Küçüködük, 2010)	35.10	3.10	4.90	56.90	3.70
Kahramanmaraş İlinin Bazı Doğal ve Kültürel Sit Alanları Florası (Tel ve ark., 2018)	23.20	6.90	10.50	59.30	18.60
Karatepe-Aslantaş Milli Parkı ve Bazı Doğal/Kültürel Koruma Alanları Üzerine Floristik Bir Araştırma (Tel ve ark., 2019)	38.40	6.10	4.30	51.20	9.10
Hatay İlinin Bazı Doğal/Kültürel Sit Alanları Florası Üzerine Bir Çalışma (Tel ve ark., 2021)	36.80	3.90	4.50	54.80	4.50
Gazihan Dede Mesire Alanı Florası (Ortaç & Tel, 2021)	14.50	6.10	29.60	49.80	4.04
Bazı mağara ve obrukların flora ve genel vejetasyon yapıları üzerine bir araştırma (Tel ve ark., 2022b)	48.69	1.73	6.08	43.47	2.60
A floristic study on some natural and cultural sites of Adana province (Tel ve ark., 2022a)	34.60	4.50	5.00	55.90	1.30
Mersin İlindeki Bazı Doğal/Kültürel Sit Alanlarının Floristik Yapısı Üzerine Bir Çalışma (Tel ve ark., 2023)	56.07	0.00	0.00	43.93	1.51
Contributions of floristic and general vegetation characteristics of the Mavga Castle (Tel ve ark., 2024)	29.32	0.00	7.01	63.15	14.03

Araştırma alanında endemik takson tespit edilmemiştir. Çalışma alanının diğer floristik çalışmalarda en yüksek endemizm oranının %18.60 ile Tel ve ark. (2018)'nin çalışmasında, en düşüğünün ise %1.30 ile Tel ve ark. (2022a)'nın çalışmasında olduğu görülmüştür. Tel ve ark. (2018)'nin çalışmasında endemizmin yüksekliği bölgenin farklı mikroklima özelliği, yükselti ve habitat çeşitliliğine sahip olması ile açıklanabilir. Tel ve ark. (2022a)'nın çalışmasında endemizmin en düşük olması ise alanın sulak alan habitatına sahip olması, ayrıca kumul ve tuzcul bitkilerden oluşan vejetasyon yapısına sahip olması ile açıklanabilir (Çizelge 10). Çalışma alanlarında ihtiva ettikleri takson sayısına göre en büyük familyalar Tarsus Dalyanı'nda *Asteraceae*, Adanalıoğlu ormanı ve sahili, Kazanlı, Alata sahili, Alata Bahçe Kültürleri Araştırma Enstitüsü'nde *Fabaceae*'dir.

Çalışma alanı ve yakın sahalarda gerçekleştirilen araştırmalar, en çok takson içeren familyalara göre mukayese edildiğinde Yıldızıtugay & Küçüködük (2010), Ortaç & Tel (2021), Tel ve ark. (2018; 2022a)'da *Asteraceae*'nin birinci, Bu çalışma, Tel ve ark. (2019; 2021; 2022b; 2023)'nin çalışmalarında ise *Fabaceae*'nin birinci sırada olduğu belirlenmiştir. Bu durumun nedeninin Türkiye Florası'nda *Asteraceae* ve *Fabaceae* familyalarının takson sayıları bakımından üst sıralarda bulunması ve bu familyaların generatif üreme kabiliyetinin yüksek olması ve çalışma alanlarının Akdeniz bölgesinde yer almasından kaynaklandığı düşünülmektedir (Çizelge 11).

Alanın genel vejetasyon yapısıyla ilgili olarak; çalışma alanlarının vejetasyon yapısı araştırılırken Braun-Blanquet metoduyla ayrıntılı incelenmediğinden bitki birlikleri oluşturulmamıştır. Araştırma sahasına yakın ve uzak bölgelerde yapılan bazı sintaksonomik çalışmalarla karşılaştırma yapıldığında araştırma alanının Tel ve ark. (2010)'un çalışmasındaki *Quercetea ilicis*, *Astragalo-Brometea* sınıfları ve *Qercetalia ilicis* ordosu ile benzerlik gösterdiği ancak daha aşağı sintaksonomik birimlerde benzerlik bulunmadığı görülmüştür (Tel ve ark., 2010; Tel

& Tak, 2012; Tel & Eğilmez, 2015). Çalışma alanı Kavak (2006)'nın çalışması ile karşılaştırıldığında vejetasyon yapısının *Juncetea maritimi* ve *Cakiletea maritimae* sınıfı, *Phragmitetalia* ve *Pistacia-Rhamnetalia* ordosu, *Phragmition communis*, *Cakilion maritimae* ve *Olea-ceratonion* alyansları ile benzerlik gösterdiği görülmüştür. Uçar (2002) ve Aksay (2006)'nın çalışmasıyla habitat farklılığından dolayı benzerlik bulunmadığı görülmüştür. Karaömeroğlu (2007)'nin çalışmasındaki *Juncetea maritimi*, *Cakiletea maritimae*, *Pragmito-Magnocaricea*, *Cisto-micromerietea* ve *Quercetea ilicis* sınıfı, *Juncetalia maritimi* ve *Cisto-micromerietalia* ordoları, *Juncion maritimi*, *Cakilion maritimae* ve *Pragmition communis* alyansları ile benzerlik gösterdiği görülmüştür. Tel & Tak (2012)'in çalışmasında *Astragalo-Brometea* sınıfının benzerlik gösterdiği alt birimlerde benzerlik bulunmadığı görülmüştür. Bunun sebebi olarak çalışma alanlarının fitocoğrafik bölgelerinin farklı olması, iklim, yükseklik ve toprak yapısının farklı olmasından kaynaklandığı söylenebilir. Yapılan değerlendirmede sınıf seviyesinde benzerlik olduğu ancak ordo ve alyans düzeyinde farklılıkların bulunduğu belirlenmiştir. Bu durumun habitat tiplerinin farklılığından kaynaklandığı düşünülmektedir. Tel ve ark. (2024), *Quercetea ilicis* sınıfı, *Quercetalia ilicis* ve *Pistachio-Rhamnetalia* ordoları *Olea-Ceratonion* alyansı ile benzerlik gösterdiği görülmüştür.

Çizelge 11 Çalışma alanı ve yakın sahalardaki çalışmalarda en fazla takson içeren familyalar

Table 11. Families containing the most taxa in studies in the study area and nearby areas

Araştırma Alanları	Fabaceae	Lamiaceae	Asteraceae
Çalışma Alanı	8	2	9
Anamur Antik Kenti ve Çevresinin Florası (Yıldızıtugay & Küçüköğüt, 2010)	35	15	40
Kahramanmaraş İlinin Bazı Doğal ve Kültürel Sit Alanları Florası (Tel ve ark., 2018)	5	4	13
Karatepe-Aslantaş Milli Parkı ve Bazı Doğal/Kültürel Koruma Alanları Üzerine Floristik Bir Araştırma (Tel ve ark., 2019)	26	13	13
Hatay İlinin Bazı Doğal/Kültürel Sit Alanları Florası Üzerine Bir Çalışma (Tel ve ark., 2021)	22	15	13
Gazihan Dede Mesire Alanı Florası (Ortaç & Tel, 2021)	27	27	43
Bazı mağara ve obrukların flora ve genel vejetasyon yapıları üzerine bir araştırma (Tel ve ark., 2022b)	32	12	4
A floristic study on some natural and cultural sites of Adana province (Tel ve ark., 2022a)	15	10	33
Mersin İlindeki Bazı Doğal/Kültürel Sit Alanlarının. Floristik Yapısı Üzerine Bir Çalışma (Tel ve ark., 2023)	12	6	9
Contributions of floristic and general vegetation characteristics of the Mavga Castle (Tel ve ark., 2024)	7	10	5

SONUÇ ve ÖNERİLER

Sonuç olarak, Akdeniz bölgesinde bulunan çalışma alanlarının floristik ve genel vejetasyon yapısını belirlemek amacıyla yapılan bu çalışmada 38 familya ve 69 cinse ait 78 takson (58 tür) tespit edilmiştir. Taksonların fitocoğrafik bölgelere göre dağılımı incelendiğinde, Akdeniz fitocoğrafik bölgesi elementlerinin %26.92'lik bir oranla ilk sırada yer aldığı görülmüştür. Bu çalışmada endemik takson belirlenmemiş olup çalışma alanlarının tamamında nadir bir takson olan *Panocratium maritimum* L. un bulunduğu tespit edilmiştir. Bu türün IUCN tehlike kategorisinin LC (En az endişe verici) olduğu belirlenmiştir.

Araştırma alanlarında EUNIS habitat tipleri sınıflandırılması sonucunda farklı düzeylerde 5 seviye 1 habitata ait 8 seviye 2 habitat tipi bulunduğu belirlenmiştir. Habitat tiplerinin belirlenmesi hem biyolojik çeşitlilik çalışmalarına hem de farklı disiplinlerin çalışmalarına katkı sağlayacaktır. Araştırma alanlarının genel vejetasyon yapısının Adana'da orman alanı ve sahili, Kazanlı ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal sit alanında kıyı kumullarında kompozisyonunu çoğunlukla kumul bitkilerinin oluşturduğu kıyı kumul vejetasyonu ve sahilden iç kesimlerde maki elemanlarının bulunduğu, Tarsus Dalyanında sulak alan vejetasyonu ve Alata sahilinin genel vejetasyon yapısının kıyı kumul vejetasyonundan teşekkül ettiği belirlenmiştir.

Çalışma Çalışma sonucunda; Adana'da orman ve sahili'nde 23 familya, 33 cins ve bu cinslere ait toplam 35, Kazanlı'da 20 familya 31 cinse ait 31, Tarsus Dalyanı'nda 19 familya 29 cinse ait toplam 30, Alata Sahili'nde 11 familya, 13 cinse ait 13 ve Alata Bahçe Kültürleri Araştırma Enstitüsü Doğal Sit Alanı'nda, 19 familya 24 cinse ait 24 takson belirlenmiştir. Alanlardaki takson sayılarının az olması alanın iklim ve istasyon tiplerinin stabil olmasıyla açıklanabilir. Araştırma alanlarından toplanan bitkilerin fitocoğrafik bölgelerinin dağılımının 1 takson Avrupa-Sibirya (%1.28), 1 İran-Turan (%1.28), 17 Geniş Yayılışlı (%21.79), 37 fitocoğrafik bölgesi bilinmeyen

(%47.43) ve 21 Akdeniz elementi (%26.92) şeklinde olduğu tespit edilmiştir. Çalışma alanlarında ihtiva ettikleri takson sayısına göre en büyük familyaların Tarsus Dalyanında *Asteraceae*, Adanalıoğlu ormanı ve sahili, Kazanlı, Alata Sahili, Alata Bahçe Kültürleri Araştırma Enstitüsü'nde *Fabaceae* olduğu görülmüştür.

Akd. Elm.: Akdeniz Fitocoğrafik Bölge Elementi; D. Akd. Elm.: Doğu Akdeniz Fitocoğrafik Bölge Elementi; Av-Sib.: Avrupa-Sibirya Fitocoğrafik Bölge Elementi; (Ir-Tur. Elm.: İran-Turan Fitocoğrafik Bölge Elementi; Ge. Yay.: Geniş Yayılışlı; End.: Endemik; VU: Zarar görebilir; EN: Tehlikede; CR: Kritik Tehlikede; NT: Tehdit altına girebilir; LC: Asgari endişe şeklindedir.

*Akd. Elm.: Akdeniz Fitocoğrafik Bölge Elementi; D. Akd. Elm.: Doğu Akdeniz Fitocoğrafik Bölge Elementi; Av-Sib.: Avrupa-Sibirya Fitocoğrafik Bölge Elementi; (Ir-Tur. Elm.: İran-Turan Fitocoğrafik Bölge Elementi; Ge. Yay.: Geniş Yayılışlı; End.: Endemik; VU: Zarar görebilir; EN: Tehlikede; CR: Kritik Tehlikede; NT: Tehdit altına girebilir; LC: Asgari endişe şeklindedir.

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KAYNAKLAR

- Aksay, C. S. (2006). *Pusat Dağı flora ve vejetasyonu (Silifke-Mersin-Türkiye)*, (Tez no 180747). [Yüksek Lisans Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Anonim, (1983). *Kültür ve tabiat varlıklarını koruma kanunu*. 23 Temmuz 1983 Tarih ve 18113 Sayılı Resmî Gazete, Ankara (Erişim tarihi: 19.05.2024).
- Anonim, (2016). *Adana, Hatay, Mersin, Kahramanmaraş, Osmaniye, Gaziantep ve Kilis illeri doğal sit alanlarının ekolojik temelli bilimsel araştırma projesi sonuç raporu*. T.C. Çevre ve Şehircilik Bakanlığı, Ankara.
- Anonim, (2022). Korunan alanların tespit, tescil ve onayına ilişkin usul ve esaslara dair yönetmelik. 19 Temmuz 2012 Tarih ve 28358 Sayılı Resmî Gazete, Ankara (Erişim tarihi: 19.05.2024).
- Anonim, (2024a). Eunis habitat type hierarchical view (Version 2012). <https://eunis.eea.europa.eu> (Alınma Tarihi: 19.05.2024).
- Anonim, (2024b, Mayıs 19). *Mersin ili haritası*. https://tr.wikipedia.org/wiki/Mersin#/media/Dosya:Latrans-Turkey_location_Mersin.svg
- Anonim, (2024c, Mayıs 19). *Mersin yer gösterim haritası*. <https://paintmaps.com/tr/bos-haritalar/58c/ornekler>
- Anonim, (2024d, Mayıs 19). *Mersin ili sıcaklık ve yağış verileri*. <https://tr.climate-data.org/asya/tuerkiye/mersin/mersin-245/>
- Anonim, (2024e, Mayıs 19). *Mersin ili sıcaklık ve yağış verileri*. <https://tr.climate-data.org/asya/tuerkiye/mersin/tarsus-19373/>
- Anonim, (2024f, Mayıs 19). *Mersin ili sıcaklık ve yağış verileri*. <https://tr.climate-data.org/asya/tuerkiye/mersin/erdemli-26441/>
- Anonim, (2024g, Mayıs 19). *Bitki isimleri kontrol*. <https://www.ipni.org>
- Anonim, (2024h, Mayıs 19). *Bitki isimleri kontrol*. <https://wfoplantlist.org/>
- Anonim, (2024i, Mayıs 19). *Bitki isimleri kontrol*. www.bizimbitkiler.org
- Anonim, (2024j, Mayıs 19). *Tehlike kategorileri*. <https://www.iucnredlist.org>
- Arslan, M. & Arslantürk, N. (2009). Avrupa doğa bilgi sistemi (EUNIS) habitat sınıflandırması. *Orman Mühendisliği*, 46(1-2-3), 48-51.
- Arslan, M., Bingöl, M. Ü., & Erdoğan, N. (2012). Avrupa doğa bilgi sistemi (EUNIS) habitat sınıflandırması ve Türkiye batı Öksin alanındaki Doğu Kayını (*Fagus orientalis* Lipsky) ormanları örneği. *Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi*, 13(2), 278-290.
- Avcı, M. (1993). Türkiye'nin Flora bölgeleri ve Anadolu Diagonali'ne coğrafi bir yaklaşım. *Türk Coğrafya Dergisi*, 28, 225-248.
- Baytop, A. (2010). Plant collectors in Anatolia (Turkey). *Phytologia Balcanica*, 16(2), 187-213.
- Boissier, E. (1867-1888). *Flora Orientalis*, Vol. 1-5. Basilae, Geneva, Switzerland.

- Çakmak, M. H. & Aytaç, Z. (2020). Determination and mapping of EUNIS habitat types of Mamak District (Ankara) Turkey. *Acta Biologica Turcica* 33(4), 227-236. <https://doi.org/10.30516/bilgesci.888297>
- Çiftçi, D. (2015). *Sündiken Dağları Staphylinine (Coleoptera: Staphylinidae) grubunun tür çeşitliliği ve EUNIS habitatları ile ilişkisi (Tez no 395753)*. [Doktora Tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Davies, C. E., Moss, D., & Hill, M.O. (2004). EUNIS Habitat classification revised 2004. http://Eunis.Eea.Eu.Int/Upload/Eunis_2004_Report.Pdf. (Alınma Tarihi: 19.05.2024).
- Davis, P. H. (Editör). (1965-1985). *Flora of Turkey and the east Aegean islands Vol. 1-9*. Edinburgh Univ. Press, Edinburgh.
- Davis P. H. & Hedge I. C. (1975). *Flora of Turkey past, present and future*. Candollea, Edinburgh.
- Davis, P. H., Mill, R. R., & Tan, K. (Editörler). (1988). *Flora of Turkey and the East Aegean Islands Vol. 10*. Edinburgh Univ. Press, Edinburgh.
- Demir, O., Kızıllırmak, A., Bozdağ, C. M., & Cabi, E. (2022). Determination of terrestrial EUNIS habitat types of Mount Ganos (Işıklar), Tekirdağ, Türkiye. *Frontiers in Life Sciences and Related Technologies*, 3(2), 69-74.
- Dinç, M. (2008). The flora of Cocakdere valley (Aslanköy-Mersin). *Ot Sistematik Botanik Dergisi*, 15(2), 87-114.
- Düzenli, A., Çakan, H., Türkmen, N., Uygur, F. N. & Orel, E. (1996). *Göksu Deltasının (Silifke-İçel) florası. Turkish Journal of Botany* 20, 173-191.
- Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., & Adıgüzel, N., 2000: *Türkiye bitkileri kırmızı kitabı (Eğrelti ve Tohumlu Bitkiler)*. Türkiye Tabiatını Koruma Derneği ve Van Yüzüncü Yıl Üniversitesi Yayınları, Ankara.
- Erdoğan, İ. (2016). *Coğrafi bilgi sistemleri ve uzaktan algılama ile Aşağı Kelkit Havzası EUNIS habitat tiplerinin tanımlanması ve potansiyel ürün yetiştirme alanlarının tespiti, (Tez no 446385)*. [Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Everest A. & Rauss, T. (2004). Investigations flora in Mersin: Kozlar highplateau south Turkey. *Pakistan Journal of Biological Sciences*, 7(5), 802-811.
- Geven, F., Özdeniz, E., Kurt, L., Bölükbaşı, A., Özbey, B.G., Özcan, A.U., & Turan, Ü. (2016). Habitat classification and evaluation of the Köyceğiz-Dalyan Special Protected Area (Muğla/Turkey). *Rendiconti Lincei. Scienze Fisiche e Naturali*, 27, 509-519. <https://doi.org/10.1007/s12210-016-0510-1>
- Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M. T. (Editörler). (2012). *Türkiye bitkileri listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.
- Güner, A. & Ekim, T. (Editörler). (2014). *Resimli Türkiye Florası 1*. Flora Araştırmaları Derneği ve Türkiye İş Bankası Kültür Yayınları, İstanbul.
- Güner, A., Kandemir, A., Menemen, Y., Yıldırım, H., Aslan, S., Ekşi, G., Güner, I. & Çimen, A. O. (Editörler). (2018). *Resimli Türkiye Florası cilt 2*. ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları, İstanbul.
- Güner, A., Kandemir, A., Menemen, Y., Yıldırım, H., Aslan, S., Çimen, A. Ö., Güner, I., Ekşi, G. & Şen, F. (Editörler). (2022). *Resimli Türkiye Florası cilt 3a*. ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları, İstanbul.
- Güner, A., Kandemir, A., Menemen, Y., Yıldırım, H., Aslan, S., Çimen, A.Ö., Güner, I., Bona, G.E. ve Gökmen, F.Ş. (edlr.) (2023). *Resimli Türkiye Florası, Cilt 4b*. ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları. İstanbul.
- Güner, A., Kandemir, A., Menemen, Y., Yıldırım, H., Aslan, S., Çimen, A.Ö., Güner, I., Bona, G.E. ve Gökmen, F.Ş. (edlr.) (2024). *Resimli Türkiye Florası, Cilt 4a*. ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları, İstanbul.
- Güner A., Özhatay N., Ekim T. & Başer K. H. C. (2000). *Flora of Turkey and the east Aegean islands Vol. 11*. Edinburgh Univ. Press, Edinburgh.
- Juan Vicedo, J. (2018). *Pancratium maritimum*. The IUCN Red List of Threatened Species 2018: e.T18990540A57467022. <http://dx.doi.org/10.2305/IUCN.UK.2018->
- Kanca, H., Terzioğlu, E., Adıgüzel, U., Erbaş, S., & Erdoğan, E. (2019). *Türkiye'nin biyolojik çeşitliliği*. T.C. Tarım ve Orman Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Ankara.
- Karaömerlioğlu, D. (2007). *Göksu deltasındaki (silifke) doğal ekosistemlerin bitki ekolojisi yönünden araştırılması, (Tez no 212532)*. [Doktora Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Karaömerlioğlu, D. & Düzenli, A. (2008). Göksu deltası (silifke) doğal alanlarında ana habitat tiplerinin araştırılması. *Çukurova Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 17(2), 2.
- Kavak, S. (2006). *Burnaz kumullarının (Adana) flora ve vejetasyonu, (Tez no 198163)*. [Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

- Lončarević, N., Liu, U., Stefanaki, A., Carapeto, A., Ensslin, A., Meade, C., Metzger, D., Peci, D., Fantinato, E., Colling, G., Pankova, H., Akman, I., Svetkov, I., Sibik, J., Szitár, K., Van Meerbeek, K., Daco, L., Boudagher, M., Klisz, M., ... Glasnović, P. (2024). Database of European vascular plants red lists as a contribution to more coherent plant. *Conservation scientific data*, 11:1138, <https://doi.org/10.1038/s41597-024-03963-0>
- Mergen, O., & Karacaoğlu, C. (2015). Tuz Lake Special Environment Protection Area, Central Anatolia, Turkey: The EUNIS habitat classification and habitat change detection between 1987 and 2007. *Ekoloji*, 24(95), 1-9. <https://doi.org/10.5053/ekoloji.2015.06>
- Moss, D. & Roy, D. (1998). *Towards a European habitat classification*. European Environment Agency, Copenhagen.
- Orcan, N., Binzet, R. & Yaylaloğlu, E. (2004). The flora of Fındıklı (Mersin-Turkey) Plateau. *Flora Mediterranea*, 14, 309-345.
- Ortaç, İ. (2017). *Doğu Akdeniz Bölgesinde Bulunan Bazı Doğal ve Kültürel Sit Alanlarının Bitki Örtüsü Üzerine Araştırmalar (Tez no 457718)*. [Yüksek Lisans Tezi, Adıyaman Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Ortaç, Z. & Tel, A. Z. (2021). Gazihan Dede mesire alanı (Adıyaman, Türkiye) florası. *Türler ve Habitatlar*, 2(1), 33-53.
- Özen, A. & Ürker, O. (2020). Avrupa doğa bilgi sistemi (EUNIS) habitat sınıflandırmasını kullanarak Işıklı Gölü ve Gököl Sulak Alanlarında habitat değişimlerinin belirlenmesi. *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 13(2), 518-531, <https://doi.org/10.18185/erzifed.64607>.
- Seçmen, Ö., Gemici, Y., Görk, G., Bekat, L., Leblebici, E. (2000). *Tohumlu bitkiler sistematigi*. Ege Üniversitesi Fen Fakültesi Yayınları, No: 116, İzmir.
- Seyfe, M. (2019). *Kazan tepeleri (Kahramankazan/Ankara) sürünen türlerinin EUNIS habitat tiplerine göre tercih ve dağılımları, (Tez no 598674)*. [Yüksek Lisans Tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Şahin, B. & Karavelioğulları, F. A. (2018a). Erzincan ili EUNIS Habitat Tipleri ve Haritalandırılması (Poster). 1st International Congress on Plant Biology, Konya, Türkiye 10-12 Mayıs 2018, ss. 440.
- Şahin, B. & Karavelioğulları, F. A. (2018b). EUNIS Habitat Types and Mapping of Bayburt (Poster). International Ecology 2018 Symposium, Kastamonu, Türkiye, 19-23 Haziran 2023, ss. 1081.
- Şen, A. N. (2019). *Anamur (Mersin) ve yaylalarının florası (Tez no 610053)*. [Doktora Tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Şenkul, Ç. & Kaya, S. (2017). Türkiye endemik bitkilerinin coğrafi dağılışı. *Türk Coğrafya Dergisi*, 69, 109-120.
- Şirin, E. (2012). *Büyükeğri Dağı (Mut, İçel) ve çevresinin florası (Tez no 315894)*. [Yüksek Lisans Tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Şirin, E. & Ertuğrul, K. (2015). Büyükeğri dağı (mut, içel) ve çevresinin endemik bitkileri. *Selçuk Üniversitesi Fen Fakültesi Dergisi*, 40:50-58.
- Tel, A. Z. (2009). Contributions to the flora of Nemrut Mountain (Adıyaman/Turkey). *BioDiCon*, 2(1), 36-60.
- Tel, A. Z. & Eğilmez, Ç. (2015). Gölbaşı gölleri (Adıyaman/Türkiye) havzası vejetasyon tiplerinin floristik kompozisyonu ve ekolojik özellikleri üzerine bir araştırma, *ADYÜTAYAM Dergisi*, 3(1), 1-28.
- Tel, A. Z. & İlçim, A. (2016). Doğu Akdeniz bölgesinde bulunan bazı doğal ve kültürel sit alanlarındaki bazı endemik ve nadir bitkiler. *ADYÜTAYAM Dergisi*, 4(2), 1-7.
- Tel, A. Z. & Tak, M. (2012). Perre (Pirin) Antik Şehri (Adıyaman) Vejetasyonu. *Biyoloji Bilimleri Araştırma Dergisi*, 5(2), 45-62.
- Tel, A. Z. & Tak, M. (2018). Karagöl (Sülüklü Göl) havzası (Gerger/Adıyaman) florası üzerine bir araştırma. *ADYÜTAYAM Dergisi*, 6(1), 40-53.
- Tel, A. Z., Ortaç, İ., & İlçim, A. (2018). Kahramanmaraş ilinin bazı doğal ve kültürel sit alanları florası üzerine bir çalışma. *Commagene Journal of Biology*, 2(2), 43-47.
- Tel, A. Z., Ortaç, İ., & İlçim, A. (2019). Karatepe-Aslantaş milli parkı ve bazı doğal/kültürel koruma alanları üzerine floristik bir araştırma (Osmaniye, Türkiye). *Commagene Journal of Biology*, 3(2), 103-109.
- Tel, A. Z., Ortaç, İ., & İlçim, A. (2021). Hatay ilinin bazı doğal ve kültürel sit alanları florası üzerine bir çalışma. *Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi*, 22(1), 9-18.
- Tel, A. Z., Ortaç, İ., & Özusu, E. (2022a). Adana ilinin bazı doğal ve kültürel sit alanları üzerine floristik bir çalışma. *Anatolian Journal of Botany* 6(2): 92-103. <https://doi.org/10.30616/ajb.1152708>
- Tel, A. Z., Ortaç, İ., & Özusu, E. (2022b). Bazı mağara ve obrukların (Mersin/Türkiye) flora ve genel vejetasyon yapıları üzerine bir araştırma. *Biological Diversity and Conservation*, 15(3), 356-368. <https://doi.org/10.46309/biodicon.2022.1180111>
- Tel, A. Z., Ortaç, İ., İlçim, A., & Özusu, E. (2023). Mersin ilindeki (Türkiye) bazı doğal ve kültürel sit alanlarının floristik yapısı üzerine bir çalışma. *KSÜ Tarım ve Doğa Dergisi*, 26(5), 1056-1065. <https://doi.org/10.18016/ksutarimdoga.vi.1196119>

- Tel, A. Z., Tatlı, A., & Varol, Ö. (2010). Phytosociological structure of Nemrut Mountain (Adıyaman/Turkey) *Turkish Journal of Botany*, 34, 417-434. <https://doi.org/10.3906/bot-0906-43>
- Tel, A. Z., Ortaç, İ., Özuslu, E., & İlçim, A. (2023). Contributions of floristic and general vegetation characteristics of the Mavga Castle (Mut/Mersin, Türkiye). *Biharean Biologist*, 18(1): 1 – 11.
- Terzioğlu, E., Güvendiren, A. D., Erdoğan, E., Erdoğan, N. M. & Ekmen Nural, İ. (2015). *Biyolojik Çeşitliliği İzleme ve Değerlendirme Raporu 2013-2014*. T.C. Orman ve Su İşleri Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Ankara.
- Tezcan, F. (1995). *Ekşiler Köyü'nün (Silifke) florası, (Tez no 38338)*. [Yüksek Lisans Tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Topal, A., Palabaş Uzun, S. & Uzun, A. (2022). Mersin ili geofit bitki zenginliği. *Turkish Journal of Forest Science*, 6(1), 229-254.
- Tug, G. N., Yaprak, A. E., Körüklü, S. T., Bingöl, U. (2018). Flora and habitat diversity of Kavuncu Saltmarsh. *Communications Faculty of Sciences University of Ankara Series C*, 27(2), 55-68. https://doi.org/10.1501/commuc_00000000198
- Ulu Agır, S., Kutbay, H.G., Karaer, F., & Surmen, B. (2014). The Classification of coastal dune vegetation in Central Black Sea Region of Turkey by numerical methods and EU habitat types. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 25, 453–460. <https://doi.org/10.1007/s12210-014-0328-7>.
- Uçar, A. H. (2002). *Balandız Yaylasının Florası ve Genel Vegetasyon Yapısı, (Tez no 128988)*. [Yüksek Lisans Tezi, Mersin Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Zeren, O. & İspirgil, Y. (2001). Mersin Üniversitesi kampüs alanı florasının tespiti. *Ekoloji*, 10(40), 12-16.
- Üzgör-Ün A., Everest, A., & Demirkuş, N. (2021). *Mersin Üniversitesi Herbaryumu'nda teşhis edilmeyen Asteraceae, Fabaceae ve Lamiaceae familyalarına ait taksonların teşhisi üzerine bir çalışma*. 13. Uluslararası Matematik, Mühendislik, Doğa ve Tıp Bilimleri Kongresi, Ekim 26-27, 2021. Kapadokya, Türkiye.
- Yıldızıtugay, E. & Küçüköyük, M. (2010a). The flora of Anamur antique city and its surroundings (Mersin-Turkey). *Biological Diversity and Conservation*, 3(3), 46-63.

EK-1: Bitki Listesi

No	Familiya Adı	Takson Adı	Fitocoğrafik Bölge	Tehlike Kategorisi	Bulunduğu Sit Alanı	Bitki Toplama Tarihi	Bitki Toplayıcı Numarası
1	Amaranthaceae	<i>Halimione portulacoides</i> (L.) Aellen	-	-	1,2	8.05.2016	Ortaç 1145
2	Amaranthaceae	<i>Salsola kali</i> L.	-	-	1,3	3.04.2016	Ortaç 1149
3	Amaryllidaceae	<i>Allium neapolitanum</i> Cyr.	Akd. Elm.	-	2	3.04.2016	Ortaç 1439
4	Amaryllidaceae	<i>Pancratium maritimum</i> L.	Akd. Elm.	LC	1,2,3,4,5	22.07.2016	Ortaç 1424
5	Anacardiaceae	<i>Pistacia palaestina</i> Boiss.	D. Akd. Elm.	-	4,5	7.04.2016	Ortaç 1017
6	Apiaceae	<i>Turgenia latifolia</i> (L.) Hoffm.	Gen. Yay.	-	1,2	7.04.2016	Ortaç 1025
7	Asparagaceae	<i>Drimia maritima</i> (L.) Stearn	-	-	3	23.10.2016	Ortaç 1456
8	Asparagaceae	<i>Muscari comosum</i> (L.) Miller	Akd. Elm.	-	5	23.07.2016	Ortaç 1448
9	Asteraceae	<i>Carduus pycnocephalus</i> L. subsp. <i>albidus</i> (Bieb.) Kazmi	Gen. Yay.	-	3	22.07.2016	Ortaç 1037
10	Asteraceae	<i>Cichorium intybus</i> L.	Gen. Yay.	-	1	3.04.2016	Ortaç 1046
11	Asteraceae	<i>Inula viscosa</i> (L.) Aiton	Akd. Elm.	-	1,2,3	7.04.2016	Ortaç 1058
12	Asteraceae	<i>Lactuca serriola</i> L.	-	-	2	3.04.2016	Ortaç 1060
13	Asteraceae	<i>Senecio vernalis</i> Waldst. & Kit.	Gen. Yay.	-	2,3	7.04.2016	Ortaç 1070
14	Asteraceae	<i>Silybum marianum</i> (L.) Gaertner	-	-	2	8.05.2016	Ortaç 1073
15	Asteraceae	<i>Sonchus oleraceus</i> L.	-	-	3	7.04.2016	Ortaç 1074
16	Asteraceae	<i>Xanthium papposa</i> L. subsp. <i>italicum</i>	Gen. Yay.	-	3	7.04.2016	Ortaç 1079
17	Asteraceae	<i>Xanthium spinosum</i> L.	-	-	3	3.04.2016	Ortaç 1078
18	Boraginaceae	<i>Anchusa azurea</i> Miller var. <i>azurea</i>	-	-	1	3.04.2016	Ortaç 1088
19	Boraginaceae	<i>Echium angustifolium</i> Miller	D. Akd. Elm.	-	2,4	7.04.2016	Ortaç 1092
20	Boraginaceae	<i>Echium parviflorum</i> Moench	Akd. Elm.	-	1,3	7.04.2016	Ortaç 1093
21	Boraginaceae	<i>Hormuzakia aggregata</i> (Lehm.) Guşul.	Akd. Elm.	-	2,5	8.05.2016	Ortaç 1087
22	Brassicaceae	<i>Cakile maritima</i> Scop.	-	-	1,2,3,5	8.05.2016	Ortaç 1105
23	Brassicaceae	<i>Capsella bursa-pastoris</i> (L.) Medik	Gen. Yay.	-	3	8.05.2016	Ortaç 1106
24	Brassicaceae	<i>Raphanus raphanistrum</i> L.	Gen. Yay.	-	1,2	8.05.2016	Ortaç 1117
25	Brassicaceae	<i>Sinapis arvensis</i> L.	-	-	1,2,5	3.04.2016	Ortaç 1119
26	Caryophyllaceae	<i>Silene colorata</i> Poiret	-	-	1,2	8.05.2016	Ortaç 1138
27	Cistaceae	<i>Helianthemum nummularium</i> (L.) Miller subsp. <i>nummularium</i>	-	-	1,2,4,5	8.05.2016	Ortaç 1155
28	Convolvulaceae	<i>Calystegia sepium</i> (L.) R.Br. subsp. <i>sepium</i>	Gen. Yay.	-	2	8.05.2016	Ortaç 1157
29	Convolvulaceae	<i>Ipomoea imperati</i> (Vahl) Griseb.	Akd. Elm.	-	3	8.05.2016	Ortaç 1160

30	Cyperaceae	<i>Bolboschoenus maritimus</i> (L.) Palla var. <i>maritimus</i>	-	-	3	8.05.2016	Ortaç 1426
31	Cyperaceae	<i>Cyperus capitatus</i> Vandelli	-	-	2,3	22.07.2016	Ortaç 1427
32	Equisetaceae	<i>Equisetum hyemale</i> L.	-	-	4	6.04.2016	Ortaç 1002
33	Euphorbiaceae	<i>Euphorbia helioscopia</i> L.	Gen. Yay.	-	5	8.05.2016	Ortaç 1175
34	Euphorbiaceae	<i>Euphorbia peplus</i> L. var. <i>peplus</i>	Gen. Yay.	-	1	8.05.2016	Ortaç 1178
35	Fabaceae	<i>Acacia nilotica</i> (L.) Willd. ex Delile subsp. <i>kraussiana</i> (Benth.) Brenan	Kü. Bit.	-	1,4	8.05.2016	Ortaç 1181
36	Fabaceae	<i>Lotus edulis</i> L.	Akd. Elm.	-	1,2,5	8.05.2016	Ortaç 1214
37	Fabaceae	<i>Melilotus albus</i> Desr.	Gen. Yay.	-	3	8.05.2016	Ortaç 1220
38	Fabaceae	<i>Melilotus officinalis</i> (L.) Desr.	Gen. Yay.	-	1,2	3.04.2016	Ortaç 1221
39	Fabaceae	<i>Ononis spinosa</i> L. subsp. <i>antiquorum</i> (L.) Briq.	Akd. Elm.	-	4	4.04.2016	Ortaç 1225
40	Fabaceae	<i>Prosopis farcta</i> (Banks & Sol.) Macbride	-	-	1,2,3	26.10.2016	Ortaç 1228
41	Fabaceae	<i>Vicia sativa</i> L. subsp. <i>sativa</i>	-	-	1,2	22.07.2016	Ortaç 1242
42	Fabaceae	<i>Vicia villosa</i> Roth subsp. <i>villosa</i>	-	-	1,5	26.10.2016	Ortaç 1243
43	Geraniaceae	<i>Erodium gruinum</i> L.	D. Akd. Elm.	-	4,5	8.05.2016	Ortaç 1250
44	Geraniaceae	<i>Geranium dissectum</i> L.	-	-	1,2	8.05.2016	Ortaç 1253
45	Geraniaceae	<i>Geranium molle</i> L.	-	-	5	22.07.2016	Ortaç 1256
46	Iridaceae	<i>Iris pseudacorus</i> L.	-	-	3	8.05.2016	Ortaç 1431
47	Juncaceae	<i>Juncus inflexus</i> L.	Gen. Yay.	-	1	22.07.2016	Ortaç 1435
48	Juncaceae	<i>Juncus maritimus</i> Lam.	-	-	2,3	22.07.2016	Ortaç 1436
49	Juncaceae	<i>Juncus rigidus</i> Desf.	Gen. Yay.	-	1	8.05.2016	Ortaç 1437
50	Lamiaceae	<i>Prasium majus</i> L.	Akd. Elm.	-	5	4.04.2016	Ortaç 1287
51	Lamiaceae	<i>Vitex agnus-castus</i> L.	Akd. Elm.	-	3	8.05.2016	Ortaç 1419
52	Malvaceae	<i>Malva sylvestris</i> L.	-	-	1,2,5	22.07.2016	Ortaç 1316
53	Moraceae	<i>Ficus carica</i> L. subsp. <i>carica</i>	Akd. Elm.	-	1	27.10.2016	Ortaç 1317
54	Myrtaceae	<i>Eucalyptus camaldulensis</i> Dehnh.	-	-	1,3	22.07.2016	Ortaç 1321
55	Myrtaceae	<i>Myrtus communis</i> L. subsp. <i>communis</i>	Akd. Elm.	-	4	7.04.2016	Ortaç 1322
56	Oleaceae	<i>Phillyrea latifolia</i> L.	Akd. Elm.	-	5	22.07.2016	Ortaç 1330
57	Onagraceae	<i>Epilobium angustifolium</i> L.	Gen. Yay.	-	3	24.07.2016	Ortaç 1331
58	Papaveraceae	<i>Fumaria asepala</i> Boiss.	İr.-Tur. Elm.	-	5	4.05.2016	Ortaç 1333
59	Papaveraceae	<i>Papaver rhoeas</i> L.	Gen. Yay.	-	1,2,5	26.10.2016	Ortaç 1336
60	Pinaceae	<i>Pinus pinea</i> L.	Gen. Yay.	-	1,3	7.04.2016	Ortaç 1014
61	Plantaginaceae	<i>Plantago maritima</i> L.	-	-	4	8.05.2016	Ortaç 1342
62	Plumbaginaceae	<i>Limonium angustifolium</i> (Tausch) Turrill	Akd. Elm.	-	1	22.07.2016	Ortaç 1344
63	Poaceae	<i>Avena sterilis</i> L. subsp. <i>ludoviciana</i> (Durieu) Gillet et Magne	-	-	4	23.07.2016	Ortaç 1463

64	Poaceae	<i>Arundo donax</i> L.	-	-	1,2,3,4,5	26.10.2016	Ortaç 1462
65	Poaceae	<i>Phragmites australis</i> (Cav.) Trin. ex Steudel	Av.-Sib. Elm.	-	1,2,3,5	8.05.2016	Ortaç 1474
66	Polygonaceae	<i>Polygonum maritimum</i> L.	-	-	1,2,3,5	22.07.2016	Ortaç 1346
67	Polygonaceae	<i>Rumex scutatus</i> L.	-	-	3	27.10.2016	Ortaç 1349
68	Primulaceae	<i>Anagallis arvensis</i> L. var. <i>arvensis</i>	-	-	5	22.07.2016	Ortaç 1351
69	Ranunculaceae	<i>Clematis cirrhosa</i> L.	Akd. Elm.	-	5	26.10.2016	Ortaç 1361
70	Ranunculaceae	<i>Ranunculus arvensis</i> L.	-	-	2	22.07.2016	Ortaç 1365
71	Ranunculaceae	<i>Ranunculus muricatus</i> L.	-	-	1	22.07.2016	Ortaç 1367
72	Rosaceae	<i>Sarcopoterium spinosum</i> (L.) Spach	D. Akd. Elm.	-	4	22.07.2016	Ortaç 1383
73	Scrophulariaceae	<i>Verbascum sinuatum</i> L. subsp. <i>sinuatum</i> var. <i>sinuatum</i>	Akd. Elm.	-	1,2	22.07.2016	Ortaç 1399
74	Solanaecae	<i>Solanum americanum</i> Mill.	-	-	5	22.07.2016	Ortaç 1405
75	Tamaricaceae	<i>Tamarix smyrnensis</i> Bunge	-	-	2,3,5	22.07.2016	Ortaç 1407
76	Urticaceae	<i>Urtica dioica</i> L.	-	-	1	23.10.2016	Ortaç 1415
77	Xanthorrhoeaceae	<i>Aloe vera</i> (L.) Burm. fil.	-	-	3	27.10.2016	Ortaç 1440
78	Xanthorrhoeaceae	<i>Asphodelus aestivus</i> Brot	-	-	3	22.07.2016	Ortaç 1443



A New Annual *Veronica* (Plantaginaceae) From Bingöl, Türkiye

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ABSTRACT

Veronica hispidula Boiss. & A. Huet subsp. *hispidula* var. *bingolense*, collected from the alpine parts of Bingöl province (Eastern Anatolia region-Türkiye), was defined as a new variety. New variety: it differs from the typical variety in terms of pedicel/bracte length ratio, leaf and bracte indumentum, leaf dimensions, and style length. *Veronica hispidula* subsp. *hispidula* var. *bingolense* grows in moist and wet habitats along stream banks in alpine high mountain meadows. This newly identified variety; description, ecological preferences, distribution map, and photographs were given.

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Bingöl (Türkiye)'den Yeni Tek Yıllık Bir *Veronica* (Plantaginaceae)

ÖZET

Bingöl ilinin (Doğu Anadolu bölgesi-Türkiye) alpinik kesimlerinden toplanan *Veronica hispidula* Boiss. & A. Huet subsp. *hispidula* var. *bingolense* yeni bir varyete olarak tanımlandı. Yeni varyete; tip varyeteden pedicel/bracte uzunluk oranı, yaprak ve bracte tüy durumu, yaprak boyutları ve style boyu bakımından farklılık gösterir. *Veronica hispidula* subsp. *hispidula* var. *bingolense* alpinik yüksek dağ çayırılarının dere kenarlarındaki nemli - sulak habitatlarda yetişmektedir. Yeni tanımlanan bu varyetenin; tanımı, ekolojik tercihleri, dağılım haritası ve fotoğrafları verildi.

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Anahtar Kelimeler

Veronica hispidula subsp. *hispidula* var. *bingolense*
Plantaginaceae
Yeni varyete

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INTRODUCTION

Bingöl province is located in the transition zone from Türkiye's harsh and long winter climate Eastern Anatolia Region to the relatively hot and drier Southeastern Anatolia Region and has interesting ecological characteristics (Behçet 2019). It has a rich plant diversity due to its location and ecology. Although this richness in biodiversity is largely determined; it is a fact that it has a richer potential than known, with many new plant taxa (Behçet et al., 2017; Behçet & İlçim, 2018; Behçet et al., 2019; Behçet & Yapar, 2020, 2021, 2023; Behçet & Çetin 2023; Behçet & Gülbasan, 2023; Hamzaoglu et al., 2020; Hamzaoglu & Behçet 2022; Doğan et al., 2015; İlçim & Behçet, 2016; Sinan et al., 2021; Yapar & Behçet, 2020 and new records (Behçet & Altınsoy, 2023; Behçet & Cengiz 2023a and 2023b; Pınar et al., 2018, Yapar & Behçet, 2021) published through floristic studies in recent years.

Veronica L. (Plantaginaceae) is a genus with a cosmopolitan distribution and approximately 450 species in the world (Albach et al. 2004, Advay et al. 2024). There are 107 taxa (77 species, 24 subspecies, and 6 varieties) of this genus distributed in Türkiye (Güner 2012, Yaylaci et al. 2018).

Some interesting *Veronica* L. members were collected during botanical excursions in Bingöl in 2023 (Figure 1). These collected samples: with the help of the keys and definitions in the 6th volume of the book Flora of Turkey and the Eastern Aegean Islands (Fischer, 1978), it was determined that they belong to the species *Veronica hispidula* Boiss. & A. Huet (Figure 2,3)



Figure 1. Scanned habits of *Veronica hispidula* subsp. *hispidula* var. *bingolense*.

Şekil 1. *Veronica hispidula* subsp. *hispidula* var. *bingolense*'nin taranmış habitusları.

It was determined that some characteristics of the collected samples (such as bract/pedice length ratio, leaf and bract indumentum feature, leaf dimensions, and style length) (Figure1, 4 and 5) differed from the description given about *Veronica hispidula* Boiss. & A. Huet in the mentioned studies (Fischer, 1978; 1981). Although the samples collected from Bingöl are more similar to the *hispidula* subspecies of the two subspecies of *Veronica hispidula* (Table 1; Figure 1–5); as mentioned above, they also differ from it in some features.

Veronica hispidula subsp. *hispidula* in Türkiye; it is an Irano-Turanian phytogeographical region element

distributed in the provinces of Erzurum, Kars, Diyarbakır, Adana, Hatay, Afyon, Uşak, Niğde, Bitlis and Muş (Fischer, 1978, Yıldırım 2012) (Figure 6). *Veronica* samples collected from Bingöl were compared with the descriptions of *Veronica hispidula* in the 6th volume of the Flora of Turkey and the 147th volume of the Flora of Iran (Fischer, 1978; 1981) and the type (Figure 2) and isotype images (Figure 3) in the PRC and HBG herbariums, and *Veronica hispidula* subsp. *hispidula* var. *bingolense* was described as a new variety.



Figure 2. The typus image of *Veronica hispidula* subsp. *hispidula* (var. *hispidula*) (syn.: *V. pusilla* Kotschy var. *pusilla*) in Herbarium Prague Charles University (PRC 453983) (from GBIF 2024)

Şekil 2. Prag Charles Üniversitesi Herbaryumundaki (PRC 453983) *Veronica hispidula* subsp. *hispidula* (var. *hispidula*) (sinonim: *V. pusilla* Kotschy var. *pusilla*)'nın typus görüntüsü (GBIF 2024'den)

MATERIALS and METHODS

Some interesting *Veronica* L. specimens were collected during botanical excursions in the mountainous parts of the west-southwest of Bingöl province in 2023. These specimens, collected from the alpine slopes of the mountainous part (south of Ortaköy village) on top of Lake Gerindal in the west-southwest of Bingöl province

(Türkiye), appeared close to *Veronica hispidula* subsp. *hispidula* at first glance. This taxon is also known to be distributed in the provinces of Erzurum, Muş (B8 square), and Diyarbakır (C8 square), which are close to Bingöl. However, with more careful examination, it was determined that the specimens collected from Bingöl were different from *V. hispidula* subsp. *hispidula* with some characteristics (Table 1). As a result of the comparisons made by considering the definitions in the relevant literature (Fischer, 1978; 1981) and the visual images of *V. hispidula* subsp. *hispidula* in the Herbarium Prague Charles University (PRC453983E-visual images!) (Figure 2) and Hamburg University Herbarium (HBG512109E-visual images!) (Figure 3), the samples collected from Bingöl were evaluated as a new variety (var. *bingolense*). Visual images of specimens belonging to the new taxon were taken with the help of a scanning device and enlarged photographs of important morphological structures were taken with the help of a stereo microscope. Type specimens are preserved in the Bingöl University Herbarium (BIN).



Figure 3. The virtual images isotype of *Veronica hispidula* subsp. *hispidula* (var. *hispidula*) (syn.: *Veronica glaberrima* var. *glanduligera* Bornm.) in Herbarium Hamburgense (HBG 519109) (B) (from GBIF 2024).

Şekil 3. Hamburgense Herbarium (HBG 519109)'undaki *Veronica hispidula* subsp. *hispidula* (var. *hispidula*) (sinonim: *Veronica glaberrima* var. *glanduligera* Bornm.) izotip görüntüleri (GBIF 2024'den)



Figure 4. Leaf sizes (A, B) and the plant indumentum (C, D) views of *Veronica hispidula* subsp. *hispidula* var. *bingolense* (from Holotype)

Şekil 4. *Veronica hispidula* subsp. *hispidula* var. *bingolense*'nin yaprak boyutları (A, B) ve tüy örtüsü (C, D) görünümleri (Holotip'ten)

RESULTS and DISCUSSION

Veronica hispidula Boiss. & A. Huet subsp. *hispidula* var. *bingolensis* Behçet, var. nov. (Figs. 1,4,5,6 and Table 1)

Type: Türkiye. B8 Bingöl: 5 km south of Ortaköy village, 25 km west-southwest of Bingöl city center, high mountain meadows, streamside, 1900-2000m a.s.l. , 03.08.2023, *L.Behçet* 21131 (holo. BIN, iso. BIN)

Diagnosis: *Veronica hispidula* Boiss. & A.Huet subsp. *hispidula* var. *bingolensis* differs from *V. hispidula* & subsp. *hispidula* (var. *hispidula*) mainly leaf to 16×5.1 mm, margins sparsely 4-6 celled glandular ciliate (not $4-12 \times 1.5-3.5$ mm and glabrous); style length 0.2-0.3 mm (not 0.3-0.6 mm); the fruit pedicel hooked vertically upwards below fruit at the tip and pedicel length 2-3 x bracts (not $(0.3-0.5-1(-1.3) \times$ bracts; pedicel length 2-3 mm (not 2-4(-6) mm) (Figure 1,4,5). The new variety is similar to *Veronica hispidula* subsp. *ixodes* (Boiss. & Balansa) M.A. Fisch. in terms of being annual, pedicel, and capsule features, but differs in some characters (Table 1).



Figure 5. Fruiting pedicels (patent and strongly hooked below capsule) (E), flower color (F), calyx, and style in mature capsule (G) seed structure and color (H) views of *Veronica hispidula* subsp. *hispidula* var. *bingolense*(from Holotype).

Şekil 5. *Veronica hispidula* subsp. *hispidula* var. *bingolense*'nin meyve pediseli (kapsülün aşağısı belirgin olarak kıvrılmış) (E) çiçek rengi (F), olgun kapsülde kaliks ve stylus (G) ile tohum yapı ve renk (H) görünümleri (Holotip'ten)

Description: Annual, erect, 8-13(-15) cm, usually branched above, **Stems** puberulent with upward-curved 0.05–0.2 mm hairs (sometimes lower parts glabrous), eglandular. **Leaves** 1(–2) pair, petiole 0–2 mm, lamina obovate – oblanceolate, or lanceolate–linear lanceolate, rarely elliptic, to 16 × 5.1 mm, entire, margins sparsely 5–6-celled glandular ciliate. **Raceme** 5–25-flowered, axis straight; eglandular–puberulent and glandular–pubescent with scattered 4–6-celled 0.3–0.5 mm glandular hairs. **Bract** lanceolate, glandular ciliate, herbaceous. **Fruiting pedicels** 2–3 mm, 2–3 × bracts, horizontally patent and hooked vertically upwards below the fruit, pubescent with ± adpressed very short eglandular and 4–6-celled 0.3–0.5 mm glandular hairs. **Calyx** 2.5–4 × to 4 mm, the lobes united at the base about 1 mm, ovate-lanceolate or lanceolate, outer surface with 4–5 celled glandular hairs, as long as (rarely slightly shorter) or slightly longer than the capsule. **Corolla** blue, 2–3 mm diam. **Style** 0.2–0.3 mm long. **Capsule** hygrophytic, 3–3.5 × 3.5–4 mm, slightly shorter or equaling calyx, brownish, glandular-ciliate, otherwise usually glabrous; sinus 1/2 × capsule; base broadly truncate to subcordate; style 1/3 × sinus or shorter.

Seeds 20-35, elliptic to oblong, 1-1.2 x 0.4-0.6 mm, ± convex, smooth, ventrally with no chalazal podium but with an eccentric minute dot-like circular *depression*; sticky when wet. *Fl.&Fr.* 6-8. High mountain meadows, stream banks, 1900-2000 m.

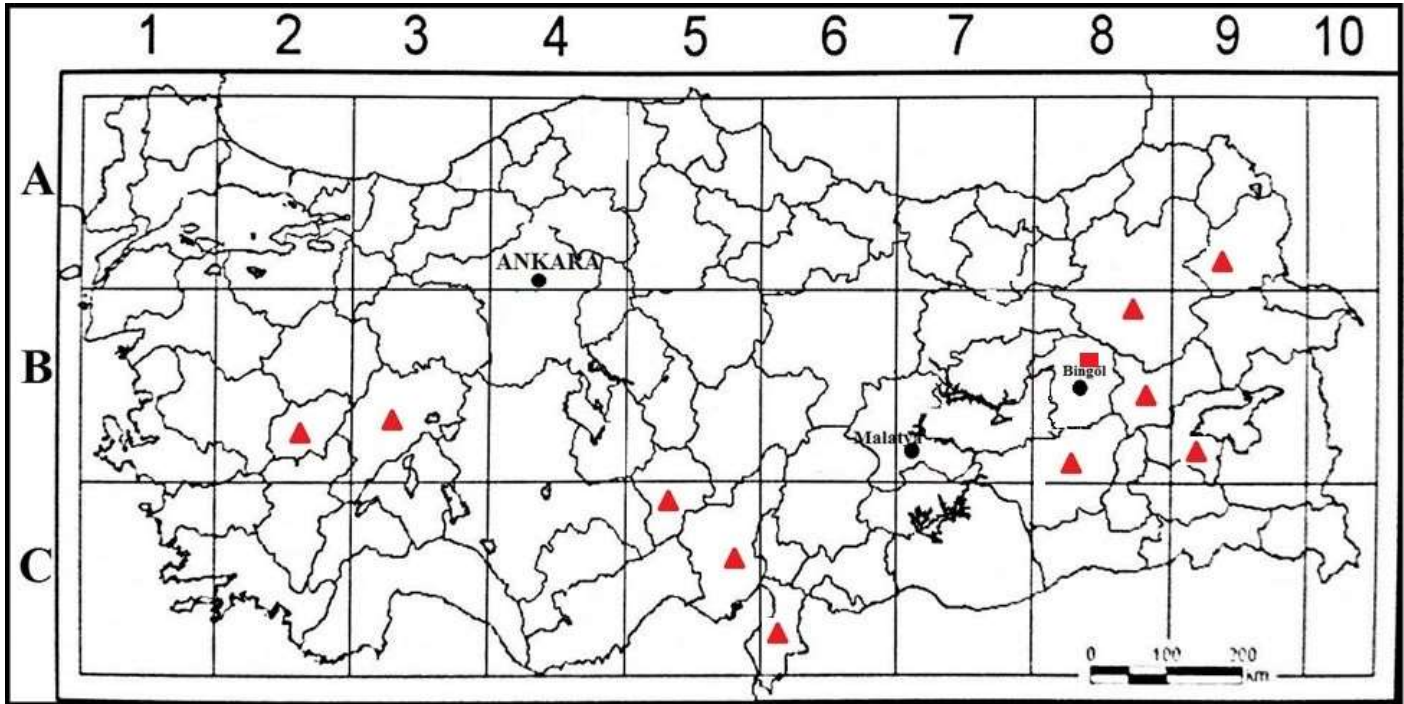


Figure 6. Distribution map of *Veronica hispidula* subsp. *hispidula* var. *bingolense* (■) and subsp. *hispidula* var. *hispidula* (▲) in Türkiye

Şekil 6. *Veronica hispidula* subsp. *hispidula* var. *bingolense* (■) ve subsp. *hispidula* var. *hispidula* (▲) 'nın Türkiye'deki dağılışı haritası

Ecological preferences: *Veronica hispidula* Boiss. & A. Huet subsp. *hispidula* var. *bingolensis* is an endemic taxon distributed in a narrow area in the alpine parts of the mountainous area in the west-southwest of Bingöl province (Figure 6). This new variety is distributed along stream banks in higher elevations (1900-2000 m). While the flowering period is June-July; the fruiting period is between the end of July and August. Other important plants that develop in the area where the new taxon spreads are as follows; *Achillea biebersteinii* Afan., *A. millefolium* L. subsp. *millefolium*, *Agrostis olympica* (Boiss.) Bor, *Alopecurus arundinaceus* Poir., *Centaurea pterocaula* Trautv., *C. spectabilis* (Fisch. & C.A.Mey.) Sch.Bip. subsp. *microlapha* (Boiss.) Wagenitz, *Cirsium pubigerum* DC. var. *coniforme* Petr., *C. pubigerum* DC. var. *glomeratum* (Freyn & Sint.) P.H.Davis & Parris, *Crepis commutata* (Spreng.) Greuter, *Dactylis glomerata* L. subsp. *hispanica* (Roth) Nyman, *Deschampsia caespitosa* (L.) P.Beauv., *Elymus elongatus* (Host) Runemark subsp. *elongatus*, *Festuca gigantea* (L.) Vill., *F. pratensis* Huds., *Hypericum perforatum* L., *Lapsana communis* L. subsp. *intermedia* (M.Bieb.) Hayek var. *intermedia*, *Lythrum salicaria* L., *Lysimachia verticillaris* Spreng., *L. vulgaris* L., *Scrophularia umbrosa* Dumort., *Clinopodium vulgare* subsp. *vulgare*, *Myosotis alpestris* F.W. Schmidt subsp. *alpestris*, *M. sylvatica* subsp. *rivularis* Vestergr., *Phleum pratense* L., *Pilosella x macrotricha* (Boiss.) F.W.Schultz & Sch.Bip, *P. verruculata* (Link) Soják, *Poa longifolia* Trin., *P. pratensis* L., *P. trivialis* L., *Prangos ferulacea* (L.) Lindl. *P. platychlaena* Boiss. subsp. *platychleana*, *Pulicaria dysenterica* (L.) Bernh. subsp. *dysenterica*, *Scrophularia umbrosa* Dumort., *Senecio othonnae* M.Bieb., *Tanacetum parthenium* (L.) Sch.Bip., *Urtica dioica* L. subsp. *dioica*, *Verbascum oreophilum* var. *joannis* (Bordz.) Hub.-Mor., *Veronica anagallis-aquatica* L., *V. biloba* L., *V. oxycarpa* Boiss.

Veronica hispidula subsp. *hispidula* var. *bingolensis* is similar to *V. hispidula* subsp. *hispidula* var. *hispidula* in that it is an annual, its general appearance, its fruit characteristics, and its fruit pedicel is hooked vertically upwards below the fruit at the tip. However, the new taxon; differs from *V. hispidula* subsp. *hispidula* (var. *hispidula*) and other close subspecies in terms of leaf characteristics, bract indumentum, pedicel/bract length ratio, and style length (Table 1 and Figure 1,4,5).

Specimens Seen: *Veronica hispidula* subsp. *hispidula* var. *bingolensis* Türkiye. B8 Bingöl: 5 km south of Ortaköy village, 25 km west-southwest of Bingöl, high mountain meadows, streamside, 1900-2000m a.s.l. , 03.08.2023,

L.Behçet 21131 (holo. BIN, iso. BIN). *Veronica hispidula* subsp. *hispidula* (var. *hispidula*) Libani, in excelsis jugi Sanin, 2200-2300 m, 16/6/1897, Bornmüller J. 1219 (BR0000035779261 Virtual image!), The isotype of *Veronica hispidula* Boiss.& A.Huet subsp. *hispidula* (var. *hispidula*) (syn.: *Veronica glaberrima* Boiss.&Balansa var. *glanduligera* Bornm.)in Herbarium Hamburgense (HBG 519109 Virtual image!)

The number of members of the *Veronica* genus in Türkiye reached 108 taxa with the addition of the new variety, and 36(33.33%) of these taxa are endemic to Türkiye (Güner,2012, Yaylaci *et al.* 2018).

Table 1. Comparison of the diagnostic features of *Veronica hispidula* subsp. *hispidula* var. *bingolensis* with the distinguishing characters between *Veronica* taxa close to this taxon.

Çizelge 1. *Veronica hispidula* subsp. *hispidula* var. *bingolensis*'in diagnostik özellikleri ile bu taksona yakın *Veronica* taksonları arasındaki ayırt edici karakterlerin karşılaştırılması

Characters / Karakterler	<i>Veronica hispidula</i> subsp. <i>hispidula</i> var. <i>bingolensis</i>	<i>V. hispidula</i> subsp. <i>hispidula</i> (var. <i>hispidula</i>)	<i>V. hispidula</i> subsp. <i>ixodes</i>
Plant height(cm) / Bitki boyu	5–11	2–8 (–15)	2–8 (–15)
Leaf lamina/ Yaprak laminası	to 16 × 5.1 mm, sparsely 5–6-celled glandular ciliated	4–12 × 1.5–3.5 mm, glabrous	4–12 × 1.5–3.5 mm, glabrous
Position of the fruit pedicel at the tip and the ratio of pedicel length to bract length / Meyve pediselinin uçta pozisyonu ve pedisel brakte oranı	hooked vertically upwards below the fruit, 2–3 × bracts	hooked vertically upwards below the fruit, (0.3–) 0.5–1(–1.3) × bracts	hooked vertically upwards below the fruit, 1–1.5 × bracts
Fruit pedicel length(mm) / Meyve pedisel uzunluğu	2–3	2–4(–6)	4–6
Corolla color/Korolla rengi	blue	blue	white
Style length(mm)/ stylus uzunluğu	0.2–0.3	0.3–0.6	0.8–1.1
Seed /Tohum	bright yellow, smooth	bright yellow, smooth	brownish

Author's Contributions

Single author

Statement of Conflict of Interest

The study has a single author.

REFERENCES

- Advay, M., Albach D.C. & Doostmohammadi M. (2024) A new species of *Veronica* (Plantaginaceae) from Western Iran. *PhytoKeys* 237, 219-230
- Albach DC, Martinez-Ortega MM, Chase MW (2004) *Veronica*: Parallel morphological evolution and phylogeography in the Mediterranean. *Plant Systematics and Evolution* 246,177–194. <https://doi.org/10.1007/s00606-004-0148-9>
- Behçet, L., (2019). Bingöl ekolojisi ve bitki çeşitliliği (Ecology and plant diversity of Bingöl)(Sözlü tam metin yayımlanmış bildiri). *Uluslararası Arıcılık Araştırmaları Ve Sürdürülebilir Kırsal Kalkınma Stratejileri Kongresi, Bingöl, Türkiye*,11-13 Ekim 2019, ss. 1-16.
- Behçet, L. & Altınsoy, İ. (2023). *Clinopodium debile* (Bunge) Kuntze (Lamiaceae), A New Record for the Flora of Türkiye. *KSU J. Agric Nat.* 26 (3), 504–510.
- Behçet, L. & Cengiz, H. (2023a). *Pimpinella major* (Apiaceae); a New Record for the Flora of Türkiye and Contributions to Its Taxonomy. *KSÜ Tarım ve Doğa Dergisi* 26 (5), 1048-1055.
- Behçet, L. & Cengiz, H. (2023b). On the Presence and Distribution of *Lycopus exaltatus* (Lamiaceae) in Türkiye. *KSU J. Agric Nat.* 26 (6), 1253-1258.
- Behçet, L. & Çetin, A. (2024) A New Taxon of *Stachys* (Lamiaceae) from Bingöl -Türkiye. *KSU J. Agric Nat.* 27 (1), 101-107
- Behçet, L. & Gülbasan, İ.H. (2024) A New Gigantic *Vicia* (perennial Wild vetch) (Fabaceae) Taxon From East Anatolia, Türkiye. *KSU J. Agric Nat.* 27 (2),325-332

- Behçet, L. & İlçim, A. (2015). *Paracaryum bingoelianum* (Boraginaceae), a New Species from Turkey. *Turkish Journal of Botany* 39 (2), 334–340.
- Behçet, L., İlçim, A. & Yapar, Y. (2017). *Centaurea bingoelensis* (Asteraceae), a New Species from Turkey. *Turkish Journal of Botany* 41, 180–188.
- Behçet, L. & Yapar, Y. (2019). Rediscovery of the lost endemic *Micromeria cymuligera* (Lamiaceae) in Eastern Anatolia-Turkey. *Nordic Journal of Botany* 37 (10), 1–6.
- Behçet, L., Yapar, Y. & Olgun, Ş. 2019. *Prangos aricakensis* (Apiaceae), a new species from eastern Turkey. *Phytotaxa* 401 (1), 055–063 . doi.org/10.11646/phytotaxa.401.1.5
- Behçet, L. & Yapar, Y. (2020). *Lactuca anatolica* (Asteraceae: Lactucinae), a New Species from Eastern Anatolia (Turkey). *Phytotaxa* 455 (4), 287–294.
- Behçet, L. & Yapar, Y. (2021). *Bromus orientalis* (Poaceae: B. sect. Bromopsis), a New Species from Turkey. *Nordic Journal of Botany* 39 (4), 1–7.
- Behçet, L. & Yapar, Y. (2023). A new species *Eremogone* (Caryophyllaceae) from Türkiye. *Phytotaxa* 633 (1), 1-8.
- Davis, P.H. & Plitmann, U. (1970). *Vicia* L. in: Davis PH (ed.) *Flora of Turkey and the east aegean islands*, vol: 3, Edinburgh Univ. Press, Edinburgh, pp. 274–325.
- Duran, A., Behçet, L. & Öztürk, M. (2015). *Diplotaenia bingolensis* (Apiaceae), New Species from East Anatolia, Turkey. *Plant Syst Evol* 301, 467–478.
- Doğan, M., Behçet, L., Sinan, A. (2015). *Pseudophleum anatolicum*, a New Endemic Species of *Pseudophleum* (Poaceae) from East Anatolia, Turkey. *Systematic Botany* 40 (2), 454–460.
- Fischer M.A. (1978). *Veronica* L. in: Davis PH (ed.) *Flora of Turkey and the east aegean islands*, Vol: 6, pp. 689–753, Edinburgh Univ. Press, Edinburgh
- Fischer M.A. (1981). *Veronica* L. in: Rechinger KH (ed.) *Flora Iranica* Vol.:147, pp. 52-165 (Akademische Druck-u. Verlagsanstalt : Graz, Austria)
- GBIF.org (2024), GBIF Home Page. Available from: <https://www.gbif.org> [26.08.2024].
- Güner, E.D. (2012) *Veronica* L. In: Güner A., Aslan, S., Ekim, T., Vural, M. ve Babaç, M. T (eds.) *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, pp. 677-684
- Hamzaoglu, E., Behçet, L. & Yapar, Y. (2020). A New Suffrutescent Taxon of *Dianthus* (Caryophyllaceae) from Bingöl, Turkey. *KSÜ Tarım ve Doğa Dergisi* 23 (6), 1529–1534.
- İlçim, A., & Behçet, L. (2016). *Astragalus topalanense* (Fabaceae), a New Species from Turkey. *Turkish Journal of Botany* 40, 74–80.
- Meise Botanic Garden (2024). Meise Botanic Garden Herbarium (BR). Version 1.32. Meise Botanic Garden. Occurrence dataset <https://doi.org/10.15468/wrthhx> accessed via GBIF.org on 2024-08-26. <https://www.gbif.org/occurrence/4072772974>
- Pınar, S. M., Fidan, M., Behçet, L. & Eroğlu, H. (2018). A New Record for The Flora of Turkey: *Onopordum cinereum* Grossh. (Asteraceae). *Erzincan University Journal of Science and Technology* 11 (1), 85-91.
- Sinan, A., Behçet, L. & Yapar, Y. (2021). *Ranunculus solhanensis* (Ranunculaceae), a new species from eastern Turkey. *Phytotaxa* 497 (2), 157–164.
- Yapar Y. & Behçet L., 2022. *Pentanema divaricatum* Cass. (Inuleae, Asteraceae), A New Record for the Flora of Turkey. *KSÜ Tarım ve Doğa Dergisi* 25 (6), 1401-1405
- Yapar Y. & Behçet L., 2020. A new subspecies of *Ornithogalum malatyanum* (Asparagaceae: Scilloideae) from eastern Anatolia, Turkey. *Nordic Journal of Botany* 38 (11), 1-5
- Yaylacı, Ö.K., Sezer, O., Özgişi, K., Öztürk, D., Potoğlu Erkara, İ., Koyuncu, O. & Ocak, A. (2018). A new *Veronica* (Plantaginaceae) species from Central Anatolia, Turkey. *Phytotaxa* 362 (1), 55-67.
- Yıldırım, Ş. (2005). The Chorology of the Turkish species of Scrophulariaceae family. *Ot Sistematiik Botanik Dergisi* 19 (1), 151-211.

The Effect of Paclobutrazol Applications on Yield, Quality, Plant Growth and Residue in Hydroponic Tomato Cultivation

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ABSTRACT

The study was conducted in 2022 in a fully automated, soilless agriculture greenhouse at the Research Centre Directorate of Tokat Gaziosmanpaşa University. The study investigated the effects of different doses of paclobutrazol (PBZ) on yield, quality, plant growth, and PBZ residue levels in tomato plants. The plant material employed in this study was the Belford F₁ (Syngenta-Türkiye) pole tomato variety. The experiment was conducted in three replicates according to a random plots factorial experiment design. The results of this study indicate that foliar applications of 50 ppm, and 100 ppm PBZ produced the most optimal outcomes. The highest marketable yield was observed in the treatment with a foliar application of 50 ppm PBZ, resulting in an average yield of 385.33 kg/ha. Subsequently, a foliar application of PBZ at a rate of 100 ppm (374.24 kg/ha) was conducted, along with a control treatment (268.55 kg/ha). The study revealed no statistically significant differences between treatments in terms of fruit wet weight, dry weight, pH, and titratable acidity. Furthermore, as the PBZ doses increased, the internode length, and plant height showed a corresponding reduction. The findings of this study indicate that, in soilless tomato cultivation, foliar applications of PBZ produce superior results compared to soil applications. Additionally, no PBZ residue was detected in the fruit.

Horticulture

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Topraksız Domates Yetiştiriciliğinde Paclobutrazol Uygulamalarının Verim, Kalite, Bitki Gelişimi ve Kalıntı Üzerine Etkisi

ÖZET

Bu çalışma 2022 yılında Tokat Gaziosmanpaşa Üniversitesi Araştırma Merkez Müdürlüğü bünyesindeki tam otomasyonlu topraksız tarım serasında yürütülmüştür. Araştırmada farklı dozlarda paclobutrazol uygulamalarının domates bitkisinin verim, kalite, bitki gelişimi ve paclobutrazol (PBZ) kalıntıları incelenmiştir. Bitkisel materyal olarak Belford F₁ (Syngenta-Türkiye) sırk domates çeşidi kullanılmıştır. Tesadüf parselleri faktöriyel deneme desenine göre 3 tekerrürlü olarak yürütülmüştür. Bu çalışmada domates bitkisinde verim özellikleri açısından yapılan gözlemlerde en iyi sonuç 50 ppm yapraktan PBZ ve 100 ppm yapraktan PBZ uygulamasından elde edilmiştir. En yüksek pazarlanabilir verim 50 ppm yapraktan PBZ uygulamasında 385.33 kg/da olmuştur. Bunu sırasıyla 100 ppm yapraktan PBZ uygulaması (374.24 kg/da) ve kontrol uygulaması (268.55 kg/da) takip etmiştir. Çalışmada meyve yaş-kuru ağırlık, pH ve titredilebilir asit miktarı bakımından uygulamalar arasında istatistiksel olarak önemli bir fark gözlenmemiştir. Ayrıca, uygulamalar arasında PBZ dozları arttıkça ve buna paralel olarak boğum arası uzunluk ve bitki boyunda kısalmalar meydana gelmiştir. Bu çalışmanın sonucunda farklı PBZ uygulamaları arasında topraksız domates yetiştiriciliğinde yapraktan PBZ uygulamalarının topraktan PBZ uygulamalarına göre daha iyi sonuç verdiği; ayrıca meyvede PBZ kalıntısı oluşturmadığı tespit edilmiştir.

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INTRODUCTION

Vegetables are an essential component of the human diet, providing specific nutrients that are vital for maintaining optimal health (Keikotlhaile & Spanoghe, 2011). One of the most significant vegetables cultivated globally is the tomato (*Lycopersicon esculentum*). The tomato, a member of the Solanaceae family, contains a variety of nutrients, including carotenoids, lipids, organic acids, alcohol-insoluble solids (Minoia et al., 2010). The tomato is employed in a multitude of culinary applications, including soup, juice, ketchup, purée, and the preparation of tomato paste, pickles, and preserves from green tomatoes (Jayaramaiah et al., 2018). The tomato is cultivated extensively in both open (field), and soilless farming areas (Kartal & Geboloğlu, 2024). The collective impact of a decline in the extent of arable land, an increase in global population, a reduction in water resources, and climate change is placing significant strain on those engaged in agricultural production. One of the most promising strategies to address this challenge is the practice of soilless agriculture (Gruda et al., 2017).

Soilless agriculture refers to the cultivation of plants without the utilisation of soil as a rooting area (Yalçın & Çimrin, 2019). The system essentially comprises a cultivation method through which absorbed root nutrients are supplied by the irrigation water (Savvas et al., 2013). Soilless agriculture comprises two distinct methodologies: water culture, and solid media culture. The solid media culture method is more prevalent, although the water culture approach has also gained prominence in recent years. The preference for solid media culture over water culture is largely attributed to the lower operating costs, and ease of application associated with the former (Talaz & Engin, 2019). In soilless agriculture, a variety of media are employed, including organic (coconut fibre, peat, sawdust, bark), inorganic (perlite, vermiculite, zeolite, sand, tuff, gravel), and synthetic-organic (polyurethane foam) (Demirsoy & Uzun, 2019). The most commonly utilised soilless cultivation substrates in Türkiye are perlite, coconut fibre, and rock wool. Of these media, only perlite is a domestic material, while coconut fibre, and rock wool are imported (Toprak & Gül, 2013). Furthermore, these media are employed as a cultivation medium for plants or plant roots, rather than as a source of nutrients (Raffar, 1990).

The term "growth regulator" is used to describe any chemical that inhibits cell division, and elongation in shoot tissues, while simultaneously regulating plant height without affecting the plant's morphology (PGRSA, 2007). The misuse of plant growth regulators (PGRs), which affect physiological events, has the potential to induce health complications. Consequently, the utilisation of these substances has undergone a process of restriction, and licensing (Kumlay & Eryiğit, 2011). The utilisation of PGRs is multifaceted, encompassing applications such as the promotion of germination, the augmentation of fruit production, the acceleration of fruit maturation, the enhancement of seedling rooting, and the management of weeds (Budak, Çalışkan, & Çaylak, 1994; Kaynak & Ersoy, 1997; Aydoğdu & Boyraz, 2005). However, it is imperative to administer these substances at the appropriate dosage and timing; otherwise, a plant growth regulator that promotes growth at low concentrations may impede growth at high doses. Misuse of these substances can lead to adverse effects on both human health, and the environment. While there is a paucity of research in this area, the available evidence suggests that plant growth regulators pose a lower risk to human health than pesticides. When administered in accordance with dosage guidelines, and at optimal timing, they are essentially innocuous. However, oversupplementation or untimely application can result in residues on fruit, which have the potential to induce health complications. Furthermore, inadequate caution during application can result in acute effects arising from eye and skin contact (Halloran & Kasım, 2002; Kumlay & Eryiğit, 2011). The most commonly utilised growth regulators in hydroponics, vegetable, fruit, and other plant cultivation are daminozide, uniconazole, chlormequat chloride, and paclobutrazol (PBZ). Among growth regulators, paclobutrazol is presently the most widely employed in aquaculture (Wang & Gregg, 1990; Whipker & Dasoju, 1998; Pasian & Bennett, 2001; Di Benedetto & Klasman, 2007). PBZ is typically employed as an efficacious plant growth regulator in a plethora of crops, including all vegetables, apple, and cherry (Quinlan, 1981), carrot (Thomas, Barnes, & Hole, 1982), english ryegrass (Hebhlethwaite, Hampton, & McLaren, 1982), tulip (Menhenett & Hanks, 1982), and chrysanthemum (McDaniel, 1983). Paclobutrazol is a compound belonging to the triazole class of plant growth regulators. It has been observed to provide protection to a range of plants against diverse environmental stresses, including drought, cold, heat, and ultraviolet radiation (Orabi, Salman, & Shalaby, 2010).

The PBZ application has been shown to positively impact several plant traits, such as plant height, stem diameter, leaf count, root structure, and yield, while also decreasing the occurrence of lodging (Syahputra et al., 2016). The

effects of PBZ on the isoprenoid pathway result in the inhibition of gibberellin synthesis, and alterations in the levels of plant hormones, characterised by an increase in the levels of cytokinin. Inhibition of gibberellin synthesis results in the accumulation of precursors in the terpenoid pathway, leading to the production of abscisic acid (Khan et al., 2009; Soumya Preman, 2017). This compound exerts a considerable impact on plant growth, and development, influencing factors such as photosynthetic rate, and phytohormone levels (Kim et al., 2012).

In studies examining the impact of PBZ application on yield, quality, and plant growth in tomato cultivation, it has been reported that PBZ application can enhance yield by augmenting fruit carrying capacity, thereby facilitating the development of a more compact, and robust structure in tomato plants (Chakrabarty, Chakraborti, & Basu, 2009; Srivastava, Dwivedi, & Srivastava, 2012). Furthermore, it has been proposed that PBZ may enhance fruit size, and improve certain quality characteristics (Hassan et al., 2017). In their research on the impact of PBZ applied to soil in apricot orchards on vegetative growth, and quality of apricot, Mir et al. (2015) reported that, in terms of fruit/plant number, and yield, a 10 g PBZ application significantly reduced vegetative growth of plants, improved fruit quality, and increased yield in both years of the study when compared to other treatments.

In a pot study on the growth, yield, and quality of white aubergine, Khandaker et al. (2020) reported that a PBZ concentration of 200 mg led to increased growth, yield, and quality. The authors thus recommended the use of PBZ for the purpose of achieving better yield, and quality in aubergine. Despite an extensive literature search, there were not many published studies on plant growth, yield, quality, and fruit residues of PBZ in tomato under soilless conditions. Given the conditions outlined above, it is crucial to evaluate the effect of PBZ on controlling plant growth, as well as its role in improving fruit yield, and quality.

The purpose of this study was two-fold: first, to investigate the effect of PBZ applications on yield, quality, and plant growth in soilless tomato cultivation; second, to ascertain whether PBZ, the active ingredient in the treatment, forms residues in tomato plants.

MATERIAL and METHOD

Place and Year of Trial

The research was conducted at the Tokat Gaziosmanpaşa University Research and Application Centre in 2022. The experimental setup was a 2000 m² heated soilless greenhouse with a fully automated system. In the course of the experiment, peat of the type known as Kekkila (brown sphagnum peat) was employed as a peat substrate. The nutrient content, and certain properties of the peat are presented in Table 1. The perlite employed is chemically inert, and has a pH of approximately 7. These characteristics render it suitable for a broad range of applications. The constituents of perlite are present in an oxide form, with a water content of 3%, and an SiO₂ content of approximately 74%. The properties of the perlite employed in the study are presented in Table 2.

Table 1. The properties of Kekkila peat

Çizelge 1. Kekkila torfunun özellikleri

Org. M. (%)	pH	EC (dS/m)	N (mg/l)	P ₂ O ₅	K ₂ O	MgO
95	5.5	2.5	140	160	180	10
SO ₃	Fe	Mn	B	Zn	Cu	Mo
187	0.9	1.6	0.3	0.4	1.5	0.5

Table 2. Chemical structure of perlite (Yıldız, 2014).

Çizelge 2. Perlitin kimyasal yapısı (Yıldız, 2014).

Si %	Al	K	Na	Fe
33.8	7.2	3.5	3.4	0.6
Ca	Mg	O	H ₂ O	Other
0.6	0.2	47.5	3.0	0.2

Method

The objective of this study was to determine whether varying doses of PBZ have a residual impact on plant yield, quality, and fruit in soilless tomato cultivation when applied via foliar or soil application. The study included five distinct PBZ treatments, including a control group (Table 3).

The study employed the Belfort F1 tomato variety, developed by Syngenta Türkiye. The seedlings were planted on 1 May 2022. The plants were cultivated in 6-foot balcony pots with a width of 25 cm, a length of 75 cm, a height of 21 cm, and a volume of 24 litres of growing medium (Figure 1).

Table 3. Different paclobutrazol (PBZ) doses applied in the study

Çizelge 3. Çalışmada uygulanan farklı paclobutrazol (PBZ) dozları

1.	Pure water application (Control)
2.	50 ppm paclobutrazol foliar application
3.	100 ppm paclobutrazol foliar application
4.	100 ppm paclobutrazol soil application
5.	200 ppm paclobutrazol soil application



Figure 1. Trial setup, and post-planting view

Şekil 1. Deneme kurulumu ve ekim sonrası görünüm

The process of fertilisation, and irrigation was conducted using a fully automated system. The requisite quantities for fertilisation were prepared by modifying the methodology set forth by Hoagland & Arnon (1950), with adjustments made according to the growth status of the plants in question. Following a ten-day period, the ratio of N:P:K:Ca: Mg was prepared as 2:1:1:3:1.5:1, and fertilisation was applied at 2.0 mmhos/cm EC until the onset of flowering, and 2.2 mmhos/cm EC thereafter. The fertilisation was applied in conjunction with the irrigation, and at each instance of irrigation. Ongoing measurements were made of the drainage outputs, and measures were taken to prevent the accumulation of salinity. Irrigation was conducted six times a day at equal intervals, with each irrigation occurring automatically for a duration of two minutes. To ascertain the impact of the treatments on tomato plants, the experiment was concluded in November. This was followed by a series of observations, including marketable yield, total yield, number of fruits, number of rejects, plant height, internode distance, pH, water-soluble dry matter content, titratable acid (TA), chlorophyll index (SPAD), fruit wet-dry weight, and leaf wet-dry weight.

Observations made in the study, marketable yield (kg/da): Non-marketable fruits were separated from the fruits collected during the harvest period, and then the remaining fruits were weighed, and recorded in kg/da. Total yield (kg/da): Tomato fruits collected from each plot were weighed, and recorded in kg/ha. Number of fruits (pcs/plant): The fruits collected at each harvest were counted according to the plots, and at the end of the harvest period, the number of fruits was divided by the number of plants in the plot, and the number of fruits was calculated in units/plant. Number of rejects (pcs/plant): The fruits that reached harvest maturity were harvested, and the number of fruits that had no marketable value after harvest was recorded. Plant height (m): The length of the plant was determined by measuring the distance from the root collar to the growing tip with a tape measure. Distance between internodes (cm): The distance between the two plant exit points on the main stem of the plant was measured with the help of a ruler. Determination of pH in fruit juice: Fruit samples were passed through a juicer, and the obtained juice was measured with a pH meter (Hanna HI-9812-5N, USA). Determination of water soluble dry matter content (%): After the beginning of the harvest, the juices of the fruits taken at the 4th and 5th harvests were squeezed with a juicer, and measured with a digital refractometer without delay after filtration. Titratable acid determination (TA) (%): The juices of the harvested fruit samples were extracted using a juicer. The juices were filtered, and then titration measurements were made using the pH metric method. Then, 10 mL of juice was taken, and titrated with 0.1 N NaOH solution until the pH reached 8.1. Titration results were calculated as % according to the methodology proposed by Cemeroglu, (2010). Chlorophyll index (Spad): Chlorophyll index (Spad) was measured using a chlorophyll meter (Minolta SPAD-502, Osaka, Japan) on the third, and fourth leaves from the bottom of the growing tip of the plants (developed leaves) during the period of the experiment. Leaf, and fruit wet-dry weights (g): Healthy leaves, and fruits were taken from the plants at the beginning, and the middle of the harvesting period, and the wet weights of the leaves, and fruits were weighed on a digital balance with a precision of 0.001 g. The wet weights of the samples were placed in paper bags, placed in

an oven, and allowed to dry at 80 °C. Then, the wet, and dry weights of the dried leaves, and fruit samples were determined with a precision balance (0.01 g).

The experiment was conducted in three replicates according to a random plots factorial experiment design. Each pot contained 3 plants, and 3 pots were used in each replicate. A one-way analysis of variance (ANOVA) was conducted on the data using the SPSS 20.0 package software. The Duncan multiple comparison test was employed to ascertain whether the means differed at the 1-5% level.

Sample collection, preparation, extraction, and clean-up

Tomato samples were collected on the 1st, 3rd, 5th, 7th, and 10th day post-spraying (EC, 2002). The extraction, and clean-up procedures based on QuEChERS AOAC Method 2007.01 were executed following the guidelines provided by (Lehotay, 2007). The process steps for QuEChERS are illustrated in Figure 2. LC-MS/MS analysis of table tomatoes was conducted in triplicate.

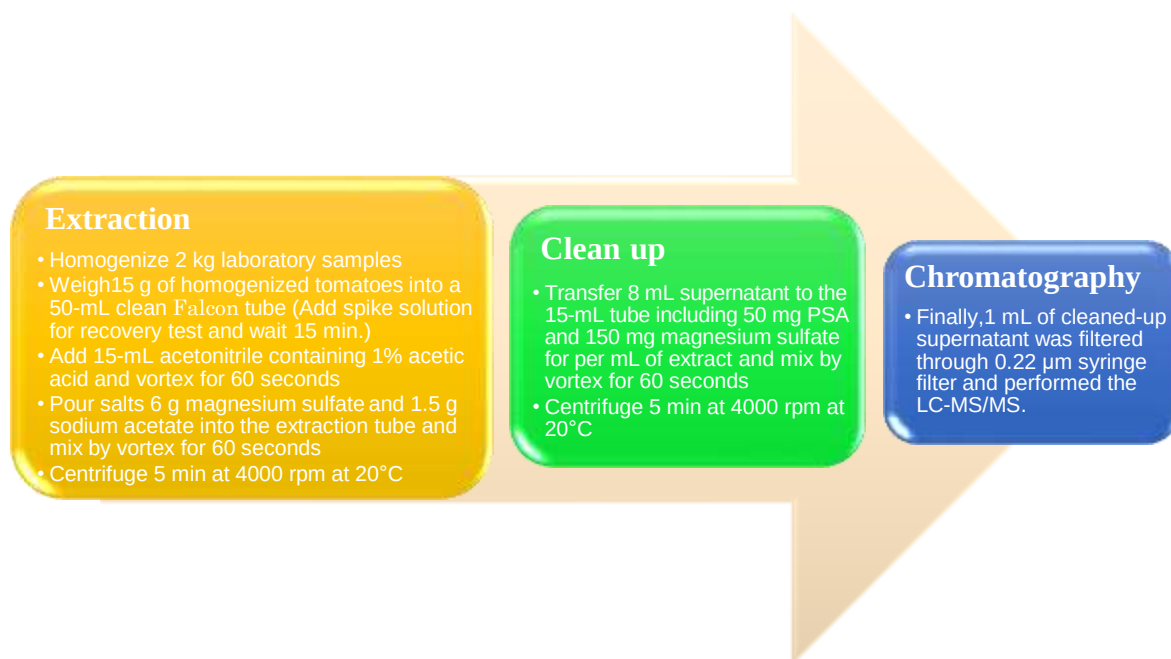


Figure 2. The analytical procedure of the QuEChERS-AOAC Official Method 2007.01.

Şekil 2. QuEChERS-AOAC Resmi Yöntemi 2007.01'in analitik prosedürü.

LC-MS/MS analyses and method verification

Analyses of PBZ were performed using a Shimadzu® LC-MS 8050 system, which is equipped with advanced UPLC, and MS/MS capabilities. The system components included a pumping device (LC-30AD), SIL-20A autosampler, degasser (DGU-20A3R), column oven (CTO-20ACV), and a tandem mass spectrometer. Chromatographic separation was achieved using a high liquid pressure chromatography column (Inertsil ODS IV C18 column, 2.1 mm x 150 mm, 3 µm particle size). A gradient elution programme was employed for the chromatographic separation, utilising eluent A, which consisted of distilled water, and 10 mM ammonium formate, and eluent B, which consisted of methanol. Gradient elution commenced with 10% of B (held for 0.5 minutes), before increasing linearly to reach 90% of B in 1 minute (held for 3 minutes), and subsequently decreasing to the initial stage (10% of B) at 4.5 minutes, where it was maintained until 5 minutes. The volume of injection for each sample was 10 µL. The flow velocity of the mobile phase was maintained at 0.4 mL/min, and the column oven was set to 40°C. Instrument parameters were precisely managed with LabSolution® software (version 5.118). Method verification was performed according to the SANTE document (SANTE, 2021; Balkan & Yilmaz, 2022).

The method validation parameters of PBZ are given in Table 4. The correlation coefficient (R²) of the PBZ calibration is 0.9993. The LOD value was calculated as 1.20, and LOQ value as 3.99. The retrieval values within the scope of the method were 97.10-104.17%, and RSD values were found in the range of 0.25-2.20% (Table 4). According to the SANTE document, the PBZ must meet 70-120% recovery, and ≤20% RSD values in order to meet the method performance criteria (SANTE, 2021). It is seen that PBZ fulfils these criteria (Table 4).

Table 4. Method optimization, and verification parameters

Çizelge 4. Metod optimizasyon ve doğrulama parametreleri

Analyte	Precursorion (m/z)	Productions (m/z, CE, eV)	RT (min)	Linear regression equation	Correlation coefficient (R ²)	LOD (µg/kg)	LOQ (µg/kg)	Fortification (µg/kg)	Reatibility Recovery % (RSD, %)	Reproducibility Recovery % (RSD, %)	U %
Paclobutrazol (PBZ)	294.4	70.1 (-21)	3.007	Y = (65059.8)X + (42256.1)	0.9993	1.20	3.99	10	104.17 (2.20)	97.10 (1.92)	7.32
		125.1 (-25)						50	97.50 (0.25)	99.68 (1.75)	
								100	100.56 (1.12)	103.72 (1.86)	

RESULTS and DISCUSSION

The impact of PBZ application on yield, quality, plant growth, and residue in tomato cultivation is presented in Tables 5, 6, 7, 8, and 9.

Table 5. Effect of PBZ applications on yield, and yield parameters of tomato

Çizelge 5. PBZ uygulamalarının domatesin verimi ve verim parametreleri üzerine etkisi

Applications	Number of fruits (pcs/plant)		Number of Discards (pcs/plant)		Marketable Yield (kg/da)		Total Yield (kg/da)	
<i>Control</i>	89.0±7.21	c	60.0±14.00	a	268.55±23.51	b	436.35±27.37	b
<i>50 ppm leaf</i>	131.0±6.24	a	62.3±07.37	a	385.33±26.76	a	516.06±18.74	a
<i>100 ppm leaf</i>	108.0±6.92	b	40.0±05.29	b	374.24±17.19	a	489.97±25.89	a
<i>100 ppm soil</i>	63.0±2.00	d	21.7±02.89	c	233.63±25.71	bc	269.78±09.01	c
<i>200 ppm soil</i>	49.7±5.51	e	13.7±02.52	c	192.80±17.39	c	226.12±12.99	d
p-value	0.000		0.000		0.000		0.000	
Level of importance	***		***		***		***	

***: significant at the P≤0.001 level.

Table 6. Effect of PBZ treatments on fruit-leaf fresh, and dry weight of tomato

Çizelge 6. PBZ uygulamalarının domatesin meyve-yaprak taze ve kuru ağırlığına etkisi

Applications	Fruit Fresh Weight (g)	Fruit Dry Weight (g)	Leaf Wet Weight (g)	Leaf Dry Weight (g)
<i>Control</i>	322.00±33.65	13.40±2.19	25.33±7.02	3.63±0.87
<i>50 ppm leaf</i>	322.67±14.74	11.12±8.64	28.67±5.03	4.13±0.35
<i>100 ppm leaf</i>	313.33±23.86	15.03±2.21	39.33±5.03	5.47±0.67
<i>100 ppm soil</i>	324.00±41.57	14.11±4.02	41.33±1.16	5.30±0.17
<i>200 ppm soil</i>	326.67±14.74	12.39±3.18	44.67±7.02	5.30±0.56
p-value	0.981	0.863	0.006	0.009
Level of importance	ns	ns	**	**

** : Significant at the P≤0.01 level; ns: not significant.

Table 7. The impact of PBZ treatments on plant height, internode length, and chlorophyll content in tomato.

Çizelge 7. Domateste PBZ uygulamalarının bitki boyu, boğum arası uzunluğu ve klorofil içeriğine etkisi.

Applications	Plant Height (m)		Internode length (cm)		Chlorophyll index (Spad)	
<i>Control</i>	2.93±0.15	a	10.00±1.00	a	51.33±3.51	c
<i>50 ppm leaf</i>	2.67±0.12	b	9.33±0.58	a	58.67±1.52	b
<i>100 ppm leaf</i>	2.47±0.10	b	8.00±0.00	b	62.00±1.00	b
<i>100 ppm soil</i>	1.69±0.14	c	4.33±0.58	c	66.67±1.16	a
<i>200 ppm soil</i>	1.60±0.11	c	3.67±0.58	c	68.67±0.58	a
p-value	0.000		0.000		0.000	
Level of importance	***		***		***	

***: Significant at the P≤0.001 level.

The administration of PBZ led to a statistically significant enhancement in the yield, and yield components of soilless tomato cultivation, with this effect being observed at the value of 0.001 according to the doses administered. The lowest number of fruits per plant was observed in the treatment group receiving 200 ppm soil-applied PBZ, while the other treatment groups exhibited a higher number of fruits than the control. Furthermore, the highest number of rejects was observed in the control group, and in the 50 ppm foliar PBZ applications. Conversely, the lowest figure was observed in the soil PBZ applications at 100, and 200 ppm.

Table 8. Effect of PBZ treatments on fruit quality characteristics of tomato

Çizelge 8. PBZ uygulamalarının domates meyvesinin kalite özelliklerine etkisi

Applications	pH	Water-soluble dry matter	Titratable Acid (TA)
Control	4.81±0.06	6.00±0.88 a	3.83±0.06
50 ppm leaf	4.89±0.03	5.97±0.37 a	3.87±0.61
100 ppm leaf	4.89±0.04	6.03±0.31 a	3.87±0.25
100 ppm soil	4.91±0.05	4.84±0.29 b	3.50±0.10
200 ppm soil	4.84±0.05	4.38±0.33 b	3.63±0.15
p-value	0.141	0.004	0.515
Level of importance	ns	***	ns

*** : Significant at the $P \leq 0.001$ level; ns: not significant.

Table 9. Paclobutrazol residue on different days

Çizelge 9. Farklı günlerdeki paclobutrazol kalıntısı

Analysis	Treatment	Residue at 1 st (µg/kg)	Residue at 3 rd (µg/kg)	Residue at 5 th (µg/kg)	Residue at 7 th (µg/kg)	Residue at the 10 th (µg/kg)
Paclobutrazol (PBZ)	Control	Nd				
	50 ppb leaf	Nd				
	100 ppb leaf	0.96 (<LOD)	2.20 (<LOQ)	0.76 (<LOD)	0.32 (<LOD)	0.10 (<LOD)
	200 ppb soil	2.54 (<LOQ)	4.43	2.88 (<LOQ)	2.77 (<LOQ)	1.04 (<LOD)

LOD: Limit of detection, LOQ: Limit of quantification Nd: Not detected

The control application yielded a marketable yield of 268.55 kg/da, while the 50, and 100 ppm foliar PBZ applications yielded 385.33 kg/da, and 374.24 kg/da, respectively. Similarly, the highest total yield was obtained from applications of 50 ppm, and 100 ppm foliar PBZ, with yields of 516.06 kg/da, and 489.97 kg/da, respectively. In general, a statistically significant difference at 0.001 level was observed between treatments in terms of number of fruits, number of rejects, marketable, and total yield (Table 5). Sudrajat et al. (2021) used PBZ (p1: 0 ml/l, p2: 0.375 ml/l, p2: 0.375 ml/l, p3: 0.750 ml/l) to increase the yield, and quality of cucumber grown in hydroponic system, the lowest number of fruits was obtained from P1 (6.50 pieces), and the highest number was obtained from P3 (12.0 pieces) among PBZ treatments. Wijayanti & Dewi, (2024) applied four different PBZ doses (0 ppm (control), 25-50-75-100 ppm) on the growth, and development of beans, the lowest number of fruits in beans was obtained from 100 ppm (2.33 plants/piece), and the highest number was obtained from control (15 plants/piece). Aydınli et al. (2021) conducted a study to ascertain the impact of foliar PBZ application on yield, and fruit quality in the apple cultivar. Although PBZ applications did not affect yield, the highest yield was observed in 10 ppm (63.26 kg), and the lowest yield was observed in 20 ppm (42.83 kg) PBZ application. Mohan et al. (2015) reported that PBZ application had positive effects on yield, and improved fruit number, and fruit quality in chilli plants. Similarly, PBZ applications have been reported to improve yield, and fruit quality in potato cultivation (Ali et al., 2018). It has been reported that PBZ application in conventional cucumber cultivation can increase yield by 36% to 58.5% more compared to cultivation without PBZ application (Budiyanto, Hajoeningtjas, & Nugroho, 2010; Harpitaningrum, Sungkawa, & Wahyuni, 2014). Khandaker et al. (2020), in a pot study on growth, yield, and quality of white aubergine, found that 200 mg PBZ concentration increased the growth, yield, and quality of aubergine under pot conditions. The application of PBZ has been demonstrated to inhibit gibberellin synthesis, and the proliferation of meristem cells, consequently inducing a decline in both flowering, and leaf area (Kim et al., 2012; Du et al., 2018). The inhibition of gibberalin synthesis has been shown to result in an increase in ABA synthesis (Soumya et al., 2017), which, in turn, has been demonstrated to cause ion loss of stomatal guard cells, leading to stomatal closure, and loss of turgor, and water (Hopkins and Hüner, 2009). The closure of stomata, and the subsequent decrease in stomatal conductance, have been shown to result in a decline in photosynthesis rates (Chaves, Flexas, & Pinheiro, 2009; Wardani et al., 2022). This, in turn, leads to a reduction in the transportation of assimilates (carbohydrates), which consequently decreases the fruit weight of the plant, and by extension, the overall yield. The effects of PBZ on yield were found to vary across different plant species in the course of studies conducted for this purpose (Giovinazzo & Souza-Machado, 2000; Singh, 2000; Samaan & Nasser, 2020). Indeed, Christov et al. (1995) observed that the time of PBZ application may cause variability in yield. According to the results obtained, it is thought that the differences between the literatures are due to differences in PBZ application technique, application time, application dose, and plant species.

In soilless tomato cultivation, a statistically significant distinction at the 0.01 level was identified between the treatments in regard to leaf wet weight, and leaf dry weight. The highest leaf wet weight was observed in treatments receiving 200 ppm soil PBZ (44.67 g), 100 ppm soil PBZ (41.33 g), and 100 ppm foliar PBZ (39.33 g), respectively. The lowest recorded leaf wet weight was 25.33 g in the control treatment, and 28.67 g in the 50 ppm foliar PBZ application. Similarly, with regard to leaf dry weight, the most favourable outcome was noted in the case of 5.47 g in the 100 ppm foliar PBZ application, and 5.30 g in the 100 ppm, and 200 ppm soil PBZ applications. The least leaf dry weight was observed in the control treatment, and in the 50 ppm foliar PBZ application. There was no statistically significant difference between the treatments in terms of fruit wet, and dry weights (Table 6). Wijayanti & Dewi, (2024) applied four different PBZ doses (0 ppm (control), 25, 50, 75, and 100 ppm) on the growth, and development of beans, the lowest fruit wet weight were obtained from 100 ppm (9.26 g), and the highest was obtained from control (21.46 g). Aydınli et al. (2021), to assess the impact of foliar PBZ application on yield, and fruit quality in apple cultivar, when the effect of treatments on fruit weight was examined; control (230.35 g), 10 ppm (233.80 g), and 20 ppm (224.19 g) doses were in the same group in terms of fruit weight, while fruit weight at 40 ppm (210.83 g) dose decreased statistically significantly compared to other treatments. Greene, (1986, 1991), and Khurshid et al. (1997a) reported that PBZ application decreased fruit weight in their studies. Similarly, another study in tomato plants reported that PBZ treatments resulted in a reduction in fruit, and leaf wet weight (Srivastava et al., 2012).

Such reductions may be attributed to the fact that PBZ controls plant growth, thereby making the plant more compact, and limiting leaf formation. However, the same study also indicated that the application of PBZ resulted in an increase in fruit, and leaf dry weight. This increase may be attributed to the fact that PBZ regulates plant growth, promotes fruit formation, and helps the plant to utilise its resources more efficiently for fruit formation. Furthermore, it can be postulated that the impact of PBZ applications on fruit, leaf wet, and dry weights may be contingent upon factors such as the dosage employed, the timing of application, and the prevailing growth conditions.

Among the PBZ treatments, the tallest plant height was achieved with the control treatment, while the shortest plant height was recorded in the 100 ppm, and 200 ppm soil PBZ treatments. In the tomato plant, the highest internode distance was obtained from the control, and 50 ppm foliar PBZ application, and the lowest was obtained from 100 ppm, and 200 ppm soil PBZ application. The highest chlorophyll content was obtained from 100 ppm, and 200 ppm soil PBZ application, and the lowest was obtained from the control application. The application of PBZ resulted in a statistically significant difference at the 0.001 level in plant height, internode length, and chlorophyll content (Table 7). Wijayanti & Dewi, (2024) applied four different PBZ doses (0 ppm (control), 25, 50, 75, and 100 ppm) on the growth, and development of beans, the lowest plant height was obtained from 100 ppm (8.60 cm) PBZ treatment, and the highest was obtained from control (251.17 cm) treatment. In our study, and other studies, plant height decreased in direct proportion as PBZ doses increased.

In PBZ-treated plants, cells can continue to divide even if gibberellin synthesis decreases. Nevertheless, cell growth was impeded as a consequence of the inhibition of gibberellin biosynthesis in the subapical meristem. In different plant species, PBZ treated plants had shorter internodes, and reduced plant height (Meena, 2014; Muneeba, 2020).

According to the PBZ application doses, a significant distinction was identified at the level of 0.001 between the water-soluble dry matter ratios in soilless tomato plants. Among the applications, better results were obtained in control, 50 ppm, and 100 ppm foliar PBZ applications than in soil PBZ applications. In addition, the impact of PBZ treatments on pH, and titratable acidity (TA) in tomato fruits was found insignificant (Table 8). Aydınli et al. (2021) reported in their study to determine the effects of foliar PBZ application on yield, and fruit quality in apple variety that the best result in terms of water-soluble solids ratio of the fruit was obtained at 40 ppm dose (15.20%) compared to the control application, and in terms of titratable acidity ratio, the best result was obtained from 20 ppm (0.42%) PBZ application compared to the control application. Suja & Anusuya, (2018) reported in their study on the growth, and quality of tomato PKM-1 with PBZ, and sridiamine, the titratable acidity ratio (TA) was found to be 19.6% to 37.2% higher compared to the control application, and the water-soluble solids content in the fruit was found to be between 4.03-5.48.

In studies on different species, it has been reported by different researchers that PBZ application causes an increase in the water-soluble dry matter content (Jain, Sing, & Misra, 2002; Yeshitela, Robbertse, & Stassen, 2004; Burondkar et al., 2013; Samaan & Nassar, 2020), that the amount of water-soluble dry matter in apples is not affected or decreases as a result of PBZ applications from soil (Johnson & Legge, 1985; Khurshid et al., 1997b), and that it increases when applied from the leaves (Naira et al., 2017; Sha et al., 2021). One of the positive effects of PBZ on fruit quality is that it reduces the acidity rate in the fruit (Webster, Quinlan, & Richardson, 1986; Khurshid et al., 1997a; Jain et al., 2002; Burondkar et al., 2013; Samaan & Nasser, 2020; Sha et al., 2021). Nevertheless, in our study, there was no difference in pH, and TA amounts, which may be due to plant species,

application time, and application doses. Paclobutrazol has been demonstrated to induce substantial metabolic alterations in plant species, culminating in a decline in TA, and pH levels. This effect is manifested by a reduction in the growth rate of plants, and the production of acidic metabolites, resulting in decreases in TA and pH (Desta & Amare, 2021; Christov, Tsvetkov, & Kovachev, 1995). The present findings suggest that plant growth regulators have significant effects on pH, and the production of acidic compounds through changes in plant metabolism. Notably, growth regulators such as Paclobutrazol have been observed to exert influence not only on pH, and acidic compound production, but also on stress responses, and plant productivity. In the context of external application, the judicious selection of the most suitable chemical, the determination of the optimal concentration, and the temporal considerations of application are of paramount importance to ensure the achievement of the desired outcome (Palavan-Ünsal, 1993; Buban, 2000). From the consumer's point of view, fruit quality characteristics of vegetables are generally assessed by criteria such as colour, size, shape, firmness, dry matter, taste and nutritional value, but the concept of quality also includes characteristics such as pH, SÇKM, and TA (Dorais, Papadopoulos, & Gosselin, 2001; Tüzel et al., 2001). Paclobutrazol has significant effects on fruit quality, particularly in fruit-eating vegetables such as tomatoes. Erman et al. (2016) reported that the application of paclobutrazol resulted in an increase in the pH level of tomato, leading to a reduction in acidity, and an enhancement in flavour. Yıldırım & Küçük, (2008) observed that the application of paclobutrazol increased the acidic components of the fruit by increasing the TA ratio, thereby improving the flavour profile. Furthermore, Kumar et al. (2015) and Şahin et al. (2010) observed that paclobutrazol increased fruit water content, and increased SÇKM, resulting in fuller, and juicier fruit tissues. These findings suggest that paclobutrazol provides multifaceted effects that improve the fruit quality of tomato. In particular, parameters such as pH, TA, and SÇKM improve the flavour, and marketability value of tomato.

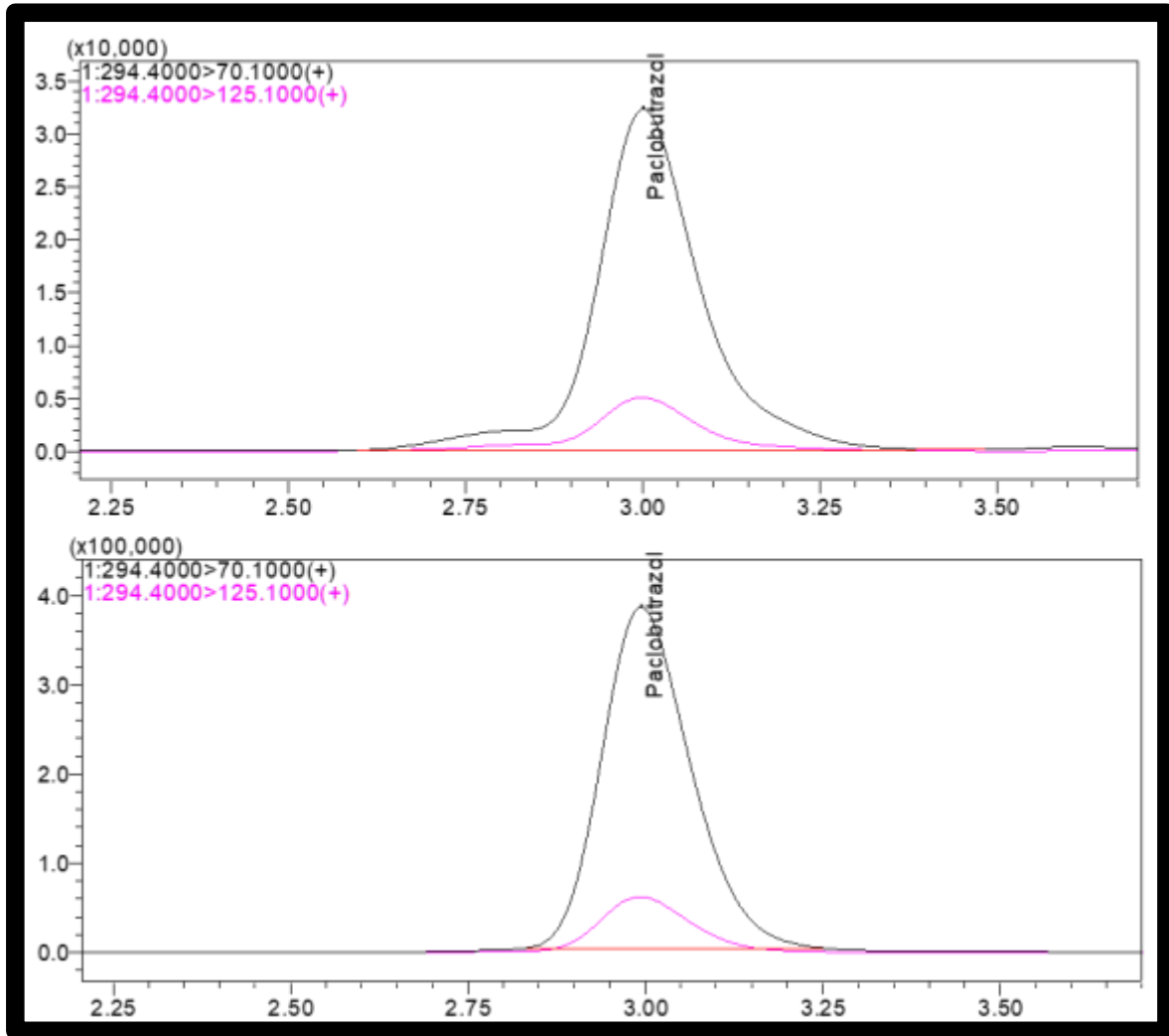


Figure 3. Paclobutrazol sample (top), and standard chromatograms (bottom)

Şekil 3. Paclobutrazol örneği (üstte) ve standart kromatogramlar (altta)

The results of paclobutrazol residue analysis are shown in Table 9. No PBZ residues were detected in the control, and 50 ppb foliar applications. PBZ residues in samples from the 100 ppb foliar application on day 3 were below the LOQ, while on the other days they were below the LOD. As a result of the 200 ppb soil application, PBZ residues in the third day samples were found to be 4.43 µg/kg, and below the LOQ on the other days (Table 9). The chromatograms of the third day sample above the LOQ, and the calibration point at a concentration of 100 µg/kg are shown in Figure 3. Çimen et al. (1999) reported that no paclobutrazol residue was found in seventh lemon in their study where they applied different doses of paclobutrazol in seventh lemon.

CONCLUSIONS

In this study on soilless tomato cultivation, it was found that foliar applications of PBZ at 50 ppm, and 100 ppm resulted in better yields compared to the control, and soil applications of PBZ. Foliar application in soilless tomato production appears advantageous for improving yield, and quality. Nevertheless, as the PBZ dosage increased, a notable decrease in plant height was observed.

Considering the study results as a whole, it would be accurate to state that PBZ applications positively affect yield, quality, and plant growth in soilless tomato production. Foliar applications of PBZ produced better results than soil applications, and no residues were detected in tomato fruits treated with PBZ according to the analytical results.

Research is required at varying ecological conditions, dosage levels, and phenological stages in order to clarify the effects of PBZ, and to contribute to the development of strategies for increasing yield, and management. Monitoring of PBZ residues is critical for ensuring food safety, and health risks can be minimised through regular residue analyses. Regulations for the use of PBZ should be established with due consideration for health, and environmental safety, while also fostering support for sustainable agricultural practices. The present study has determined that doses of 50 ppm, and 100 ppm are optimal for soilless greenhouse tomato cultivation, exhibiting favourable outcomes during the flowering, and fruit setting periods. In open field cultivation, it was determined that paclobutrazol application was more effective during active growth stages such as the spring period, considering factors such as plant species, application dose and time.

In conclusion, it is imperative for farmers to pay close attention to the precise dosage, and application timing of PBZs in order to optimise plant growth, and yield. Farmers are advised to employ the correct application techniques at the appropriate phenological stages for each plant species, and to undertake regular residue analyses to prevent any detrimental effects on environmental health. The implementation of these practices will ensure the enhancement of productivity, and facilitate the establishment of sustainable, and healthy production methodologies.

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Authorship Contributions

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

Conflict of Interest

No conflict of interest was declared by the authors.

REFERENCES

- Ali, S., Asghar, A., Akhter, J., & Khan, S.A. (2018). Growth and yield response of potato (*Solanum tuberosum* L.) to paclobutrazol application. *Pure and Applied Biology*, 7(2), 510-515. <https://doi.org/10.3329/bjar.v43i2.37330>.
- Aydoğdu, M., & Boyraz N. (2005). Bitki büyüme düzenleyicileri (hormon) ve hastalıklara dayanıklılık. *Bitkisel Araştırma Dergisi*, 1, 35-40.
- Aydın, M., Kaçal, E., Gür İ., Koçal, H., Yalçın, B., & Öztürk, G. (2021). Paclobutrazol'un fuji elma çeşidinde verim ve meyve kalitesi üzerine etkisi. *Meyvecilik Araştırma Enstitüsü Müdürlüğü, Cilt/Vol. 8* (2), 46-52. Eğirdir, 32500/Isparta. <https://doi.org/10.51532/meyve.1040158>.
- Balkan, T., & Yılmaz, Ö. (2022). Investigation of insecticide residues in potato grown in Türkiye by LCMS/MS and GC-MS and health risk assessment. *Türkiye entomoloji dergisi*, 46 (4), 481-500. <http://dx.doi.org/10.16970/entoted.1201475>
- Buban, T. (2000). The use of benzyladenine in orchard fruit growing: a mini review. *Plant Growth Regulation*, 32, 381-390. <https://link.springer.com/article/10.1023/A:1010785604339>
- Budak, N., Çalışkan, C.F., & Çaylak, Ö. (1994). Bitki büyüme regülatörleri ve tarımsal üretimde kullanımı. *Ege Üniv. Zir. Fak. Dergisi*, 31, 289-296.

- Budiyanto, O., Hajoeningtjas, D., & Nugroho, B. (2010). Effect of Pruning Branches and Levels of Paclobutrazol on Cucumber Yield (*Cucumis sativus* L.). *Agritech*, 12 (2), 100–113. <https://doi.org/10.13057/cellbioldev/v010103>.
- Burondkar, M.M., Rajan, S., Upreti, K.K., Reddy, Y.T.N., Singh, V.K., Sabale, S.N., Naik, M.M., Ngade, P.M., & Saxena, P. (2013). Advancing Alphonso mango harvest season in lateritic rockysoils of Konkan region through manipulation in time of paclobutrazol application. *Journal of Applied Horticulture*, 15, 178-182. <https://doi.org/10.37855/jah.2013.v15i03.34>
- Cemeroğlu, B. (2010). *Gıda Analizleri genişletilmiş 2. baskı*. Gıda Teknolojisi Derneği. Yayın No: 34.
- Chaves, M.M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, 103 (4), 551-560. <https://doi.org/10.1093/aob/mcn125>.
- Chakrabarty, D., Chakraborti, S.P., & Basu, M. (2009). Paclobutrazol Induced Leaf Senescence and Antioxidant Activities in Pigeonpea (*Cajanus cajan* L. Millsp.). *American Journal of Plant Physiology*, 4 (2), 41-47.
- Christov, C., Tsvetkov, I., & Kovachev, V. (1995). Paclobutrazolün üzüm asmalarının (*Vitis vinifera* L.) vejetatif büyümeyi kontrol etmek ve meyve verimini artırmak için kullanımı. *Bulg. J. Plant Pysiol.* 21, 64–71. <https://api.semanticscholar.org/CorpusID:15628345>
- Çimen, İ., Güngör, T., & Kemal, G. (1999). Gelişme engelleyici paclobutrazol'un yediveren limonunda sürgün büyümesi, verim ve kalite üzerine etkileri ile kalıntı durumlarının araştırılması. *Bitki Koruma Bülteni*, 39 (1-2), 77-90. <https://hdl.handle.net/11468/25506>
- Demirsoy, M., & Uzun, S. (2019). The quantitative effects of different growing media on the growth of aubergine (*Solanum melongena* L.) and cucumber (*Cucumis sativus* L.) in autumn. *International Journal of Environmental Trends (IJENT)*, 3 (2), 151-158. <https://5e3a8d3692851c7f7f1d45cb.pdf>
- Desta, B., & Amare, G. (2021). Paclobutrazol as a plant growth regulator. *Chemical and Biological Technologies in Agriculture*, 8, 1. <https://doi.org/10.1186/s40538-020-00199-z>
- Di Benedetto, A., & Klasman, R. (2007). The effects of plug cell volume paclobutrazol height control and the transplant stage on the post-transplant growth of Impatiens wallerana pot plant. *European Journal of Horticultural Science*, 193-197.
- Dorais, M., Papadopoulos, A.P., & Gosselin, A. (2001). Influence of electric conductivity management on greenhouse tomato yield and fruit quality. *Agronomic*, 21, 367-383. <https://doi.org/10.1051/agro:2001130>
- Du, F., Guan, C., & Jiao, Y. (2018). Molecular mechanisms of leaf morphogenesis. *Molecular plant*, 11 (9), 1117-1134. <https://doi.org/10.1016/j.molp.2018.06.006>.
- EC, (2002). Commission Directive 2002/63/EC of 11 July 2002 establishing community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing directive 79/700/EEC. *Official Journal of the European Communities*, 2, 30–43.
- Erman, M., Yıldız, M., & Gökçe, M. (2016). "Effects of paclobutrazol on growth and quality parameters of tomato (*Lycopersicon esculentum* Mill.)." *Journal of Agricultural Sciences*, 22 (2), 164-171. <https://doi.org/10.15832/jagri.2016.22.2.164>
- Giovinazzo, R., & Souza-Machado, V. (2000, June). Paclobutrazol responses with processing tomato in France. In *VII International Symposium on the Processing Tomato*, 542 , 355-358. <https://doi.org/10.17660/ActaHortic.2001.542.46>
- Greene, D.W. (1986). Effect of paclobutrazol and analogs on growth, yield, fruit quality, and storage potential of 'Delicious' apples. *Journal of American Society for Horticultural Science*, 111 (31), 328-332. <https://doi.org/10.21273/JASHS.111.3.328>
- Greene, D.W. (1991). Reduced Rates and Multiple Sprays of Paclobutrazol Control Growth and Improve Fruit Quality of "Delicious" Apples. *Journal of the American Society for Horticultural Science*, 116 (5), 807-812. <https://doi.org/10.21273/JASHS.116.5.807>
- Gruda, N., Caron, J., Prasad, M., & Maher, M. (2017). *Growing media* (1053-1058 pp.). In: *Encyclopedia of soil science* (3rd ed.) R. Lal (Ed.). CRC Press. Boca Raton.
- Halloran, N., & Kasım, M.U. (2002). Meyve ve sebzelerde büyüme düzenleyici madde kullanımı ve kalıntı düzeyleri. *Gıda*, 27(5), 351-359.
- Harpitaningrum, P., Sungkawa, I., & Wahyuni, S. (2014). Effect of Paclobutrazol Concentration on Growth and Yield of Cucumber (*Cucumis sativus* L.). *Venus cultivar J. Agrijati*, 25(1), 1-17.
- Hassan, A.M.A., El-Miniawy, S.M., Ahmed, A.M., & Shalaby, T.A. (2017). Response of tomato plants to paclobutrazol application under different irrigation intervals. *Scientia Horticulturae*, 225, 379-385.
- Hebhlhwaite, P.D., Hampton, J.G., & McLaren, J.S. (1982). The chemical control of growth development and yield of *Lolium perenne* grown for seed. J.S. McLaren (Editor). *Chemical Manipulation of Crop Growth and Development*. Butterworths, 505-523. <https://www.cabidigitallibrary.org/doi/full/10.5555/19820739442>.

- Hopkins, W.G., & Hüner, N.P.A. (2009). Introduction to Plant Physiology, Hoboken: John Wiley and Sons.
- Jain, S.K., Sing, R., & Misra K.K. (2002). Effect of paclobutrazol on growth, yield and fruit quality of lemon (*Citrus lemon*). *The Indian Journal Agricultural Science*, 72, 488-490.
- Jayaramaiah, R., Nataraja, A., Kumar, B.T.N., & Pramod, G. (2018). Effect of Calcium Metalosate on Growth and Yield of Tomato (*Lycopersicon esculentum* Mill.). *Int. J. Curr. Microbiol. App. Sci.* 7 (10), 1371–1375. <https://doi.org/10.20546/ijcmas.2018.710.152>
- Johnson, D.S., & Legge, A.P. (1985). Effects of preharvest agronomic factors on the storage quality of fruit. *Report of East Malling Research Station*, 108-109.
- Kartal, H., & Geboloğlu, N. 2024. Topraksız Tarımda Hıyar Yetiştiriciliğinde Farklı Kompost Materyallerinin Yetiştirme Ortamı Olarak Kullanım Olanaklarının Araştırılması. *KSÜ Tarım ve Doğa Dergisi*, 28 (1). <https://doi.org/10.18016/ksutarimdog.vi.1469754>
- Kaynak, L., & Ersoy, N. (1997). Bitki büyüme düzenleyicilerinin genel özellikleri ve kullanım alanları. *Akd. Üniv. Zir. Fak. Dergisi*, 10, 223-236.
- Keikotlhaile, B.M., & Spanoghe, P. (2011). Pesticide residues in fruits and vegetables. *Pesticides-formulations, effects, fate*, 2011, 243-252.
- Khan, M.S.H., Wagatsuma, T., Akhter, A., & Tawaraya, K. (2009). Sterol biosynthesis inhibition by paclobutrazol induces greater aluminum (Al) sensitivity in Al-tolerant rice. *American Journal Plant Physiological*, 4, 89–99. <https://doi.org/10.3923/ajpp.2009.89.99>
- Khandaker, M.M., Syafiq, M., Abdulrahman, M.D., Mohd, K.S., Yusoff, N., Sajili, M.H., & Badaluddin, N.A. (2020). Influence of Paclobutrazol on Growth, Yield and Quality of Eggplant (*Solanum melongena*). *Asian J. Plant Sci.* 19(4), 361-371, 2020. ISSN 1682-3974. <https://doi.org/10.3923/ajps.2020.361.371>
- Khurshid, T., McNeil, D.L., Trought, M.C.T., & Hill, G.D. (1997a). The response of young ‘Braeburn’ and ‘Oregon Spur Delicious’ apple trees growing under an ultra-high density planting system to soilapplied paclobutrazol: II. Effect on fruit quality at harvest and during storage. *Scientia horticulturae*, 71, 189-196.
- Khurshid, T., McNeil, D.L., Trought, M.C.T., & Hill, G.D. (1997b). The response of young ‘Braeburn’ and ‘Oregon Spur Delicious’ apple trees growing under an ultra-high density planting system to soilapplied paclobutrazol: I. Effect on reproductive and vegetative growth. *Scientia horticulturae*, 72(1), 11- 24.
- Kim, J., Wilson, R.L., Case, J.B., & Binder, B.M. (2012). A comparative study of ethylene growth response kinetics in eudicots and monocots reveals a role for gibberellin in growth inhibition and recovery. *Plant Physiology*, 160, 1567–1580. <https://doi.org/10.1104/pp.112.205799>
- Kondhare, K.R., Hedden, P., Kettlewell, P.S., Farrell, A.D., & Monaghan, J.M. (2014). Use of the hormone-biosynthesis inhibitors fluridone and paclobutrazol to determine the effects of altered abscisic acid and gibberellin levelson pre-maturity amylase formation in wheat grains. *Journal of Cereal Science*, 60, 210–216. <https://doi.org/10.1016/j.jcs.2014.03.001>
- Kumlay, A.M., & Eryiğit, T. (2011). Bitkilerde Büyüme ve Gelişmeyi Düzenleyici Maddeler: *Bitki Hormonları*. *Journal of the Institute of Science and Technology*, 1 (2), 47-56.
- Kumar, S. (2015). Effect of paclobutrazol on growth, yield, and quality of tomato (*Lycopersicon esculentum* Mill.) under greenhouse conditions. *Agricultural Sciences*, 6 (3), 252-257. <http://doi.org/10.4236/as.2015.63028>
- Lehotay, S.J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative Study. *Journal of AOAC International*, 90 (2), 485–520.
- McDaniel, G.L. (1983). Growth retardation activity of paclobutrazol on chrysanthemum. *Horticulture Science*, 18, 199-200.
- Meena, R.K. (2014). Effect of paclobutrazol on growth and yield of cashew (*Anacardium occidentale* L.). *Vegetos*, 27(1), 11-16. <https://doi.org/10.5958/j.2229-4473.27.1.003>
- Menhenett, R., & Hanks, G.R. (1982). Comparisons of a new triazole retardant PP 333 with ancymidol and other compounds on pot-grown tulips. *Plant Growth Regulation*, 1, 173-181.
- Mr, M.M., Baba, J.A., Umar, I., Rather, G.H., Rehman, M.U., Banday, S.A., Kumar, A., & Nazir, N. (2015). Effect of soil applied paclobutrazol on vegetative and quality attributes of apricot. *Green Farming*, (4), 813-816.
- Minoia, S., Petrozza, A., D'Onofrio, O., Florence, P., & Mosca, C. (2010). A new mutant genetic resource for tomato crop improvement by tilling technology. *BMC research notes*, 3 (1), 69.
- Mohan, D., Pal, M., & Biswas, A.K. (2015). Paclobutrazol induced fruit retention and quality in bell pepper (*Capsicum annuum* L.) cv. California Wonder. *International Journal of Vegetable Science*, 21 (3), 224-233.
- Muneeba Banoo, B., Sinha, K., Chand, G., Sharma, M.K., Rai, G.K., Gupta M., & Reena M. (2020). Effect of Paclobutrazol and Partial Root Drying on Growth and Yield Attributes of Tomato (*Solanum lycopersicum* L.). *International Journal of Current Microbiology and Applied Sciences*, 9, 10 (2020). <https://doi.org/10.20546/ijcmas.2020.910.245>

- Naira, A., Moieza, A., Bhat, M.Y., & Sharma, M.K. (2017). Paclobutrazol and summer pruning influences fruit quality of red delicious apple. *International Journal of Agriculture, Environment and Biotechnology*, 10 (3), 349-356.
- Orabi, S.A., Salman, S.R., & Shalaby, M.A.F. (2010). Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. *World Journal Agricultural Sciences*, 6 (3), 252-259. <https://api.semanticscholar.org/CorpusID:14878958>.
- Palavan-Ünsal, N. (1993). *Hormonlar ve meyvelenme. Bitki büyüme maddeleri*. İstanbul Üni. Basım Evi ve Film Merkezi., Üniversite Yayın No: 3677, 197-211.
- Pasian, C.C., & Bennett, M.A. (2001). Paclobutrazol soaked marigold, geranium, and tomato seeds produce short seedlings. *Horticulture Science*, 36 (4), 721-723.
- PGRSA, (2007). Plant growth regulation handbook of the Plant Growth Regulation Society of America (4th ed.). Athens: *The Plant Growth Regulation Society of America*.
- Quinlan, J.D. (1981). New chemical approaches to the control of fruit tree form and size. *Acta Horticulture*, 120, 95-106. <https://doi.org/10.17660/ActaHortic.1981.120.12>
- Raffar, K.A. (1990). Hydroponics in tropica. International Seminar on Hydroponic Culture of High Value Crops in the Tropics in Malaysia. November 25-27. 1990.
- Samaan, M., & Nasser, M.A. (2020). Effect of Spraying Paclobutrazol (PP333) on Yield and Fruit Quality of Crimson Seedless Grape. *Journal of Plant Production*, 11, 1031-1034.
- SANTE, (2021). SANTE/11312/2021 Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. 1-55.
- Savvas, D., Gianquinto, G., Tuzel, Y., & Gruda, N. (2013). *Soiless culture*. pp. 303-354. In: Good agricultural practices for greenhouse vegetable crops. Principles for Mediterranean climate areas. Rome, Italy.
- Sha, J., Ge, S., Zhü, Z., Dü, X., Zhang, X., Xü, X., Wang, F., Chen, Q., Tian, G., & Jiang, Y. (2021). Paclobutrazol regulates hormone and carbon-nitrogen nutrition of autumn branches, improves fruit quality and enhances storage nutrition in 'Füji'apple. *Scientia Horticulturae*, 282, 110022.
- Singh, Z. (2000). Effect of (2RS, 3RS) paclobutrazol on tree vigour, flowering, fruit set and yield in mango. *Acta Horticulturae*, 525, 459-462.
- Srivastava, M.K., Dwivedi, A.P., & Srivastava, S. (2012). Effect of paclobutrazol on growth flowering fruiting yield and quality of tomato (*Lycopersicon esculentum* Mill.). Cv. Pusa Ruby. *Progressive Horticulture*, 44 (1), 126-131.
- Soumya Preman, R. (2017). "Paclobutrazol: a novel plant growth regulator and multi-stress ameliorant. *Indian Journal of Plant Physiology*, 22, 267-278.
- Soydan, K.D., Turgut, N., Yalçın, M., Turgut, C., & Karakuş, P.B.K. (2021). Evaluation of pesticide residues in fruits and vegetables from the Aegean region of Turkey and assessment of risk to consumers. *Environmental Science and Pollution Research*, 28, 27511-27519. <https://doi.org/10.1007/s11356-021-12580-y>.
- Sudrajat, A., Frasetya, B., & Daniswara, F.G. (2021). Application of paclobutrazol and electrical conductivity value of nutrient solutions to improve yield and quality *Cucumis Sativus* L. var *Japanese* on the hydroponic system. *The 5th Annual Applied Science and Engineering Conference* (AASEC 2020). <https://doi.org/10.1088/1757-899X/1098/5/052003>
- Suja, S., & Anusuya, N. (2018). Influence of Paclobutrazol (PP333) and Sridiamin (Human hair-derived aminoacid mixture) on growth and quality of Tomato PKM-1. IOP Conf. Series: *Earth and Environmental Science*, 131, (2018) 012002. <https://doi.org/10.1088/1755-1315/131/1/012002>.
- Syahputra, B.S.A., Sinniah, U.R., Ismail, M.R., & Swamy, M.K. (2016). Optimization of paclobutrazol concentration and application time for increased lodging resistance and yield in field-grown rice. *Philippine Agricultural Sciences*, 99, 221-228 sy. <http://www.pas-uplbc.edu.ph/article.php?id=543>
- Şahin, F., Yıldırım, E., & Küçük, M. (2010). "Effect of paclobutrazol on growth, yield, and fruit quality of tomato." *Acta Horticulturae*, 877, 163-168. <http://doi.org/10.17660/ActaHortic.2010.877.23>
- Talaz, A., & Engin, N. (2019). Topraksız tarım uygulama yöntemi ile domates üretiminin Bafra ovasında gerçekleştirilebilirliğinin araştırılması. *Erciyes Tarım ve Hayvan Bilimleri Dergisi*, 2 (1), 20-34.
- Thomas, T.H., Barnes, A., & Hole, C.C. (1982). Modification of plant part relationships in vegetable crops. *Proceedings-Easter School in Agricultural Science, University of Nottingham*, 297-311.
- Toprak, E., & Gül, A. (2013). Topraksız tarımda kullanılan ortam domates verimi ve kalitesini etkiliyor mu? *International Journal of Agricultural and Natural Sciences (IJANS)*, 6 (2), 41-47. E-ISSN: 2651-3617.
- Tüzel, İ.H., & Meriç, M.K. (2001). Evapotranspiration of tomato plants grown in different soiless culture systems, *Acta Horticulturae*, 559, 555-562.
- Wang, Y.T., & Gregg, L.L. (1990). Uniconazole controls growth and yield of greenhouse tomato. *Scientia Horticulturae*, 43 (1), 55-62.

- Wardani, E.C., Murti, R.H., Sulistyanisgih, T., & Rogumulyo, R. (2022). Keragaan Tanaman Tomat Apokarpel (*Solanum lycopersicum* L.) sebagai Tanaman Hias dalam Pot dengan Pengaplikasian Paklobutrazol. *Vegetalika*, 11 (2), 163-173. <http://doi.org/10.22146/veg.66539>.
- Webster, A.D., Quinlan, J.D., & Richardson, P.J. (1986). The influence of paclobutrazol on growth and cropping of sweet cherry cultivar. The effect of annual soil treatments on the growth and cropping of cultivar 'early rivers'. *Journal of Horticultural Science*, 61, 471-478. <https://api.semanticscholar.org/CorpusID:82115708>.
- Whipker, B.E., & Dasoju, S. (1998). Potted sunflower growth and flowering responses to foliar applications of daminozide paclobutrazol and uniconazole. *Horticulture Technology*, 8(1), 86-88.
- Wijayanti, J.S., & Dewi, K. (2024). Growth and Development of Winged Bean (*Psophocarpus tetragonolobus* L.) DC.) Treated with Paclobutrazol. *Journal of Tropical Biodiversity and Biotechnology*, Volume 09, Issue 02 (2024). <http://doi.org/10.22146/jtbb.86238>.
- Yalçın, M., & Çimrin, K.M. (2019). Şanlıurfa-Siverek'te Yaygın Toprak Gruplarının Besin Elementi Durumları ve Bunların Bazı Toprak Özellikleri ile İlişkileri. *KSÜ Tarım ve Doğa Dergisi*, 22 (1), 1-13. <https://doi.org/10.18016/ksutarimdog.vi.22i39650.412922>
- Yeshitela, T., Robbertse, P.J., & Stassen, P.J.C. (2004). Paclobutrazol suppressed vegetative growth and improved yield as well as fruit quality of 'Tommy Atkins' mango (*Mangifera indica*) in Ethiopia. *New Zealand Journal of Crop and Horticultural Science*, 32 (3), 281-293. <https://doi.org/10.1080/01140671.2004.9514307>
- Yıldırım, E., & Küçük, M. (2008). "Effect of paclobutrazol on growth, yield and fruit quality of tomato (*Lycopersicon esculentum* Mill.)." *Horticultural Science*, 33 (4), 37-43. <http://doi.org/10.21273/HORTSCI.33.4.37>
- Yıldız, N. (2014). Yalıtımda doğal çözüm: Perlit. *Madencilik Türkiye Dergisi*, 39.

Determination of Pomological and Chemical Properties and Molecular Docking Analysis of *Crataegus orientalis* and *Crataegus orientalis* subsp. *orientalis* Species and Subspecies in Sandıklı (Afyonkarahisar)

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ABSTRACT

This study investigates the pomological, chemical, and molecular properties of *Crataegus orientalis* and *Crataegus orientalis* subsp. *orientalis* genotypes grown in the Sandıklı region of Afyonkarahisar, Türkiye. A total of ten genotypes were evaluated based on fruit dimensions, antioxidant activities, organic acid contents, and molecular docking properties. Pomological characteristics, including fruit width, length, and weight, were measured. The fruit weight ranged between 1.78 g⁻¹ and 6.30 g⁻¹, fruit width varied from 11.74 mm to 15.75 mm, and fruit length ranged between 13.26 mm and 23.18 mm, highlighting significant differences among genotypes. Chemical analyses revealed notable variations in antioxidant and phenolic contents among genotypes. The pH values ranged from 2.63 to 3.12, and DPPH radical scavenging activity was observed between 10.3% and 45.0%. Total phenolic content varied from 17.20 µg GAE 100 g⁻¹ to 49.70 µg GAE 100 g⁻¹, while total flavonoid content ranged from 63 mg CE 100 g⁻¹ to 348 mg CE 100 g⁻¹, demonstrating considerable diversity in antioxidant capacities among genotypes. Organic acid analyses also revealed significant differences in the levels of citric acid, malic acid, succinic acid, and ascorbic acid. Citric acid content ranged from 86.14 mg 100 g⁻¹ to 91.05 mg 100 g⁻¹, while malic acid content was measured between 739 mg 100 g⁻¹ and 821 mg 100 g⁻¹. Succinic acid levels varied from 287.9 mg 100 g⁻¹ to 301.7 mg 100 g⁻¹, and ascorbic acid content ranged from 63.9 mg 100 g⁻¹ to 70.25 mg 100 g⁻¹. These organic acids were correlated with biological activities, contributing to the understanding of the potential therapeutic effects of these genotypes. Molecular docking studies assessed the potential interactions of ascorbic acid, citric acid, malic acid, and succinic acid with the human erythrocyte catalase enzyme. Simulation results indicated binding energies of -6.8 kcal mol⁻¹ for ascorbic acid, -6.5 kcal mol⁻¹ for citric acid, -5.1 kcal mol⁻¹ for malic acid, and -4.9 kcal mol⁻¹ for succinic acid. These findings highlight the richness of *Crataegus* genotypes in phenolic and flavonoid content, supported by their strong antioxidant activities. The strong interactions of compounds such as ascorbic acid and citric acid with the catalase enzyme suggest that these genotypes hold promise as natural therapeutic agents for treating oxidative stress-related diseases.

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Sandıklı (Afyonkarahisar) Bölgesindeki *Crataegus orientalis* ve *Crataegus orientalis* subsp. *orientalis* Tür ve Alttürlerinin Pomolojik ve Kimyasal Özelliklerinin Belirlenmesi ve Moleküler Docking Analizi

ÖZET

Bu çalışma, Afyonkarahisar, Türkiye'nin Sandıklı bölgesinde yetişen *Crataegus orientalis* ve *Crataegus orientalis* subsp. *orientalis* genotiplerinin pomolojik, kimyasal ve moleküler özelliklerini kapsamlı bir şekilde araştırmaktadır. On genotip, meyve boyutları,

Bahçe Bitkileri

Araştırma Makalesi

antioksidan aktiviteleri, organik asit içerikleri ve moleküler bağlanma özellikleri açısından değerlendirilmiştir. Çalışmada meyve eni, boyu ve ağırlığı gibi pomolojik özellikler ölçülmüş; meyve ağırlığının 1.78 g⁻¹ ile 6.30 g⁻¹ arasında, meyve eninin 11.74 mm ile 15.75 mm arasında ve meyve boyunun 13.26 mm ile 23.18 mm arasında değiştiği belirlenmiştir. Bu parametreler, genotipler arasında belirgin farklılıklar olduğunu ortaya koymuştur. Kimyasal analizler, genotipler arasındaki antioksidan ve fenolik içerik farklarını ortaya çıkarmıştır. pH değerleri %2.63 ile %3.12 arasında değişirken, DPPH radikal süpürme aktivitesi %10.3 ile %45.0 arasında gözlemlenmiştir. Toplam fenolik içerik 17.20 µg GAE 100g⁻¹ ile 49.70 µg GAE 100g⁻¹ arasında değişiklik göstermiştir. Toplam flavonoid içerikleri ise genotipler arasında önemli farklılıklar göstermiş ve 63 mg CE 100 g⁻¹ ile 348 mg CE 100 g⁻¹ arasında ölçülmüştür. Bu sonuçlar, genotiplerin antioksidan kapasitelerinde önemli bir çeşitlilik olduğunu ve farklı genotiplerin farklı biyolojik aktiviteler gösterebileceğini ortaya koymaktadır. Organik asit analizleri, genotipler arasında sitrik asit, malik asit, süksinik asit ve askorbik asit içeriklerinin önemli farklılıklar gösterdiğini ortaya koymuştur. Sitrik asit miktarı 86,14 mg 100 g⁻¹ ile 91,05 mg 100 g⁻¹ arasında değişirken, malik asit içerikleri 739 mg 100 g⁻¹ ile 821 mg 100 g⁻¹ arasında ölçülmüştür. Süksinik asit miktarı 287,9 mg 100 g⁻¹ ile 301,7 mg 100 g⁻¹ arasında değişiklik göstermiştir. En dikkat çekici sonuçlardan biri, askorbik asit miktarının genotipler arasında 63,9 mg 100 g⁻¹ ile 70,25 mg 100 g⁻¹ arasında değişmesi olmuştur. Bu organik asitlerin biyolojik aktivitelerle ilişkilendirilmesi, genotiplerin potansiyel terapötik etkilerini daha iyi anlamamıza katkı sağlamıştır. Moleküler yerleştirme çalışmaları, askorbik asit, sitrik asit, malik asit ve süksinik asit gibi bileşiklerin insan eritrosit katalaz enzimi ile potansiyel etkileşimlerini değerlendirmiştir. Moleküler docking simülasyonları sonucunda, askorbik asidin -6.8 kcal mol⁻¹, sitrik asidin -6.5 kcal mol⁻¹, malik asidin -5.1 kcal mol⁻¹ ve süksinik asidin -4.9 kcal mol⁻¹ bağlanma enerjilerine sahip olduğu belirlenmiştir. Bu bulgular, *Crataegus* genotiplerinin zengin fenolik ve flavonoid içerikleriyle dikkat çektiğini ve bu özelliklerin güçlü antioksidan aktivitelerle desteklendiğini göstermektedir. Özellikle askorbik asit ve sitrik asit gibi bileşiklerin katalaz enzimiyle güçlü etkileşimleri, bu genotiplerin oksidatif stresle ilişkili hastalıkların tedavisinde kullanılabilecek doğal terapötik ajanlar olma potansiyelini ortaya koymaktadır.

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INTRODUCTION

Hawthorn (*Crataegus* spp.) is a fruit naturally distributed in most regions of our country. It is included in the *Crataegus* genus belonging to the *Rosaceae* family, and has about 3500 species, and it is thought that there are more than 200 species in our country. (Tomar, 2020). Our country is rich in hawthorn (*Crataegus* spp.) genotypes, and 21 species can grow naturally in our country. (Fikret Balta et al., 2015). Along with Turkey, the temperate regions of Northern Europe, Africa, and Asia are the homelands of wheat. (Keleş, 2018). It is an oval, edible, pome, juicy fruit with yellowish-green, yellow, orange, red, dark purple or black coloured fruits. (Kayacık, 2019). The most common hawthorn species in Anatolia is *C. monogyna*, although *C. azarolus* and *C. orientalis* species are also

frequently encountered. It can be said that cultivation with large-fruited genotypes within the *C. azarolus* species has become increasingly widespread. (Çaliskan et al., 2012; Caliskan et al., 2016).

With the spread of modern agricultural techniques, the use of chemical inputs, soil destruction, and single-variety breeding have increased, and single-variety breeding has increased, especially due to yield and quality concerns, causing a decrease in genetic differences and causing erosion in the gene pool. (Miller & Schaal, 2006). For these reasons, identifying, collecting, and protecting plant genetic resources is important for our country. In our country, we have many types of cultivated and wild fruits. Our people know most of them and use their fruits, leaves, and wood for various purposes. One of these is *Crataegus* spp. It is the fruit popularly known as hawthorn in our country.

The term 'pomological characteristics' is used in the field of fruit science or pomology, which studies topics such as the cultivation, maintenance, storage, and trade of fruits. Therefore, the term 'pomological characteristics' refers to the physical attributes, growth properties, taste profile, color, yield, and storage life of a particular fruit type or variety. (El Hamzaoui et al., 2014; Kurnaz et al., 2024).

It is reported that antioxidant compounds such as vitamins, organic acids, and phenolic compounds found in the flowers and fruits of the hawthorn are beneficial for human health. The antioxidants found in hawthorn fruit prevent the formation of free radicals and ensure the regular functioning of the heart. Of these compounds. It is reported that it protects the heart against rhythm disturbances by increasing blood flow to the heart and brain, balancing the contraction power of the heart and heart pressure, and preventing high blood pressure formation. Dried flowers and fruits of hawthorns are brewed as a tea and used against upper respiratory tract infections, cough, heart failure, kidney diseases, arteriosclerosis, and liver diseases (Ljubuncic et al., 2005). In addition, hawthorn has high mineral levels such as Ca, P, Mg, and Fe (Özcan et al., 2005). In our country, hawthorn is generally consumed fresh, its fruits are used to make marmalade, jam, and vinegar, its flowers, leaves, and fruits are used as medicinal plants, pears can be grafted onto wild hawthorns, it is a source of food for wildlife, and it is used to make walking sticks due to the durability of its wood (Caliskan et al., 2016). Today, the use of natural plants continues to increase, and considering the potential of hawthorn in the pharmaceutical industry, apart from its use as food, it is thought that hawthorn fruit cannot be utilised sufficiently. All characteristics of fruits vary depending on the variety, the conditions of the region where they grow, and other processes.

Oxidative stress is defined as a fundamental mechanism in various pathologies and toxicities caused by xenobiotics (El-Demerdash et al., 2018). The balance between oxidation and reduction in cells plays a significant role by affecting the signalling pathways of hydrogen peroxide (H_2O_2) (Grilo et al., 2020). Despite its essential cellular signalling functions, H_2O_2 can become hazardous at high concentrations (Di Marzo et al., 2018). The accumulation of oxidative damage can contribute to chronic inflammation, accelerated aging, and the development of cardiovascular diseases, neurodegenerative disorders, and certain types of cancer (Singh et al., 2019).

Catalase (CAT) activity is the fundamental mechanism for detoxifying and regulating H_2O_2 levels (Bukowska et al., 2000). CAT is an important enzyme that protects biomolecules from oxidative damage. This enzyme breaks down hydrogen peroxide (H_2O_2) into water and oxygen. CAT is found in all aerobic microorganisms, animals, and plants (Hadwan et al., 2024). CATs are found in the peroxisome as it is the center of H_2O_2 production due to oxidative stress (Aftab and Hakeem, 2021). CATs also exist in other cellular structures such as mitochondria (Baker et al., 2023). Specific inhibitors that reduce CAT activity decrease cellular resistance to ROS (Zhao et al., 2019).

In this study, the richness of hawthorn genotypes in Afyonkarahisar province, Sandıklı district in terms of pomological characteristics (Fruit width, Fruit height, Fruit weight) and chemical properties (organic acid, vitamin C, total phenolic substance amount, total flavonoid amount, DDPH, pH) was investigated. Additionally, a molecular docking study was conducted to identify the potential biological interactions of the fruit.

MATERIAL and METHOD

Plant Material

In the study, plant material; In 2022-2023, Afyonkarahisar province, Sandıklı district, the northern direction of the Akdağ nature park artificial pond (1 = *Crataegus orientalis* subsp. *orientalis*), the Bekteş Village of Sandıklı close to the Akdağ nature park (2 = *Crataegus orientalis* subsp. *orientalis*), the northwestern aspect of the Akdağ nature park artificial pond (3 = *Crataegus orientalis*), Kızık Village (4 = *Crataegus orientalis*), near Samura Mountain (5 = *Crataegus orientalis* subsp. *orientalis*), east side of Akdağ nature park artificial pond (6 = *Crataegus orientalis* subsp. *orientalis*), west side of Akdağ nature park artificial pond (7 = *Crataegus orientalis* subsp. *orientalis*), Reşadiye Village (8 = *Crataegus orientalis* subsp. *orientalis*), Menteş Village (9 = *Crataegus orientalis*) and south side of Akdağ nature park artificial pond (10 = *Crataegus orientalis*) Hawthorn fruits collected from.

Authors confirm in this study that the use of plants is in accordance with international, national, and or institutional regulations. We obtained necessary permissions from relevant garden authorities and or owners for the collection of plant samples.

Dr. Alper DURMAZ identified the collected field samples, and the identified specimens are preserved in the Ondokuz Mayıs University Herbarium (OMUB) with the accession number 5332-5333.

There are 10 genotypes in total. For pomological characteristics of hawthorn genotypes, fruit weight (g^{-1}), fruit length (mm), and fruit width (mm) were determined in 30 fruits of each genotype. To determine the chemical properties, 20 g of fruit samples from each genotype were homogenized with 80 ml of pure water with an electric hand blender for 1 minute. The homogenized sample was passed through a cheesecloth and the juice was filtered.

Total Phenolic Content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 minutes, 1 mL of 2% Na_2CO_3 solution was added. The mixture was then left to incubate in the dark at room temperature for 1 hour, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in microgram per gram of dried extract ($\text{mg GAE } 100\text{g}^{-1}$ extract) (Singleton & Rossi, 1965).

Total Flavonoid Content

This method was assessed using the AlCl_3 method with minor adjustments, following the procedure by (Zhishen et al., 1999). Extracts were mixed with distilled water, followed by NaNO_2 (5%) addition and a standing period. Subsequently, AlCl_3 (10%) was added, and the solution underwent incubation. NaOH (1M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE g^{-1} extract).

2,2-Diphenyl-1-Picrylhydrazyl Assay

Modifications were made based on the procedure of (Dorman et al., 2003). The free radical scavenging potential of the extract was determined using the DPPH assay. For this test, various concentrations of 1 mL extract were mixed with a methanol solution of DPPH radical (0.1 mM) in a tube. The mixture was left to incubate in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a UV spectrophotometer against a blank

Organic Acid Content

Organic Acid Contents (mg g^{-1}) using the method developed by (Bevilacqua and Califano, 1989). A reflectoquant device (RQflex® 20 reflectometer) was used in vitamin C analysis. The ascorbic decisive test kit (Merck 116981) was immersed in the prepared fruit juices for 2 seconds, allowed to oxidize outside for 8 seconds, and then placed into the test adapter of the reflectoquant device for 5 seconds. Then, the values read on the device were recorded in mg L^{-1} .

Molecular Docking Studies

Molecules were drawn using Chem-Draw Ultra 18.0 software, and their minimal energy forms were obtained using Chem 3D 18.0. This process aims to minimize the energy levels of the molecules to achieve their most stable conformations, with the results saved in Mol2 format. Enzyme structures were recorded using the Protein Data Bank (PDB). The structure of human erythrocyte catalase, with PDB-ID 1DGB (2.20 Å), was selected and preserved in PDB format. PDB is a widely used database for storing and sharing three-dimensional structures of biomolecules. Molecule-enzyme interactions were analyzed using AutoDock Vina 1.5.7 software, and binding energies (kcal mol^{-1}) were calculated. AutoDock Vina is a powerful software used for molecular docking analyses, providing high accuracy in determining binding energies (Biovia, 2021; Trott & Olson, 2010). 2D and 3D visualizations were generated using BIOVIA Discovery Studio Visualizer software. This software is used for detailed visualization of molecular structures and interactions, presenting the analysis results visually (Biovia, 2019).

Statistical Analysis

To determine whether there are differences in the means of genotypes analyzed by the examined variables, a One-Way Analysis of Variance (ANOVA) was applied. To identify significant differences among the groups found to be

significant in the ANOVA results, Dunnett's multiple comparison test was utilized. A minimum significance level of ($p < 0.005$) was accepted. The data were analyzed using SPSS 20 software.

RESULTS and DISCUSSION

The average fruit width ranged from 11.74 mm to 15.75 mm, while the average fruit length ranged from 13.26 mm to 23.18 mm. The fruit weights varied between 1.78 g and 6.30 g⁻¹. The genotype with the largest average fruit width was genotype 7 (15.75 mm), followed closely by genotype 2 (15.72 mm). Genotype 10 had the smallest average fruit width (11.74 mm). Genotype 2 exhibited the greatest average fruit length (23.18 mm), significantly longer than the other genotypes. The shortest average fruit length was observed in genotype 10 (13.26 mm). Genotype 2 also had the highest average fruit weight (6.30 g⁻¹), while genotype 10 had the lowest (1.78 g⁻¹). The pomological characteristics of hawthorn genotypes are summarized in Table 1.

Table 1. Pomological characterization of hawthorn genotypes: measurements of fruit width, length, and weight (mean $\pm$ SD, n = 20)

Çizelge 1. Ahç genotiplerinin pomolojik karakterizasyonu: meyve eni, boyu ve ağırlığı ölçümleri (mean $\pm$ SS, n = 20)

Genotype	Fruit Width (mm)	Fruit Length (mm)	Fruit Weight (g ⁻¹)
1 <i>C. orientalis</i> subsp. <i>orientalis</i>	14.92 $\pm$ 0.5 ^{ab}	17.13 $\pm$ 0.4 ^c	3.50 $\pm$ 0.3 ^c
2 <i>C. orientalis</i> subsp. <i>orientalis</i>	15.72 $\pm$ 0.3 ^a	23.18 $\pm$ 0.9 ^a	6.30 $\pm$ 0.9 ^a
3 <i>C. orientalis</i>	13.58 $\pm$ 0.7 ^c	17.30 $\pm$ 0.5 ^c	3.29 $\pm$ 0.3 ^c
4 <i>C. orientalis</i>	13.56 $\pm$ 0.7 ^c	17.76 $\pm$ 0.5 ^c	3.48 $\pm$ 0.3 ^c
5 <i>C. orientalis</i> subsp. <i>orientalis</i>	14.24 $\pm$ 0.5 ^{bc}	18.66 $\pm$ 0.7 ^c	3.78 $\pm$ 0.3 ^c
6 <i>C. orientalis</i> subsp. <i>orientalis</i>	15.03 $\pm$ 0.3 ^{ab}	18.05 $\pm$ 0.6 ^c	3.84 $\pm$ 0.3 ^c
7 <i>C. orientalis</i> subsp. <i>orientalis</i>	15.75 $\pm$ 0.3 ^a	20.55 $\pm$ 0.8 ^b	5.44 $\pm$ 0.5 ^b
8 <i>C. orientalis</i> subsp. <i>orientalis</i>	15.03 $\pm$ 0.4 ^{ab}	17.61 $\pm$ 0.5 ^c	3.24 $\pm$ 0.3 ^c
9 <i>C. orientalis</i>	14.23 $\pm$ 0.5 ^{bc}	17.29 $\pm$ 0.3 ^c	3.47 $\pm$ 0.3 ^c
10 <i>C. orientalis</i>	11.74 $\pm$ 1.2 ^{cd}	13.26 $\pm$ 1.0 ^d	1.78 $\pm$ 1.1 ^d

In the same column, the difference between the means with different letters is significant at the $p < 0.05$ level ($p=0.047$).

The pH values ranged from 2.63 to 3.12. Genotypes 8 and 9 had the highest pH (3.01 and 3.12, respectively), while genotype 5 had the lowest (2.63). The DDPH values, representing free radical scavenging activities, varied significantly among the genotypes. Genotype 10 showed the highest DDPH value (45.0%), indicating the strongest antioxidant activity. Genotype 3 had the lowest DDPH value (10.3%). The total phenolic content ranged from 17.20 μ g GAE 100g⁻¹ (genotype 6) to 49.70 μ g GAE 100g⁻¹ (genotype 9). The total flavonoid content varied widely, with genotype 7 having the highest content (348 mg CE 100 g⁻¹) and genotype 6 the lowest (63 mg CE 100 g⁻¹) (Table 2).

Table 2. Genotype, total phenolic content Mean $\pm$ SD), DPPH activity (% $\pm$ SD), and total flavonoid content (mg CE 100 g⁻¹ $\pm$ SD)

Çizelge 2. Genotip, toplam fenolik içerik (Mean $\pm$ SS), DPPH Aktivitesi (% $\pm$ SS) ve toplam flavonoid içeriği (mg CE 100 g⁻¹ $\pm$ SD)

Genotype	Ph %	DDPH %	Total Phenolic Content (μ g GAE 100g ⁻¹)	Total Flavonoid Content (mg CE 100 g ⁻¹)
1 <i>C. orientalis</i> subsp. <i>orientalis</i>	2.84 $\pm$ 0.5 ^b	19.1 $\pm$ 1.25 ^h	27.00 $\pm$ 0.2 ^g	203 $\pm$ 1.3 ^e
2 <i>C. orientalis</i> subsp. <i>orientalis</i>	2.88 $\pm$ 0.9 ^b	19.2 $\pm$ 0.5 ^h	30.50 $\pm$ 0.3 ^f	78 $\pm$ 1.1 ^h
3 <i>C. orientalis</i>	2.93 $\pm$ 0.4 ^b	10.3 $\pm$ 1.5 ⁱ	30.20 $\pm$ 0.3 ^f	178 $\pm$ 1.4 ^f
4 <i>C. orientalis</i>	2.69 $\pm$ 0.2 ^b	33.0 $\pm$ 0.7 ^e	37.10 $\pm$ 0.4 ^e	293 $\pm$ 1.6 ^c
5 <i>C. orientalis</i> subsp. <i>orientalis</i>	2.63 $\pm$ 0.2 ^b	28.6 $\pm$ 1.8 ^g	19.10 $\pm$ 0.2 ^h	143 $\pm$ 1.2 ^g
6 <i>C. orientalis</i> subsp. <i>orientalis</i>	3.09 $\pm$ 0.1 ^a	31.9 $\pm$ 0.8 ^f	17.20 $\pm$ 0.1 ⁱ	63 $\pm$ 1.0 ⁱ
7 <i>C. orientalis</i> subsp. <i>orientalis</i>	2.89 $\pm$ 0.5 ^b	35.2 $\pm$ 0.5 ^d	48.80 $\pm$ 0.9 ^b	348 $\pm$ 2.3 ^a
8 <i>C. orientalis</i> subsp. <i>orientalis</i>	3.01 $\pm$ 0.3 ^a	38.4 $\pm$ 0.8 ^c	40.40 $\pm$ 0.5 ^d	333 $\pm$ 2.5 ^b
9 <i>C. orientalis</i>	3.12 $\pm$ 0.3 ^a	41.7 $\pm$ 0.5 ^b	49.70 $\pm$ 1.0 ^a	298 $\pm$ 1.8 ^c
10 <i>C. orientalis</i>	2.83 $\pm$ 0.5 ^b	45.0 $\pm$ 0.7 ^a	45.10 $\pm$ 0.8 ^c	253 $\pm$ 1.3 ^d

In the same column, the difference between the means with different letters is significant at the $p < 0.05$ level ($p=0.038$).

The citric acid content ranged from 86.14 mg 100 g⁻¹ (genotype 2) to 91.05 mg 100 g (genotype 9). The malic acid content was highest in genotype 6 (821 mg 100 g⁻¹) and lowest in genotype 5 (739 mg 100 g⁻¹). Succinic acid levels varied from 287.9 mg 100 g⁻¹ (genotype 5) to 301.7 mg 100 g (genotype 9). Ascorbic acid content ranged from 63.9 mg 100 g⁻¹ (genotype 8) to 70.25 mg 100 g⁻¹ (genotype 9) (Table 3).

Table 3. Comparative analysis of organic acids in different hawthorn genotypes: citric, malic, succinic, and ascorbic acid levels (mean $\pm$ SD, n = 3)

Çizelge 3. Farklı alıç genotiplerinde organik asitlerin karşılaştırmalı analizi: sitrik, malik, suksinik ve askorbik asit düzeyleri (ortalama $\pm$ SS, n = 3)

Genotype	Citric acid (mg 100g ⁻¹)	Malic acid (mg 100g ⁻¹)	Succinic acid (mg 100g ⁻¹)	Ascorbic acid (mg 100g ⁻¹)
1 <i>C. orientalis</i> subsp. <i>orientalis</i>	87.56 $\pm$ 0.4 ^b	786 $\pm$ 1.50 ^c	295.6 $\pm$ 1.6 ^b	66.5 $\pm$ 0.3 ^{bc}
2 <i>C. orientalis</i> subsp. <i>orientalis</i>	86.14 $\pm$ 0.5 ^b	778 $\pm$ 3.5 ^d	293.5 $\pm$ 1.8 ^b	68.7 $\pm$ 0.2 ^{ab}
3 <i>C. orientalis</i>	88.34 $\pm$ 0.3 ^{ab}	782 $\pm$ 2.2 ^c	298.7 $\pm$ 1.6 ^{ab}	69.2 $\pm$ 0.2 ^a
4 <i>C. orientalis</i>	89.62 $\pm$ 0.2 ^a	759 $\pm$ 7.1 ^e	301.4 $\pm$ 1.5 ^a	65.8 $\pm$ 0.5 ^{bc}
5 <i>C. orientalis</i> subsp. <i>orientalis</i>	86.57 $\pm$ 0.5 ^b	739 $\pm$ 5.5 ^f	287.9 $\pm$ 2.0 ^c	69.4 $\pm$ 0.4 ^a
6 <i>C. orientalis</i> subsp. <i>orientalis</i>	87.44 $\pm$ 0.6 ^b	821 $\pm$ 1.5 ^a	295.7 $\pm$ 1.6 ^b	67.7 $\pm$ 0.2 ^b
7 <i>C. orientalis</i> subsp. <i>orientalis</i>	88.21 $\pm$ 0.4 ^{ab}	815 $\pm$ 5.8 ^a	296.8 $\pm$ 1.5 ^{ab}	68.5 $\pm$ 0.4 ^{ab}
8 <i>C. orientalis</i> subsp. <i>orientalis</i>	89.69 $\pm$ 0.3 ^a	767 $\pm$ 4.1 ^e	289.5 $\pm$ 2.0 ^c	63.9 $\pm$ 0.5 ^c
9 <i>C. orientalis</i>	91.05 $\pm$ 0.2 ^a	793 $\pm$ 0.5 ^b	301.7 $\pm$ 0.8 ^a	70.25 $\pm$ 0.9 ^a
10 <i>C. orientalis</i>	89.61 $\pm$ 0.2 ^a	786 $\pm$ 0.9 ^c	296.1 $\pm$ 0.5 ^{ab}	67.5 $\pm$ 1.5 ^b

In the same column, the difference between the means with different letters is significant at the p < 0.05 level (p=0.025).

This study evaluates the binding interactions and energetics of four compounds ascorbic acid, malic acid, citric acid, and succinic acid with a target protein. Molecular docking simulations were performed to determine the binding energy, specific amino acid interactions, and the types of bonds involved (Table 4).

Ascorbic acid demonstrated a binding energy of -6.8 kcal mol. It formed multiple conventional hydrogen bonds with the amino acids ASN385, ASN397, GLN398, GLN395, ASP396, and ASP389, with interaction distances ranging from 1.94 Å to 2.77 Å. Additionally, carbon hydrogen bonds were observed with HIS372 and ARG388, with interaction distances between 1.65 Å and 2.78 Å. Malic acid exhibited a binding energy of -5.1 kcal mol. It primarily formed conventional hydrogen bonds with ASN385, GLN398, ASP389, and GLN387, with distances from 1.86 Å to 2.74 Å, and carbon hydrogen bonds with HIS372 and ASN397, with distances of 2.26 Å and 2.74 Å. (Figure 1). Citric acid had a binding energy of -6.5 kcal mol and formed conventional hydrogen bonds with ASN385, ASN397, ASP389, and GLN387, with distances from 1.86 Å to 2.86 Å, as well as carbon hydrogen bonds with HIS372, ARG388, and ASN397, with distances from 1.83 Å to 2.92 Å. Lastly, succinic acid exhibited a binding energy of -4.9 kcal mol. It formed conventional hydrogen bonds with ASN385, GLN398, GLN387, and ASP389, with interaction distances from 1.91 Å to 2.67 Å, and carbon hydrogen bonds with HIS372 and ASN397, with distances of 2.08 Å to 2.64 Å (Figure 2).

These results indicate that ascorbic acid and citric acid exhibit strong binding affinities with the target protein, as evidenced by their lower binding energies compared to malic acid and succinic acid. The diversity and strength of interactions, particularly the number of conventional hydrogen bonds, contribute significantly to the binding stability of these compounds.

In the study conducted by Sorkun (2012), the average fruit width among the genotypes was 14.28-20.87 mm and the average fruit length was 14.38-17.43 mm (Özgen and Sorgun, 2010). Türkoğlu et al. (2005) in their study in the Van region, the average fruit width of the genotypes was 13.44-14.48 mm and the average fruit length was 12.45-12.89 mm (Türkoğlu et al., 2005); Gerçekcioğlu et al. (2022) fruit length 13.1-17.7 mm, fruit width 14.7-20.6 mm; Okatan et al. (2017) determined the fruit width as 12.53-19.84 mm and the fruit length as 10.48-17.43 mm in hawthorn genotypes grown in Uşak (Oktan et al., 2017). Fruit size may vary depending on the variety, rootstock, periodicity, strength of fruit branches, cultural practices such as pruning, thinning, fertilization and irrigation, and the ecological conditions of the region (Atay et al., 2009). It is thought that the differences between in this findings and the results of the researchers are due to ecological factors as well as genetic factors.

Table 4. Docking scores and report of predicted interactions of docked conformations of compounds against human erythrocyte catalase.

Çizelge 4. Bileşiklerin insan eritrosit katalazına karşı bağlanma skorları ve bağlanmış konformasyonlarının tahmin edilen etkileşim raporu.

Component	Binding Energy (kcal mol)	Amino acid	Interacting	Distance
Ascorbic acid	-6.8	C: ASN385:HD21: O6	Conventional Hydrogen Bond	1.94
		C: ASN397:HN: O2	Conventional Hydrogen Bond	2.52
		C: GLN398:HN: O3	Conventional Hydrogen Bond	2.07
		C: GLN398:HE22: O6	Conventional Hydrogen Bond	2.67
		C: GLN395:OE1:H1	Conventional Hydrogen Bond	2.13
		C: GLN395:OE1:H2	Conventional Hydrogen Bond	2.77
		C: ASP396:OD1:H2	Conventional Hydrogen Bond	2.59
		C: ASP389:O:H6	Conventional Hydrogen Bond	2.30
		C: HIS372:HE1: O4	Carbon Hydrogen Bond	2.59
		C: ARG388: HA: O5	Carbon Hydrogen Bond	2.74
		C: GLN395:OE1:H3	Carbon Hydrogen Bond	1.72
		C: HIS372:NE2:H8	Carbon Hydrogen Bond	1.65
		C: GLN395:OE1:H8	Carbon Hydrogen Bond	2.78
Malic acid	-5.1	C: ASN385:HD21:O1	Conventional Hydrogen Bond	1.86
		C: GLN398:HE22: O1	Conventional Hydrogen Bond	2.26
		C: ASP389:O:H2	Conventional Hydrogen Bond	2.07
		C: GLN387:OE1:H5	Conventional Hydrogen Bond	2.74
		C: HIS372:HE1: O3	Carbon Hydrogen Bond	2.74
		C: ASN397: HA: O3	Carbon Hydrogen Bond	2.26
Citric acid	-6.5	C: ASN385:HD21: O5	Conventional Hydrogen Bond	2.17
		C: ASN397:HN: O3	Conventional Hydrogen Bond	2.81
		C: ASN397:HD21: O7	Conventional Hydrogen Bond	2.04
		C: ASN397:HD22: O3	Conventional Hydrogen Bond	2.29
		C: ASP389:O:H7	Conventional Hydrogen Bond	2.86
		C: GLN387:O:H8	Conventional Hydrogen Bond	1.86
		C: HIS372:HE1: O3	Carbon Hydrogen Bond	2.73
		C: ARG388: HA: O7	Carbon Hydrogen Bond	2.92
		C: ASN397: HA: O3	Carbon Hydrogen Bond	1.83
		C: ASN385:HD21: O4	Conventional Hydrogen Bond	1.91
Succinic acid	-4.9	C: GLN398:HE22: O4	Conventional Hydrogen Bond	2.67
		C: GLN387:OE1:H1	Conventional Hydrogen Bond	2.23
		C: ASP389:O:H6	Conventional Hydrogen Bond	2.56
		C: HIS372:HE1: O2	Carbon Hydrogen Bond	2.64
		C: ASN397: HA: O2	Carbon Hydrogen Bond	2.08

Balta et al. (2015), in their study in Çorum, the average fruit weight of the genotypes they examined was 1.54-4.75 g (Balta et al., 2015); In the study conducted by Karadeniz and Kalkamış (1996) in Edremit and Gevaş districts, the average fruit weight of the genotypes was 0.81-2.14 g⁻¹ (Karadeniz and Kalkamış, 1996); Sorkun (2012), 2.63 g⁻¹ in hawthorns grown in Hakkâri province (Sorkun, 2012.); Gündoğdu et al. (2014) in their study in Erzincan, 0.58-3.48 g (Gundogdu et al., 2014); Taylan (2015) 2.605-3.082 g⁻¹ in hawthorn genotypes grown in Hakkâri (Taylan, 2015); Gürsoy (2016) found 0.38-2.41 g⁻¹ in hawthorn genotypes in Bahçesaray (Van) region (Gürsoy, 2016); Bektas et al. (2017) 0.98-5.91 g⁻¹ in hawthorn genotypes grown in Hekimhan and Akçadağ (Malatya) regions (Bektas et al., 2017); In the study conducted by Koşar (2017) in Malatya, 0.94-4.07 g⁻¹ (Koşar, 2017); Okatan et al. (2017) 0.96-4.03 g⁻¹ in hawthorn genotypes grown in Uşak; Bağran (2018) 1.48-7.67 g⁻¹ in hawthorns examined in the Orta Kelkit valley; Gürlen (2018) found it to be 0.29-4.20 g⁻¹ in hawthorns grown in the Bolu region, and Keles (2018) found it to be 3.24-6.36 g⁻¹ in his study in Yozgat (Keles, 2018; Ağlar et al. (2020) has an average of 0.68-6.35 g⁻¹ in 20 genotypes in Su City (Ağlar et al., 2020) and Dokumacı et al. (2021) have an average of 0.93 g⁻¹ in genotypes in the central Anatolia region (Dokumacı et al., 2021.); Gerçekçioğlu et al. (2022) determined fruit

weights as 3.0-6.2 g⁻¹ in their study in Sivas province (Gerçekcioğlu et al., 2022). Our study is parallel to the previous studies.

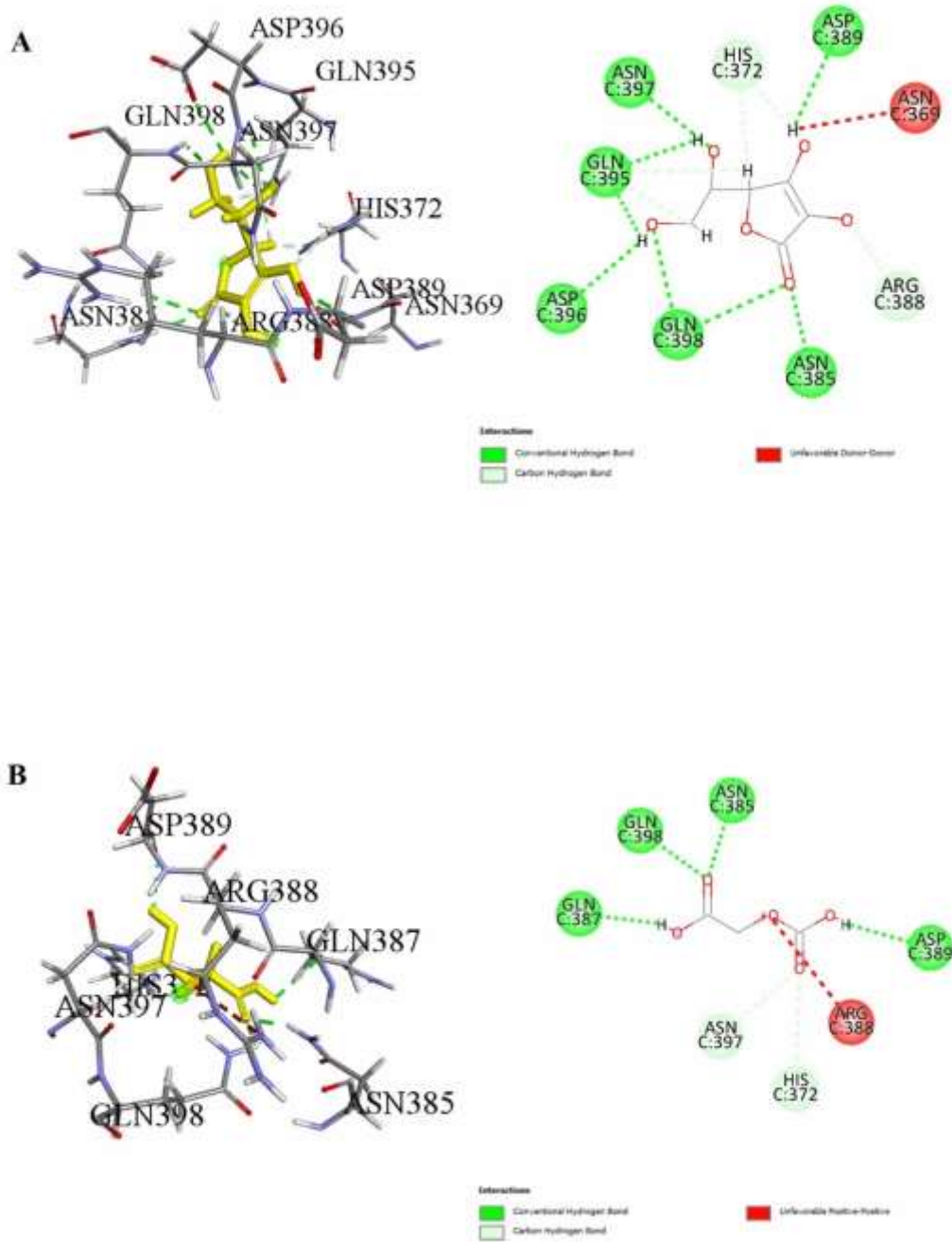


Figure 1. Molecular docking process of A) ascorbic acid and B) malic acid with human erythrocyte catalase
Şekil 1. Askorbik asit (A) ve malik asidin (B) insan eritrosit katalazı ile moleküler bağlanma süreci

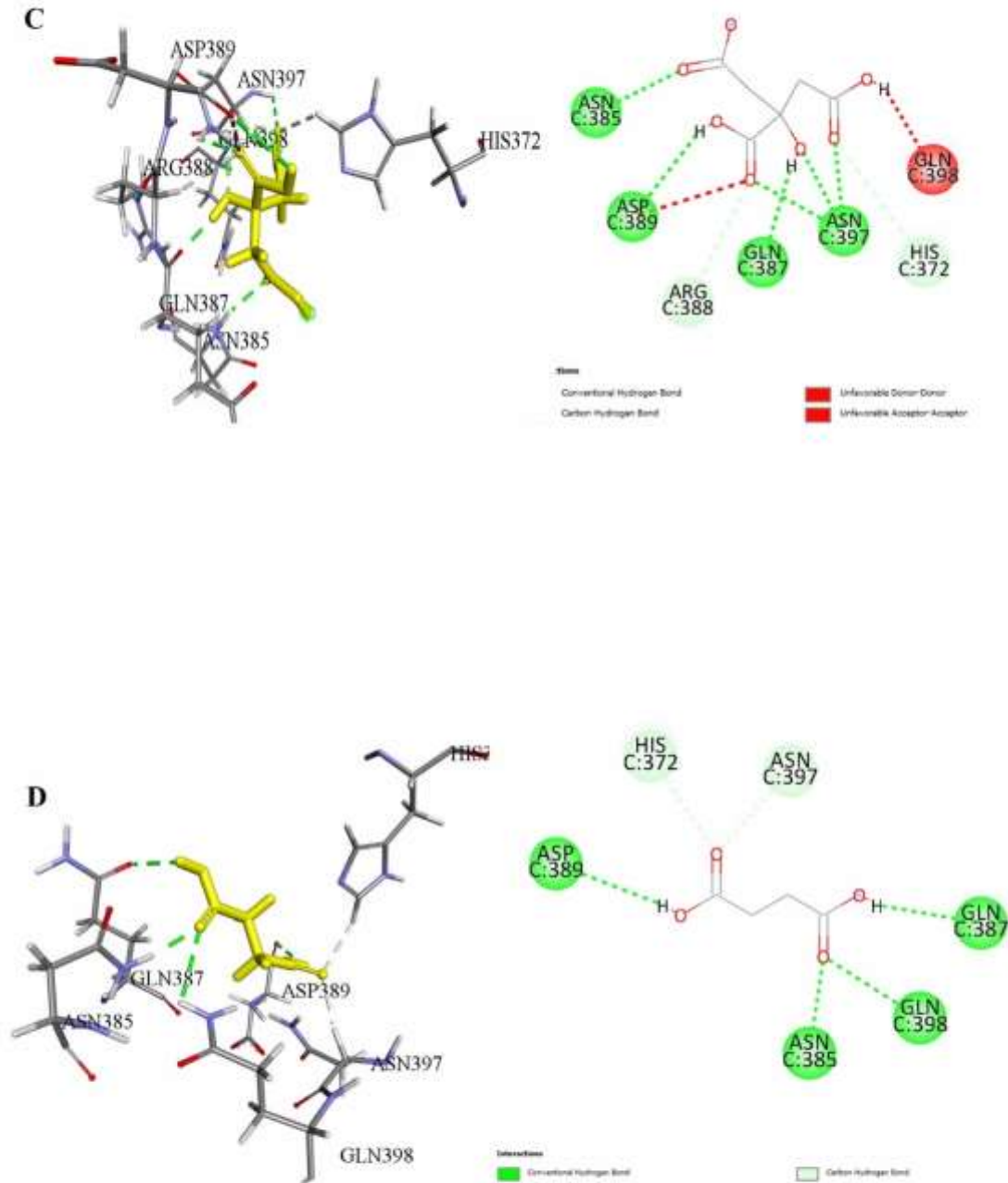


Figure 2. Molecular docking process of C) citric acid and D) succinic acid with human erythrocyte catalase
Şekil 2. Sitrik asit (C) ve suksinik asidin (D) insan eritrosit katalazı ile moleküler bağlanma süreci

Yavic et al. (2016) in their study in Hakkâri province, the pH values of the hawthorn genotypes they investigated were 3.04-4.06 (Yaviç et al., 2016); In their study in Sivas province, Gerçekcioğlu et al. (2022) found pH 3.30-3.85 (Gerçekcioğlu et al., 2022); Ercişli et al. (2015) found the pH value to be 2.88-3.65 in 18 hawthorn genotypes in the Malatya region (Ercişli et al., 2015); Mironeasa et al. (2017) determined the pH value of hawthorns grown in the

Gura Humorului region of Romania between 5.90-6.00 (Mironcica et al., 2016). Caliskan et al. (2012) in their study among hawthorn genotypes, the percentage of DDPH was 21.4-33.2% (Caliskan et al., 2016); Volkan et al. (2017) found the average DPPH amount among the genotypes they examined in Uşak province to be 19.24-59.24% (Volkan et al., 2017). Kostic et al. (2012) in their research on hawthorn in Serbia Kostić et al., (2012), they determined the total phenolic substance rate of the samples to be between 2.12-30.63 $\mu\text{g GAE } 100\text{g}^{-1}$. Bahorun et al. (2003) found the total phenolic substance amount as 47.40 $\mu\text{g GAE } 100\text{g}^{-1}$ in their study on hawthorn (Bahorun et al. 2003). In their study, Iskakova et al. (2023) reported the following findings for *Crataegus songarica*: Vitamin C content was $43.34 \pm 0.30 \text{ mg } 100 \text{ g}^{-1}$, total phenolic content was $669.57 \pm 5.00 \text{ mg GAE } 100 \text{ g}^{-1}$, and DPPH activity had an IC_{50} value of 2.5 ± 0.05 . The values we obtained are like the results found by researchers.

In her research on hawthorn, Nihal Güzel (2021) found the total amount of flavonoids to be between 78.7-272.6 mg CE 100 g^{-1} . This value we found was higher than the value found by the researchers in their study (Güzel, 2021).

It was found between mg 100 g^{-1} . In Italy, in 5 genotypes of the *Crataegus azarolus* species, malic acid in the fruit is between 1.19-2.27% (1190-2270 mg 100 g^{-1}) and citric acid is between 0.19-0.64% (190-640 mg 100 g^{-1}) on a fresh weight basis (Bignami et al., 2004); In a study conducted on 22 varieties of 3 different hawthorn species in China, the most abundant organic acids in all samples were citric, quinic and malic acid, while ascorbic acid was found in trace amounts; On a dry weight basis, citric acid is between 2.0-8.4 $\text{g}^{-1} 100 \text{ g}^{-1}$, quinic acid is between 0.5-5.6 $\text{g}^{-1} 100 \text{ g}^{-1}$, and malic acid is between 0.3-1.1 $\text{g}^{-1} 100 \text{ g}^{-1}$ (Liu et al., 2009); In fruit samples of 11 different hawthorn species growing in Otlukbeli, Kemaliye, Çayırılı and İliç districts of Erzincan, on a fresh weight basis, citric acid was 1.953-23.688 $\text{g}^{-1} 100 \text{ g}^{-1}$ (1953-23688 mg 100 g^{-1}), malic acid was 1.045-2.671 $\text{g}^{-1} 100 \text{ g}^{-1}$ (1045-2671 mg 100 g^{-1}) and 1.080-2.581 $\text{g}^{-1} 100 \text{ g}^{-1}$ (1080-2581 mg 100 g^{-1}) of succinic acid (Gundogdu et al., 2014); In a total of 9 hawthorn varieties, including 5 clones belonging to Korea and 4 Chinese varieties, grown in Korea, citric acid in the fruits is 36.84-157.50 $\text{g}^{-1} 100 \text{ g}^{-1}$, malic acid is 11.81-34.12 $\text{g}^{-1} 100 \text{ g}^{-1}$ and shikimic acid varies between 0.22-0.67 $\text{g}^{-1} 100 \text{ g}^{-1}$ and according to these results, citric, malic and shikimic acids are the most important organic acids in hawthorn fruits (Park and Kim 2018). Citric acid content of fruits of 18 genotypes belonging to 4 hawthorn species growing in nature in Bahçesaray district of Van, on fresh weight basis, is 0.424-4.74 $\text{g}^{-1} 100 \text{ g}^{-1}$ (424-4740 mg 100 g^{-1}), malic acid content is 1.51-4.76 $\text{g}^{-1} 100\text{g}^{-1}$ (1510-4760 mg 100 g^{-1}), succinic acid content between 1.50-10.68 $\text{g}^{-1} 100 \text{ g}^{-1}$ (1500-10680 mg 100 g^{-1}) (Muradoğlu et al., 2019); 'Xintai Tianhong', also known as 'Tianhongzi', which is a wild variant of wild hawthorn in China, contains 2.00 mg g^{-1} (2.00 mg g^{-1}) of acid, the most abundant of which consists of oxalic acid, tartaric acid, malic acid, acetic acid, citric acid, and succinic acid. 200 mg 100 g^{-1} (47.32%), followed by 1.13 mg g^{-1} (113 mg 100 g^{-1}) (26.63%) and citric acid, 0.62 mg g^{-1} (62 mg 100 g^{-1}) (14.76%), with malic acid, 0.21 mg g^{-1} (21 mg 100 g^{-1}) (4.98%) with oxalic acid, 0.20 mg g^{-1} (20 mg 100 g^{-1}) with (4.71%) tartaric acid and 0.07 mg g^{-1} (7 mg) 100 g^{-1} (1.60%), followed by acetic acid (Wei et al., 2019). As can be seen from previous studies, organic acid content in hawthorns varies significantly according to species, genotypes, and ecology; It is observed that ascorbic and citric acids, especially malic and succinic acid, are also found at significant levels. In our study, it was determined that malic, succinic, and citric acids, respectively, are important organic acids.

CONCLUSION

In this study, we have shown that Akdağ Nature Park and its immediate surroundings have a rich potential in terms of hawthorn genotypes. Differences were observed in the examined characteristics of the genotypes in our study, especially their chemical properties, and it is thought that these differences may be due to the genetic structure of the genotype examined, the climate and soil characteristics of the region, the maturity of the fruit, and the altitude and direction where the tree is located. This study we have conducted is important in terms of determining the hawthorn population existing in the region. We think that the hawthorn genotypes we determined because of the study can be used as genetic material with their distinctive characteristics.

Compounds such as ascorbic acid and citric acid found in *Crataegus* spp. with high binding affinity to human erythrocyte catalase may play a significant role in the treatment of diseases associated with oxidative stress. Oxidative stress arises from an imbalance between the production of free radicals and the antioxidant defense system in cells, contributing to the pathogenesis of various diseases including cardiovascular diseases, diabetes, and neurodegenerative disorders. Compounds like ascorbic acid and citric acid possess antioxidant properties, capable of neutralizing free radicals in cells and thereby mitigating the effects of oxidative stress. Thus, the catalase enzyme, which exhibits high binding affinity to these compounds, represents a promising target for the treatment of oxidative stress-related diseases. A deeper understanding of the effects of these compounds on catalase could facilitate the development of more effective and natural-based drugs for these diseases. On the other hand, compounds such as malic acid and succinic acid, showing low binding affinity to human erythrocyte catalase, may not directly contribute to the treatment of diseases associated with oxidative stress. Consequently, their use in therapy may be limited.

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 among them.

REFERENCES

- Aftab, T., & Hakeem, K. R. (Eds.). (2021). *Frontiers in plant–soil interaction: Molecular insights into plant adaptation*. Academic Press.
- Ağlar, E., Sümbül, A., Karakaya, O., & Ozturk, B. (2020). Determination of the quality characteristics of naturally growing Hawthorn in Susehri. *Acta Scientiarum Polonorum. Hortorum Cultus*, 19(1), 61-70. <https://doi.org/10.24326/asphc.2020.1.6>
- Atay, E., Pırlak, L., & Atay, A. N. (2009). Elmalarda meyve büyüklüğünü etkileyen faktörler. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 46(2), 137-144.
- Baker, A., Lin, C. C., Lett, C., Karpinska, B., Wright, M. H., & Foyer, C. H. (2023). Catalase: A critical node in the regulation of cell fate. *Free Radical Biology & Medicine*, 199, 56–66. <https://doi.org/10.1016/J.FREERADBIOMED.2023.02.009>
- Balta, M., Karakaya, O., & Ekici, G. K. (2016). Çorum'da Yetişen alıçların (*Crataegus* Spp.) fiziksel özellikleri. *Ordu Üniversitesi Bilim ve Teknoloji Dergisi*, 5(2), 35-41.
- Bektaş, M., Bükücü, Ş. B., Özcan, A., & Sütyemez, M. (2017). Akçadağ ve Hekimhan ilçelerinde yetişen alıç (*Crataegus* spp.) genotiplerinin bitki ve pomolojik özellikleri. *Türk Tarım ve Doğa Bilimleri Dergisi*, 4(4), 484-490.
- Bevilacqua, A. E., & Califano, A. N. (1989). Determination of organic acids in dairy products by high performance liquid chromatography. *Journal of Food Science*, 54(4), 1076–1076. <https://doi.org/10.1111/J.1365-2621.1989.TB07948.X>
- Bignami, C., Paolocci, M., Scossa, A., & Bertazza, G. (2004). Preliminary evaluation of nutritional and medicinal components of *Crataegus azarolus* fruits. *Acta Horticulturae*, 597, 95–100. <https://doi.org/10.17660/ACTAHORTIC.2003.597.11>
- Biovia, D. S. (2019) Discovery studio visualizer. v16, 1, 15350.
- Bukowska, B., Chajdys, A., Duda, W., & Duchnowicz, P. (2000). Catalase activity in human erythrocytes: effect of phenoxyherbicides and their metabolites. *Cell Biology International*, 24(10), 705–711. <https://doi.org/10.1006/CBIR.2000.0553>
- Caliskan, O., Bayazit, S., & Gunduz, K. (2016). Hawthorn species from Turkey and potential usage for horticulture 17, 330-336
- Caliskan, O., Bayazit, S., & Gunduz, K. (2016). Hawthorn species from Turkey and potential usage for horticulture. VII International Scientific Agriculture Symposium, "Agrosym 2016", 6-9 October 2016, Jahorina, Bosnia and Herzegovina. *Proceedings*, 17, 330-336.
- Çaliskan, O., Gündüz, K., Serçe, S., Toplu, C., Kamiloglu, Ö., Sengül, M., & Ercisli, S. (2012). Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey. *Pharmacognosy Magazine*, 8(29), 16. <https://doi.org/10.4103/0973-1296.93305>
- Davis, P. H. (1970). *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, 3.
- Di Marzo, N., Chisci, E., & Giovannoni, R. (2018). The Role of hydrogen peroxide in redox dependent signaling: homeostatic and pathological responses in mammalian cells. *Cells*, 7(10). <https://doi.org/10.3390/CELLS7100156>
- Dokumacı, K., Uslu, N., Haciseferoğulları, H., & Örnek, M. N. (2021). Determination of some physical and chemical properties of common hawthorn (*Crataegus Monogyna* Jacq. Var. *Monogyna*). *Erwerbs-Obstbau*, 63(1), 99-106. <https://doi.org/10.1007/s10341-021-00545-x>
- Dorman, H. J. D., Koşar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from mentha species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry*, 51(16), 4563–4569. <https://doi.org/10.1021/JF034108K>
- El Hamzaoui, A., Oukabli, A., & Moumni, M. (2014). Morphological and molecular diversity and genetic structure of Moroccan cultivated almond (*Prunus dulcis* Mill.) beside some foreign varieties. *Plant Genetic Resources*, 12(3), 308–316. <https://doi.org/10.1017/S1479262114000094>
- El-Demerdash, F. M., Tousson, E. M., Kurzepa, J., & Habib, S. L. (2018). Xenobiotics, oxidative stress, and antioxidants. *Oxidative Medicine and Cellular Longevity*, 2018, 9758951. <https://doi.org/10.1155/2018/9758951>
- Ercisli, S., Yanar, M., Sengul, M., Yildiz, H., Topdas, E. F., Taskin, T., & Yilmaz, K. U. (2015). Physico-chemical and biological activity of hawthorn (*Crataegus* spp. L.) fruits in Turkey. *Acta scientiarum Polonorum. Hortorum cultus*, 14(1), 83-93.

- Gerçekcioğlu, R., Türkoğlu, E., & Aydemir, M. (2022). Sivas İli Yıldızeli ve Şarkışla Yörelerinde doğal olarak yetişen alıç (*Crataegus* spp.) genotiplerinin seleksiyonu. *Meyve Bilimi*, 9(2), 61-67.
- Grilo, L. F., Martins, J. D., Cavallaro, C. H., Nathanielsz, P. W., Oliveira, P. J., & Pereira, S. P. (2020). Development of a 96-well based assay for kinetic determination of catalase enzymatic-activity in biological samples. *Toxicology in Vitro*, 69, 104-996.
- Gundogdu, M., Ozrenk, K., Ercisli, S., Kan, T., Kodad, O., & Hegedus, A. (2014). Organic acids, sugars, vitamin C content and some pomological characteristics of eleven hawthorn species (*Crataegus* spp.) from Turkey. *Biological Research*, 47(1), 1-5. <https://doi.org/10.1186/0717-6287-47-21> TABLES 3
- Gürsoy, S. (2016). *Bahçesaray yöresi alıç (Crataegus) türlerinin pomolojik ve biokimyasal özelliklerinin belirlenmesi* (Tez No:526614). [Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Güzel, N. (2021). Alıç: Biyoaktif bileşenlerin termosonikasyon ile ekstraksiyonu ve fiziko-kimyasal özelliklerinin değerlendirilmesi. *Gıda*, 46(4), 939-948.
- Hadwan, M. H., Hussein, M. J., Mohammed, R. M., Hadwan, A. M., Al-Kawaz, H. S., Al-Obaidy, S. S. M., & Al Talebi, Z. A. (2024). An improved method for measuring catalase activity in biological samples. *Biology Methods and Protocols*, 9(1), bpae025. <https://doi.org/10.1093/BIOMETHODS/BPAE015>
- Iskakova, J., Oskonbaeva, Z., & Smanalieva, J. (2023). Evaluation of pomological characteristics and bioactive compounds of Wild Sea Buckthorn (*Hippophae Rhamnoides* L.) and hawthorn (*Crataegus songarica*) from Walnut-Fruit Forest Kyzyl-Unkur, Kyrgyzstan. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 26(5), 1146-1155.
- Karadeniz, T., & Kalkışım, Ö. (1996). Edremit ve Gevaş ilçelerinde yetişen alıç (*Crataegus Azarolus* L.) tiplerinin meyve özellikleri ve ümitvar tiplerin seçimi. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 6(1), 27-33.
- Kayacık H., Orman ve Park Ağaçlarının Özel Sistematiği, Cilt II, 4. Baskı, İ.Ü. Orman Fakültesi Yayın No: 2766, Bozak Matbaası, İstanbul, 1981.7.
- Keles, H (2018). *Yozgat ili ve ilçelerinde bulunan alıç (Crataegus spp.) genetik kaynaklarının seleksiyonu morfolojik, biyokimyasal ve moleküler karakterizasyonu* (Tez No:514371)[Doktora Tezi Atatürk Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı Meyve Yetiştirme ve Islahı Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Koşar, B. (2017). *Akçadağ (Malatya) İlçesinde yetişen alıç genotiplerinin (Crataegus spp.) karakterizasyonu* (Tez No: 495000) [Yüksek Lisans Tezi, Ordu Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Kostić, D. A., Velicković, J. M., Mitić, S. S., Mitic, M. N., & Randelović, S. S. (2012). Phenolic content, and antioxidant and antimicrobial activities of *Crataegus Oxyacantha* L (Rosaceae) fruit extract from Southeast Serbia. *Tropical Journal of Pharmaceutical Research*, 11(1), 117-124. <https://doi.org/10.4314/TJPR.V11I1.15>
- Kurnaz, O. C., Ozturk, A., Faizi, Z. A., Ates, U., & Ozturk, B. (2024). Pomological, bioactive compounds, and antioxidant activity of selected superior genotypes from a highly diversified loquat population. *Genetic Resources and Crop Evolution*, 71(4), 1507-1520. <https://doi.org/10.1007/S10722-023-01711-8> FIGURES 4
- Liu, P., Kallio, H., Lü, D., Zhou, C., Ou, S., & Yang, B. (2009). Acids, sugars, and sugar alcohols in Chinese Hawthorn (*Crataegus* spp.) Fruits. *Journal of Agricultural and Food Chemistry*, 58(2), 1012-1019. <https://doi.org/10.1021/JF902773V>
- Ljubuncic, P., Portnaya, I., Cogan, U., Azaizeh, H., & Bomzon, A. (2005). Antioxidant activity of *Crataegus aronia* aqueous extract used in traditional Arab medicine in Israel. *Journal of Ethnopharmacology*, 101(1-3), 153-161. <https://doi.org/10.1016/J.JEP.2005.04.024>
- Miller, A. J., & Schaal, B. A. (2006). Domestication and the distribution of genetic variation in wild and cultivated populations of the Mesoamerican fruit tree *Spondias purpurea* L. (Anacardiaceae). *Molecular Ecology*, 15(6), 1467-1480. <https://doi.org/10.1111/J.1365-294X.2006.02834.X>
- Mironeasa, S., Todosi, E. S., & Mădălina, I. U. G. A. (2017). Physico-chemical characteristics, antioxidant activity and mineral content of hawthorn fruits from Suceava County. *Food and Environment Safety Journal*, 15(2), 108-116.
- Oktan, V., Gündoğdu, M., & Çoalk, A. M. (2017). Uşak'ta yetişen farklı alıç (*Crataegus* spp.) genotipi meyvelerinin bazı kimyasal ve pomolojik karakterlerinin belirlenmesi. *Journal of the Institute of Science and Technology*, 7(3), 39-44.
- Özcan, M., Haciseferoğulları, H., Marakoğlu, T., & Arslan, D. (2005). Hawthorn (*Crataegus* spp.) fruit: Some physical and chemical properties. *Journal of Food Engineering*, 69(4), 409-413. <https://doi.org/10.1016/J.JFOODENG.2004.08.032>

- Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative Stress: A key modulator in neurodegenerative diseases. *Molecules (Basel, Switzerland)*, 24(8), 1583. <https://doi.org/10.3390/MOLECULES24081583>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. <https://doi.org/10.5344/AJEV.1965.16.3.144>
- Sorkun, E. (2012). *Farklı renkteki alıç meyvelerinin pomolojik ve fitokimyasal özelliklerinin belirlenmesi*. (Tez No: 322719) [Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Taylan, A. (2015). *Hakkâri ili Şemdinli yöresi üstün nitelikli alıç (Crataegus spp.) genotiplerinin belirlenmesi*. (Tez No: 450398) [Yüksek Lisans Tezi, Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Tomar, O., Çağlar, A., Akarca, G., & Vatansever, H. (2020). Physicochemical and sensory quality properties of yellow hawthorn fruit (*Crataegus tanacetifolia*) vinegar produced by traditional fermentation method. *Avrupa Bilim ve Teknoloji Dergisi*, (19), 176-181. <https://doi.org/10.31590/ejosat.715699>
- Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461. <https://doi.org/10.1002/JCC.21334>
- Türkoğlu, N., Kazankaya, A., & Şensoy, R. İ. (2005). Pomological characteristics of hawthorns species found in Van region. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 15(1), 17-21.
- Wei, S., & Wang, S. (2019). High performance liquid chromatography analysis of sugars and acid components in 'Xintai Tianhong' Hawthorn Fruit. *Agricultural Biotechnology*, 8(4), 163-164.
- Yaviç, A., Taylan, A., Balcı, H., & Encu, T. (2016). Biochemical and pomological characteristics of hawthorn (*Crataegus* spp.) fruits grown in Şemdinli, Hakkâri ili Şemdinli Yöresi alıç (*Crataegus* spp.) meyvelerinin biyokimyasal ve pomolojik özellikleri. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 26(4), 500-504.
- Zhao, M. X., Wen, J. L., Wang, L., Wang, X. P., & Chen, T. S. (2019). Intracellular catalase activity instead of glutathione level dominates the resistance of cells to reactive oxygen species. *Cell Stress & Chaperones*, 24(3), 609–619. <https://doi.org/10.1007/S12192-019-00993-1>
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

How do AMF and Biochar Affect Pepper Growth and Nutrient Content under Biotic and Abiotic Stress?

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ABSTRACT

Salt stress is a significant abiotic stress that adversely affects pepper plant growth which can accelerate the development of plant pathogens and increase plant susceptibility to diseases. *Verticillium dahliae*, which causes pepper wilt disease, is an important biotic stress factor. *Funneliformis mosseae* and biochar organic wastes help to take nutrients from the soil by establishing symbiotic connections with plant roots and, are effective in treating plant diseases, plant growth, and stress tolerance. This study aims to determine the effects of *F. mosseae* (Fm) and 2% biochar (Bc) against *V. dahliae* (Vd) on some plant physiological properties, plant nutrient uptake, soil pH, and EC value in pepper plants grown under salt stress (50mM, 100mM, 150mM). As a result of the study, the use of *F. mosseae* alone or in interaction with 2% biochar significantly increased some physiological parameters and some minerals (P, K, Mg, and Mn) contents of the plant. Moreover, pepper plants showed remarkable resistance to salt and stress factors caused by *V. dahliae*. In addition, the interaction between *F. mosseae* and biochar significantly lowered the soil EC value under conditions of severe salt stress. On the other hand, biochar was more effective than *F. mosseae* in terms of soil pH and Ca/Na ratio. The results showed that biochar and *F. mosseae* were beneficial in reducing biotic (*V. dahliae*) and abiotic stress (salt stress) damage while enhancing plant growth and nutrient absorption. Therefore, this study yields excellent and novel results, particularly in the field of employing beneficial microorganisms for sustainable agriculture.

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AMF ve Biyoçar Biyotik ve Abiyotik Stres Altında Biber Gelişimini ve Besin İçeriğini Nasıl Etkiler?

ÖZET

Tuz stresi, biber bitkisinin büyümesini olumsuz etkileyen, bitki patojenlerinin gelişimini hızlandırabilen ve bitkinin hastalıklara karşı duyarlılığını artırabilen önemli bir abiyotik strestir. Biber solgunluğu hastalığına neden olan *Verticillium dahliae* önemli bir biyotik stres faktörüdür. *Funneliformis mosseae* ve biyoçar organik atıkları, bitki kökleriyle simbiyotik bağlantılar kurarak topraktan besin maddesi alınmasına yardımcı olur ve bitki hastalıklarının kontrolünde, bitki büyümesinde ve stres toleransında etkilidir. Bu çalışma, tuz stresi (50mM, 100mM, 150mM) altında yetiştirilen biber bitkisinde *V. dahliae* (Vd)'ye karşı *F. mosseae* (Fm) ve %2 biyoçarın (Bc) bazı bitki fizyolojik özellikleri, bitki besin elementi alımı, toprak pH'sı ve EC değeri üzerindeki etkilerini belirlemeyi amaçlamaktadır. Çalışma sonucunda, *F. mosseae*'nin tek başına veya %2 biyoçar ile etkileşimli olarak kullanılması, bitkinin bazı fizyolojik parametrelerini ve bazı mineral (P, K, Mg ve Mn) içeriklerini önemli ölçüde artırmıştır. Ayrıca, biber bitkileri tuza ve *V. dahliae*'nin neden olduğu stres faktörlerine karşı kayda değer bir dayanıklılık göstermiştir. Buna ek olarak, *F. mosseae* ve biyoçar arasındaki etkileşim, şiddetli tuz stresi koşulları altında toprak EC değerini önemli ölçüde düşürmüştür. Öte yandan, biyoçar, toprak pH'sı

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Anahtar Kelimeler

Funneliformis mosseae

Biyoçar

Biber

Tuz stresi

Verticillium dahliae

ve Ca/Na oranı açısından *F. mosseae*'den daha etkili olmuştur. Sonuçlar, biyoçar ve *F. mosseae*'nin biyotik (*V. dahliae*) ve abiyotik stres (tuz stresi) hasarını azaltmada faydalı olduğunu ve bitki büyümesini ve besin emilimini artırdığını göstermiştir. Dolayısıyla bu çalışma, özellikle sürdürülebilir tarım için faydalı mikroorganizmaların kullanılması alanında mükemmel ve yeni sonuçlar ortaya koymaktadır.

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Graphical Abstract



INTRODUCTION

Pepper (*Capsicum annuum* L.) is an annual herbaceous plant native to South America and a member of the Solanaceae family (Guevara et al. 2021). According to Coskun et al. (2023), pepper ranks third among fruits in terms of production with a global production of 2.5 million tons, and between 6 to 9% of this is produced in Türkiye (Food and Agriculture Organization of the United Nations 2020). Pepper is a very important source of income for growers. On the other hand, biotic and abiotic stress factors that diminish pepper production are a significant issue (Geleta & Labuschagne, 2006). Plant diseases associated with fungi are responsible for 14% of the yield loss in world vegetable production (Tripathi et al., 2024). In areas where peppers are grown, fungi such as *Alternaria* spp., *Fusarium* spp., *Phytophthora capsici*, *Pythium* spp., *Botrytis cinerea* and *Verticillium dahliae* cause major diseases like fruit and root rot and wilt (Nguyen et al. 2010; Coşkun et al. 2021). Of these diseases, *V. dahliae* is one of the most damaging pathogens that cause vascular wilt (Güneş et al. 2024).

Soil-borne fungi *Verticillium dahliae* infection causes symptoms such as discoloration of root and stem structures (dark brown, black in places), stunting, yellowing, and wilting of leaves in pepper plants (Schnathorst, 1981; Tyvaert et al. 2019). It blocks the vascular tissue of the plant, prevents the transport of water and dissolved minerals from the root, and leads to plant death (Tjamos et al. 2000; Pegg & Brady 2002).

The growth and survival of *V. dahliae* may be adversely affected by the presence of salt in the soil (Geleta & Labuschagne, 2006). According to Kacjan et al. (2021), up to a certain point, pepper is salt-sensitive, and pepper plants have a salinity threshold of 1.5 dS m⁻¹, making them moderately salt-sensitive plants. Even at low salt concentrations, a salt-sensitive pepper plant cannot attain the osmotic pressure value created in the solution by the roots (Kotuby-Amacher et al. 2000). Additionally, salt stress alters the ion and water balance in pepper plant cells, causing dryness and adverse effects on plant growth (Etesami & Beattie, 2018).

Therefore, environmentally friendly strategies that promote plant growth, protect natural resources, and aim to reduce waste and environmental impacts should be used to combat such abiotic and biotic stress factors in pepper (Khriebe et al. 2019). Recently, there has been interest in the effects of arbuscular mycorrhizal fungi (AMF) and biochar, which are incorporated into sustainable agricultural practices, against biotic and abiotic stress factors (Elmer et al. 2011; Gunes et al. 2023).

In nutrient-poor conditions such as dry regions, soils containing heavy metals, and salty soils, AMF functions in symbiosis with plant roots. Furthermore, in the case of soil-borne pathogens such as *V. dahliae*, the host plant can be effectively defended by AMF. It can even increase the tolerance or resistance of the plant root at various levels under different conditions (Akköprü & Demir, 2005; Güneş et al. 2022). The combination of AMF + organic waste supports the use of environmentally friendly, economically viable, and sustainable technologies (Demirel et al. 2024). Recent studies have demonstrated the potential of biochar organic waste to control plant pathogens and abiotic stress conditions by altering signaling mechanisms between plants and symbiotic microorganisms (Kolton et al. 2011; Ogundeji et al. 2021).

Providing a safe habitat for protecting AMF against pests, biochar has a direct effect on nutrient uptake and an indirect effect on plant growth (Zhuo et al. 2020). Biochar, which is pyrolysis in an oxygen-free environment, is rich in carbon and possesses an abundant amount of plant nutrients (Ippolito et al., 2012). In addition to inhibiting the development of many plant pathogens, biochar increases water retention capacity and adsorption ability and provides effectiveness against biotic and abiotic stress factors (Graber et al., 2014; Palansooriya et al., 2020). By activating defense systems in response to biotic and abiotic stress signals, AMF+ biochar develops a local and systemic antimicrobial defense (Lamb & Dixon 1997; Low & Merida, 1996). However, studies on the combined effects of pepper grown under the effects of salt and *V. dahliae* stress factors with AMF and biochar have not been sufficiently comprehensive.

Environmentally friendly practices such as AMF and biochar are important in plant-pathogen-environment interaction. The efficacy of single or combined applications of biochar and arbuscular mycorrhizal fungi (AMF) on various parameters of pepper plants against biotic (*V. dahliae*) and abiotic stress (salt stress) factors were evaluated for the first time in this study. Therefore, in this study, the effects of combined treatments on some plant growth parameters, macro and micronutrient contents, soil pH, and EC of pepper under different salt concentrations and *V. dahliae* stress conditions were determined.

The main hypothesis of this study is that the addition of AMF and biochar will have a synergistic effect on the development of pepper plants, as well as on leaf mineral matter content, soil pH, and EC. In particular, this would increase the tolerance of plants to conditions caused by biotic (*V. dahliae*) and abiotic (salt) stress. A controlled experiment was set up to investigate this theory.

MATERIAL and METHOD

Materials

In this study, the commercial cultivar Sera Demre 8 (İklim Agricultural Products INC., Mersin, Türkiye) was utilized. Virulence (80%) *V. dahliae* isolates (Coşkun et al., 2023) and the highly active *Funneliformis mosseae* FMYU1 (Fm) AMF inoculum previously isolated (Gunes et al., 2023) from pepper was taken from the culture stock of Van YÜ Faculty of Agriculture, Department of Plant Protection (Van/Türkiye). *Medicago sativa* L., the host of AMF, was used to obtain *F. mosseae* inoculum samples, which were then kept at +4 °C. The biochar organic material (Bc) was obtained from Single Carbon Barbecue Coal Production Inc. in a 100% natural form as oak powder at 450 degrees Celsius (Medium pyrolysis type; medium heating rate.). Based on earlier pepper plants were subjected to salt stress at various NaCl (Merck, Germany) concentrations (0 mM, 50 mM, 100 mM, and 150 mM) research (Akay Rastgeldi, 2010). The place of the collection is Gevaş in Van province (Van/Turkey, 38°30'2998 "N 43°113'178"E). The soil physico-chemical properties were as follows: pH 7.19, EC 64.6, lime 2.26, organic matter content 0.87/0.044, sand 67.2, clay 17.6, and silt 15.2. The soil is classified as sandy loam by the USDA. pH 8.10, EC 3.42 (dS m⁻¹), moisture 2.48 (%), organic matter content 26.61 (%), organic carbon 15.43 (%), and carbon/nitrogen 23.74 (%) are some of the parameters of biochar.

Design of experiment

Pepper seeds were first sown for seedling growth in plastic vials (4.7 x 6.0 cm) containing a 2:1 ratio of peat and perlite. Four weeks later, the pepper seedlings were moved into plastic pots weighing three kilograms. After one hour of autoclaving at 121 °C, the soil was rendered sterile. Considering the results of previous studies (Gunes et al., 2023), the prepared soil was mixed with the best biochar rate of 2% (24 g biochar was mixed for 3 kg pots) before placing the peppers in biochar pots. In the treatments without biochar, only soil was used. For the AMF application, the application method of Schüßler and Walker, (2010) was followed. For *F. mosseae* (150 spores per

1 g soil) treatments, 10 g was deposited in each seedbed, and seedlings were transplanted. The sand was used for all treatments except AMF.

Furthermore, to test for pathogenic applications, the *V. dahliae* isolate was cultured for seven days at 25°C on Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany). Roots of pepper seedlings were immersed in 1×10^6 conidia ml⁻¹ *V. dahliae* spore suspension for 5 minutes, while control plants were only immersed in water (Coşkun et al., 2023). Pepper seedlings were exposed to salt stress at the three-five-leaf stage and five days after planting. Salt treatments were done at doses of 0, 50, 100, and 150. To maintain salt stress levels, a salt solution of 25 mM was prepared for each pot (73.05 g of salt was mixed into 1250 liters of water, and the solution was ready for application). Thus, to avoid osmotic shock in the root zone, 25 mM NaCl (25 ml water) was added to the growth medium every two days until the targeted concentrations were reached (2 times 25 mM for 50 mM, four times for 100 mM, and six times for 150 mM) and the highest salt concentration application was completed on day 12. The control group received the same volume of unsalted water. The research was carried out in a climate chamber at Van Yüzüncü Yıl University Faculty of Agriculture. According to a completely randomized experimental design with six replications, 32 application groups of plants were cultivated in a growth chamber under controlled conditions at 60-70% RH, 22 ± 2 °C, and 16 light/8 dark photoperiods. The implementation design of the study is given in Table 1 below.

Table 1. The study's implementation design

Çizelge 1. Çalışmanın uygulama tasarımı

Treatments
Control
Fm
Bc
50 mM
100 mM
150 mM
Vd
Fm+ Bc
Fm +50 mM
Fm + 100 mM
Fm +150 mM
Bc + 50 mM
Bc + 100 mM
Bc + 150 mM
Fm + Vd
Bc+ Vd
Vd+50 mM
Vd+100 mM
Vd+150 mM
Fm +Bc+50 mM
Fm +Bc+100 mM
Fm +Bc+150 mM
Fm +Bc+Vd
Fm +50 mM+Vd
Fm +100 mM+Vd
Fm +150 mM+Vd
Bc + 50 mM+ Vd
Bc + 100 mM+ Vd
Bc + 150 mM+ Vd
Fm +Bc+50 mM+ Vd
Fm +Bc+100 mM+ Vd
Fm +Bc+150 mM+ Vd

Bc: Biochar Fm: *F. mosseae* Vd: *V. dahliae*

A total of 576 plants were used for the study, with six replications in each treatment group and three plants in each replicate. The experiment was terminated at week 8 because the first symptoms appeared 7 weeks after sowing, and pathogen symptoms started to appear 1 week after the end of salt stress.

Assessment of the Parameters

Before harvesting, leaf number (young and middle), shoot diameter (at the root collar of the plant) parameters (digital caliper Insize, China), leaf area (LICOR, Model: LI-3100, Lincoln, NE, USA), and chlorophyll (taken systematically from the 4th true leaf of each plant) were determined (SPAD 502 Plus), followed by soil pH, soil EC, macro and micronutrients. Tap water was used as irrigation water.

For K, Ca, Mg, Fe, Cu, Zn, Mn, and Na content measurement, dry samples (0.5 g) collected from plant leaves (middle) were burned (Kacar 1984). Using the vanadomolybo-phosphoric yellow technique, the total phosphorus content was determined. To do this, 0.5 g of dried leaf and 1 mL of ethanol were combined (Merck 818,760, Germany). Following the addition of 4 mL of hydrochloric acid, the samples were incubated at 90 °C for 15 minutes (Merck 160 1.05590.2500, Germany). The extracts were filtered before being measured at 430 nm using a spectrophotometer (Jenway 6505 161 UV/vis, UK).

The pH of the soil samples was measured using a 1:2.5 soil-water mixture (Jackson 1958). Soil salinity (EC) was measured using conductivity equipment (U.S. Salinity Laboratory Staff 1954).

Data Analysis

The differences between AMF, biochar, salt concentrations, and *V. dahliae* were assessed using Duncan's multiple comparison test in SAS 9.4 (SAS Institute Inc., Cary, NC, USA 2012). Correlations between the studied traits were determined via Pearson's pairwise correlations using the PAST3 program. The Principal Component Analysis (PCA) was used in conjunction with the XLSTAT statistical program to highlight any similarities or differences that emerged from the applications and to determine how much of these differences could be accounted for by the features taken into consideration in the study. In addition, heatmap clustering (ClustVis) was used to cluster the dependent variable parameters that correspond to the treatments (independent variables).

RESULTS and DISCUSSION

RESULTS

Effects of *F. mosseae* and biochar on plant growth parameters under salt and *V. dahliae* stresses

The effects of *F. mosseae* (Fm) and biochar (Bc) treatments on leaf number (number), shoot diameter (caliper), leaf area (tool), and chlorophyll (Spad) parameters in plants infected with *V. dahliae* (Vd) and grown under salt stress are shown in Table 2. It was determined that there was a statistically significant difference ($p < 0.05$) between the treatments ($p < 0.05$). The highest values of leaf number, shoot diameter, leaf area, and chlorophyll parameters were found in Fm+Bc and Fm treatments (36.83 plants⁻¹, 4.20 mm, and 413.65 cm², respectively), i.e. in treatments without salt and disease stress (Table 2). Vd and conditions with 150 mM salt content were observed as the lowest values. However, despite both stress conditions, it was determined that the value of biochar was higher than AMF, especially in leaf number and shoot diameter, and the opposite situation was observed in leaf area and chlorophyll values. As the salt concentration increased in all Bc, Fm, and Vd applications, a decrease was observed in terms of the number of leaves, shoot diameter, leaf area, and chlorophyll values (Table 2).

Effects of *F. mosseae* and biochar on macro and micronutrient content under salt and *V. dahliae* stresses

The effects of AMF and biochar applications on K, P, Ca, and Mg content in pepper shoots grown under the presence of salt and *V. dahliae* are given in Table 3. As the salt concentration increases, P, Ca, and Mg values change according to the applications, while potassium values decrease. The highest values were found for P in Fm (3264.38 ppm), K in Fm + Bc (768.36 ppm), Ca in Fm + 100 mM (436.14 ppm), and Mg in Fm + 50 mM + Vd (211.07 ppm), respectively.

Therefore, the P, Ca, and Mg values of Fm and Bc alone increased in comparison to the control, and the difference between them was statistically significant ($p < 0.05$), especially in P and K mineral substances. When Fm and Bc were compared only in terms of salt concentrations, Fm + 100 mM had the highest value of all mineral substances, while in Bc, the content of all nutrients except P decreased as the salt dose increased (Table 3). In Fm + Bc + Vd treatment, only P, K, and Mg values were found to be statistically insignificant ($p > 0.05$), while the Ca value was significant ($p < 0.05$). Under high-stress conditions (with salt and Vd interaction), the different treatments of Fm and Bc, Fm + Bc + 100 mM + Vd, were highest for Phosphorus (P), while the combination Fm + Bc + 150 mM + Vd was highest for other nutrients (K, Ca, and Mg) (Table 3).

Table 2. The effects of AMF, biochar, and *V. dahliae* on the number of leaves (pieces/plant), shoot diameter (mm), leaf area (cm²) and chlorophyll in pepper grown under salt stress (mean±standard deviation)

Çizelge 2. AMF, biyokömür ve *V. dahliae*'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve *V. dahliae*'nin yaprak sayısı (adet/bitki), sürgün çapı (mm), yaprak alanı (cm²) ve klorofil üzerine etkileri (ortalama±standart sapma)

Treatments	Number of Leaves (number plant ⁻¹)	Shoot Diameter (mm)	Leaf Area (cm ²)	Chlorophyll (SPAD)
Control	22.33±0.42 e-h	2.87±0.10 b-d	221.63±7.20 d	32.16±1.56 d-j
Fm	33.83±1.07 ab	3.98±0.07 a	367.31±6.47 b	38.54±1.52 a-e
Bc	30.83±0.79 bc	3.12±0.11 bc	287.16±9.83 c	37.28±0.54 a-g
50 mM	26.33±1.99 de	2.52±0.19 d-g	71.11±8.55 ı-k	43.73±1.75 a
100 mM	20.66±2.10 g-k	2.10±0.25 g-ı	67.12±9.51 ı-k	27.85±3.96 h-j
150 mM	15.16±1.66 mn	1.60±0.25 j-l	43.20±7.97 k-m	30.03±2.82 g-j
Vd	20.33±0.84 g-l	2.44±0.08 d-h	104.04±6.87 gh	41.89±1.70 ab
Fm+ Bc	36.83±0.60 a	4.20±0.21 a	413.65±14.84 a	36.13±0.83 a-g
Fm +50 mM	22.83±1.81 e-g	2.44±0.15 d-h	122.44±10.82 fg	34.17±3.33 b-ı
Fm + 100 mM	19.00±1.71 g-m	2.09±0.18 g-ı	69.63±7.92 ı-k	25.63±2.74 j
Fm +150 mM	16.66±1.20 j-n	2.12±0.20 g-ı	68.51±3.08 ı-k	30.23±3.62 f-j
Bc + 50 mM	21.50±1.70 f-i	2.56±0.11 d-g	91.17±6.07 hı	36.35±2.24 a-g
Bc + 100 mM	16.00±1.41 l-n	2.12±0.08 g-ı	58.90±6.63 j-l	28.15±2.39 h-j
Bc + 150 mM	17.33±2.76 i-m	2.35±0.11 e-h	56.86±7.18 j-l	26.59±3.04 ıj
Fm + Vd	27.66±0.71 cd	3.25±0.15 b	221.47±16.64 d	39.77±0.79 a-d
Bc+ Vd	20.00±0.57 g-l	2.46±0.09 d-g	125.05±20.65 fg	40.11±2.72 a-d
Vd+50 mM	21.66±0.71 g-ı	2.21±0.07 f-ı	75.90±3.23 h-j	39.39±1.69 a-e
Vd+100 mM	17.50±1.05 ı-m	2.08±0.09 gh	57.62±2.48 j-l	32.65±0.91 d-j
Vd+150 mM	8.33±1.85 o	1.10±0.14 m	27.32±7.30 m	14.30±2.44 k
Fm +Bc+50 mM	25.83±0.60 d-f	2.07±0.10 g-ı	132.88±9.16 f	41.02±1.61 a-c
Fm +Bc+100 mM	20.00±1.94 g-l	1.33±0.09 k-m	75.79±7.17 h-j	38.33±2.88 a-e
Fm +Bc+150 mM	18.00±0.73 h-m	2.14±0.08 g-ı	70.88±1.44 ı-k	35.24±1.33 b-h
Fm +Bc+Vd	26.33±0.80 de	2.79±0.15 c-e	170.30±8.90 e	35.97±0.76 a-g
Fm +50 mM+Vd	21.83±0.54 f-ı	2.32±0.17 e-h	92.38±2.55 hı	40.20±1.97 a-d
Fm +100 mM+Vd	21.16±0.79 g-j	2.34±0.04 e-h	87.76±3.99 hı	38.53±1.94 a-e
Fm +150 mM+Vd	20.83±0.47 g-k	2.42±0.05 d-h	84.01±6.75 h-j	37.16±1.79 a-g
Bc + 50 mM+ Vd	16.50±0.67 k-n	1.79±0.13 ı-k	67.75±5.93 ı-k	38.07±3.36 a-f
Bc + 100 mM+ Vd	12.50±1.83 n	1.52±0.24 j-m	56.98±7.58 j-l	33.37±2.91 c-j
Bc + 150 mM+ Vd	7.66±2.09 o	1.16±0.30 lm	37.81±7.84 lm	16.94±4.51 k
Fm +Bc+50 mM+ Vd	21.16±0.47 g-j	1.93±0.12 h-j	100.89±6.27 gh	39.65±1.60 a-e
Fm +Bc+100 mM+ Vd	21.66±1.22 f-ı	2.68±0.08 c-f	85.16±7.80 h-j	36.53±1.62 a-g
Fm +Bc+150 mM+ Vd	21.66±1.22 f-ı	2.39±0.09 d-h	82.93±4.29 h-j	31.56±1.73 e-j
p ^{treatment}	p=0.0001	p=0.0001	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level

All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

In this study, it was determined that the phosphorus content of the Fm (3264.38 ppm) was higher than in most applications and that Bc had a positive effect with Fm in phosphorus intake in particular being under 50 mM and 100 mM salt conditions (Table 3). In terms of the P nutrient element, Fm + Vd (2552.75 ppm) showed a 63% increase compared to Vd (1566.90 ppm), and Bc + Vd (1357.98 ppm) showed a 13% decrease compared to Vd (1566.90 ppm). There was a 126% increase in K between the lowest value (Bc + 100 mM + Vd) and the highest value (Fm + Bc). Single binary combinations [Fm + 100 mM (0.86 ppm) and Bc + 100 mM (1.76 ppm)] slightly increased the potassium (K) content, while the triple interactions of Fm and Bc [Fm + Bc + 100 mM (2.32 ppm)] led to the significant increase in potassium content (Table 3). In the Ca parameter, a 40% increase was observed between Vd (212.76 ppm) and Fm + Vd (297.52 ppm), and a 7% increase was seen between Bc + Vd (226.83 ppm) (Table 3). This shows that the effect of Fm on the Ca value under the Vd stress was more than that of Bc. There was a 74% increase in Mg between the lowest (Fm+Bc+Vd) and the highest (Fm+150 mM+Vd) treatments (Table 3).

Table 3. The effects of AMF, biochar and *V. dahliae* on content of macro nutrient in pepper grown under salt stress (mean±standard deviation)

Çizelge 3. Tuz stresi altında Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve *V. dahliae*'nin makro besin içeriği üzerine etkileri (ortalama±standart sapma)

Treatments	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Control	2722.49±322.73 a-c	593.02±20.48 b-e	194.26±7.57 h	123.35±6.2 d
Fm	3264.38±577.98 a	626.05±33.04 a-d	236.65±2.79 e-h	152.86±12.73 a-d
Bc	2343.83±91.32 b-f	527.82±17.02 b-g	220.09±17.74 f-h	129.24±6.63 cd
50 mM	2050.03±309.08 c-ı	485.34±26.82 d-j	255.63±25.33 b-h	136.98±14.57 b-d
100 mM	1802.59±522.85 d-k	413.96±49.88 f-j	364.66±47.68 a-d	155.67±16.83 a-d
150 mM	2226.31±54.75 b-h	408.39±33.01 f-j	378.64±53.86 a-b	156.87±15.11 a-d
Vd	1566.90±122.95 e-k	444.14±41.76 e-j	212.76±13.37 g-h	128.65±11.31 cd
Fm + Bc	2951.00±757.11 ab	768.36±23.92 a	284.55±14.20 b-h	171.63±13.69 a-d
Fm +50 mM	1416.74±241.79 g-k	406.06±30.74 f-j	325.53±12.46 a-g	162.07±5.03 a-d
Fm + 100 mM	1815.00±45.85 e-k	513.40±36.17 b-ı	436.14±51.79 a	197.03±16.96 a-b
Fm +150 mM	1397.15±100.86 h-k	457.58±24.46 e-j	319.67±44.61 a-h	177.84±9.89 a-d
Bc + 50 mM	2095.51±102.35 c-ı	500.00±12.41 c-j	234.65±35.64 e-h	151.78±18.13 a-d
Bc + 100 mM	1495.09±133.32 f-k	434.72±49.84 e-j	319.47±22.37 a-h	141.14±26.40 b-d
Bc + 150 mM	1906.40±142.24 c-k	427.09±47.35 f-j	307.34±28.97 b-h	131.66±15.45 cd
Fm + Vd	2552.75±329.87 a-d	565.64±70.26 b-f	297.52±40.22 b-h	155.10±9.78 a-d
Bc + Vd	1357.98±77.61 h-k	540.98±84.52 b-g	226.83±20.93 b-h	136.21±12.08 b-d
Vd +50 mM	1436.33±216.40 g-k	391.14±73.45 g-j	259.72±8.26 b-h	142.02±15.83 b-d
Vd +100 mM	1371.04±412.98 h-k	438.71±87.03 e-j	336.05±70.59 a-g	187.29±41.63 a-c
Vd +150 mM	1044.60±108.72 k	359.48±43.83 h-j	358.93±64.45 a-e	169.55±10.99 a-d
Fm + Bc +50 mM	1978.21±200.13 c-j	526.63±47.67 b-g	259.38±11.98 b-h	125.74±8.69 cd
Fm + Bc +100 mM	1638.72±107.34 e-k	509.12±33.05 b-ı	300.83±29.20 b-h	152.49±4.14 a-d
Fm + Bc +150 mM	1299.22±116.97 ı-k	375.67±26.58 g-j	303.91±34.43 b-h	136.30±5.81 b-d
Fm + Bc +Vd	2304.65±256.17 b-g	662.13±18.22 ab	246.61±24.59 c-e	121.62±12.14 d
Fm +50 mM+Vd	1958.63±218.23 c-g	653.46±79.25 a-c	293.06±37.97 b-h	150.03±14.88 a-d
Fm +100 mM+Vd	1403.68±106.81 h-k	532.27±8.13 b-g	276.13±29.82 b-h	179.91±21.08 a-d
Fm +150 mM+Vd	1808.47±142.78 d-k	524.02±79.12 b-h	369.09±75.69 a-c	211.07±53.17 a
Bc + 50 mM+ Vd	1083.77±25.00 jk	422.75±46.71 f-j	305.13±28.52 b-h	158.78±15.47 a-d
Bc + 100 mM+ Vd	1606.08±192.05 e-k	340.60±58.62 j	256.03±58.36 b-h	119.79±17.28 d
Bc + 150 mM+ Vd	2441.76±52.77 b-e	353.59±40.38 ı-j	286.00±26.63 b-h	140.18±8.63 b-d
Fm + Bc+50 mM+ Vd	1207.82±98.50 ı-k	380.67±28.66 g-j	242.37±8.50 d-h	147.97±7.82 b-d
Fm + Bc +100 mM+ Vd	1749.71±91.71 d-k	417.89±48.86 f-j	232.98±9.49 e-h	127.74±14.37 cd
Fm + Bc +150 mM+ Vd	1318.81±185.12 ı-k	419.01±45.37 f-j	340.08±24.37 a-f	156.00±17.19 a-d
p ^{treatment}	p=0.0001	p=0.0005	p=0.0014	p=0.0459

Bc: Biochar application Fm: *F. mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level

All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

The different combinations of AMF, biochar, salt and *Verticillium* wilt significantly affected (p≤0.05) the Zn, Mn and Fe contents of pepper shoots (Table 4). The lowest Zn value was in the Fm + Bc+ 100 mM (0.16 ppm) application, while the highest value was observed in the Fm + Bc+ Vd (0.58 ppm) application with an increase of 263% (Table 4). A 110% difference was found between the highest Mn value Bc + 150 mM + Vd (4.40 ppm) and the lowest Fm + Vd (2.09 ppm) applications (Table 4). Applications of Bc and Fm showed an increase in Mn compared to the control group (Table 4). While Fm (15.02 ppm) and Bc (16.42 ppm) values in the Fe parameter were higher than in the control (7.43 ppm), there was no statistically significant difference between their interactions [Fm + Bc (7.66 ppm)] (p> 0.05). Bilateral interactions of Fm and Bc in both biotic and abiotic conditions (such as Fm + salt, Fm + Vd, Bc + salt, Bc + Vd) increased the Fe value (Table 4).

Table 5 displays the effects of applying AMF, biochar, and *V. dahliae* on sodium, potassium/sodium ratio, and calcium/sodium ratio in pepper plants grown under salt stress. Table 5's treatment group discrepancies were statistically different (p< 0.05). The highest value in Na was in Fm + 100 mM (588.31 ppm), and the lowest value was in the control (31.95 ppm) group. The highest value in the K/Na ratio content was in the control (18.64), and the lowest value was in the Fm+100 mM (0.86 ppm) application, with a 95% decrease (Table 5). The highest Ca/Na ratio was in the biochar treatment group (6.61 ppm), and the lowest value was in the Fm +100 mM (0.73 ppm) applications, and the 89% decrease between them was determined to be statistically significant (p<0.05) (Table 5).

Table 4. The effects of AMF, biochar and *V. dahliae* on content of micronutrient in pepper grown under salt stress (mean±standard deviation)

Çizelge 4. Tuz stresi altında AMF, biyokömür ve *V. dahliae*'nin tuz stresi altında yetiştirilen biberde mikro besin elementi içeriği üzerine etkileri (ortalama±standart sapma)

Treatments	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
Control	0.34±0.05 b-d	0.08±0.00 a	2.54±0.04 d-f	7.43±0.28 d
Fm	0.17±0.02 cd	0.12±0.01 a	3.77±0.22 a-e	15.02±3.54 b-d
Bc	0.21±0.05 b-d	0.08±0.00 a	3.10±0.12 a-f	16.42±3.02 b-d
50 mM	0.25±0.01 b-d	0.12±0.03 a	4.26±0.59 ab	14.99±4.87 b-d
100 mM	0.23±0.01 b-d	0.13±0.03 a	4.10±0.31 a-c	11.11±1.03 b-d
150 mM	0.25±0.01 b-d	0.13±0.05 a	3.14±0.08 a-f	9.83±0.51 b-d
Vd	0.32±0.05 b-d	0.11±0.02 a	4.02±0.08 a-d	27.41±7.87 a
Fm + Bc	0.27±0.07 b-d	0.11±0.01 a	3.82±0.63 a-e	7.66±0.11 cd
Fm +50 mM	0.19±0.00 cd	0.10±0.00 a	3.50±0.13 a-f	8.37±1.10 cd
Fm + 100 mM	0.20±0.02 b-d	0.09±0.02 a	3.14±0.10 a-f	8.82±1.08 cd
Fm +150 mM	0.21±0.03 b-d	0.14±0.04 a	2.52±0.18 d-f	8.19±0.96 cd
Bc + 50 mM	0.23±0.02 b-d	0.07±0.00 a	3.10±0.12 a-f	18.43±3.88 a-d
Bc + 100 mM	0.23±0.02 b-d	0.08±0.00 a	2.72±0.80 c-f	9.35±1.22 b-d
Bc + 150 mM	0.22±0.01 b-d	0.10±0.01 a	2.76±0.15 c-f	8.54±1.82 cd
Fm + Vd	0.26±0.05 b-d	0.10±0.02 a	2.09±0.48 f	17.47±5.07 a-d
Bc + Vd	0.22±0.03 b-d	0.10±0.02 a	2.88±0.18 b-f	14.75±2.96 b-d
Vd +50 mM	0.22±0.45 b-d	0.09±0.01 a	4.01±0.80 a-d	17.20±4.29 a-d
Vd +100 mM	0.21±0.06 b-d	0.08±0.01 a	3.91±0.44 a-e	14.71±2.13 b-d
Vd +150 mM	0.27±0.07 b-d	0.07±0.00 a	4.37±0.41ab	19.22±4.43 a-c
Fm + Bc +50 mM	0.38±0.07 bc	0.09±0.01 a	2.96±0.28 a-f	12.06±3.72 b-d
Fm + Bc +100 mM	0.16±0.01 d	0.10±0.01 a	2.74±0.06 c-f	7.37±1.14 d
Fm + Bc +150 Mm	0.22±0.01 b-d	0.08±0.00 a	3.58±0.37 a-f	12.52±1.98 b-d
Fm + Bc +Vd	0.58±0.18 a	0.07±0.00 a	2.48±0.19 e-f	7.65±0.67 cd
Fm +50 mM+Vd	0.37±0.08 bc	0.11±0.03 a	2.94±0.15 a-f	6.97±0.24 d
Fm +100 mM+Vd	0.18±0.00 cd	0.10±0.02 a	2.89±0.18 b-f	7.79±1.39 cd
Fm +150 mM+Vd	0.24±0.04 b-d	0.13±0.04 a	2.61±0.39 c-f	8.05±1.78 cd
Bc + 50 mM+ Vd	0.25±0.03 b-d	0.07±0.00 a	2.92±0.51 a-f	8.69±1.32 cd
Bc + 100 mM+ Vd	0.27±0.07 b-d	0.07±0.02 a	2.93±0.40 a-f	8.9±1.48 cd
Bc + 150 mM+ Vd	0.29±0.06 b-d	0.11±0.02 a	4.40±0.81 a	20.64±7.32 ab
Fm + Bc+50 mM+ Vd	0.21±0.01 b-d	0.10±0.03 a	4.38±0.37 ab	17.92±7.30 ad
Fm + Bc +100 mM+ Vd	0.40±0.10 b	0.10±0.01 a	3.23±0.66 a-f	9.59±2.07 bd
Fm + Bc +150 mM+ Vd	0.22±0.03 b-d	0.14±0.02 a	2.93±0.27 a-f	7.97±0.71cd
p ^{treatment}	p=0.007	p=0.863	p=0.0010	p=0.0042

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level

All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

In the current study, as a result of Bc and Fm interactions, the Fm + Bc + 100 mM (237.34 ppm) application decreased salt stress compared to the Fm + 100 mM (588.31 ppm) application. It was determined that multiple interactions (Fm + Bc + 100 mM + Vd (150.06 ppm)) had much greater effects on Na stress. In general, the Na value of Fm applications was higher than that of Bc (Table 5). In addition, there is a 67% difference between the Fm + Bc (139.31 ppm) application and the Fm + Bc + Vd (45.66 ppm) interaction (Table 5). As the salt concentration increased, the K/Na ratio between applications changed (Table 5). The K/Na ratio of salt treatments was lower than those treated with Vd, and the difference was statistically significant (p<0.05) (Table 5). The effectiveness of Fm and Bc on the K/Na parameter differed according to both salt and Vd stress factors. It was determined that the combined use of Fm and Bc was more effective in the K/Na ratio when the ratios in salt treatments were considered in isolation. Fm alone was ineffective. The K/Na ratio of Fm+Vd (10.68) and Bc+Vd (10.86) increased by 23% and 25%, respectively, compared to Vd (8.68). Especially between Fm + Bc + 50 mM + Vd (4.66 ppm) and Fm + 50 mM + Vd (4.82 ppm), a 3% decrease was observed, while Bc + 50 mM + Vd (1.98 ppm) increased by 135%. While the Ca / Na value in Fm + 100 mM (0.73) and Bc + 100 mM (1.53) increased by 110%, it was determined that Bc was more effective than Fm under salt-stress conditions. However, the Ca/Na value of Fm was higher than Bc in the treatments which involved interaction with Vd.

Table 5. The effects of AMF, biochar and *V. dahliae* on Na (ppm), K / Na (ppm), and Ca / Na (ppm) in pepper grown under salt stress (mean±standard deviation)

Çizelge 5. AMF, biyokömür ve *V. dahliae*'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve *V. dahliae*'nin Na (ppm), K/Na (ppm) ve Ca/Na (ppm) üzerine etkileri (ortalama±standart sapma)

Treatments	Na(ppm)	K / Na(ppm)	Ca / Na(ppm)
Control	31.95±1.50 ı	18.64±0.81 a	6.09±0.20 ab
Fm	39.21±3.23 ı	16.19±1.18 b	6.13±0.42 ab
Bc	33.62±2.79 ı	15.92±0.95 b	6.61±0.49 a
50 mM	232.58±53.68 d-g	2.35±0.43 h-j	1.22±0.21 e-ı
100 mM	380.13±4.04 bc	1.20±0.16 ıj	1.04±0.11 g-ı
150 mM	316.87±12.44 b-e	1.29±0.09 ıj	1.18±0.14 e-ı
Vd	53.81±5.07 ı	8.68±1.57 d	3.99±0.20 c
Fm + Bc	139.31±10.84 gı	5.62±0.50 e	2.06±0.21 e-g
Fm +50 mM	168.87±34.19 f-ı	2.76±0.62 f-j	2.22±0.50 de
Fm + 100 mM	588.31±16.27 a	0.86±0.04 j	0.73±0.07 ı
Fm +150 mM	434.32±25.13 b	1.05±0.06 j	0.75±0.13 ı
Bc + 50 mM	232.81±29.80 d-g	2.25±0.29 h-j	1.10±0.27 f-ı
Bc + 100 mM	338.58±115.56 b-d	1.76±0.47 h-j	1.53±0.59 e-ı
Bc + 150 mM	379.28±43.79 bc	1.12±0.01 j	0.82±0.05 ı
Fm + Vd	53.35±1.39 ı	10.68±1.53 c	5.59±0.75 b
Bc + Vd	51.41±6.27 ı	10.86±1.91 c	4.46±0.17 c
Vd +50 mM	231.65±43.60 d-g	1.88±0.50 h-j	1.23±0.21 e-ı
Vd +100 mM	189.92±54.84 e-h	2.54±0.45 g-j	1.90±0.26 e-h
Vd +150 mM	340.75±18.51 b-d	1.05±0.11 j	1.05±0.16 g-ı
Fm + Bc +50 mM	150.98±12.45 f-ı	3.55±0.42 e-ı	1.75±0.07 e-ı
Fm + Bc +100 mM	237.34±37.96 d-g	2.32±0.42 h-j	1.38±0.29 e-ı
Fm + Bc +150 mM	273.31±25.85 c-g	1.40±0.14 ıj	1.14±0.16 f-ı
Fm + Bc +Vd	45.66±1.71 ı	14.54±0.55 b	5.38±0.42 b
Fm +50 mM+Vd	138.72±18.03 gı	4.82±0.48 e-f	2.16±0.22 d-f
Fm +100 mM+Vd	150.94±37.11 f-ı	4.00±0.65 e-h	1.98±0.21 e-g
Fm +150 mM+Vd	279.45±50.93 c-f	1.94±0.20 h-j	1.34±0.16 e-ı
Bc + 50 mM+ Vd	239.49±57.62 d-g	1.98±0.35 h-j	1.44±0.25 e-ı
Bc + 100 mM+ Vd	149.80±23.89 f-ı	2.28±0.14 h-j	1.68±0.15 e-ı
Bc + 150 mM+ Vd	283.07±50.89 c-f	1.34±0.21 ıj	1.16±0.30 e-ı
Fm + Bc+50 mM+ Vd	85.48±10.93 h-ı	4.66±0.60 e-g	3.05±0.57 d
Fm + Bc +100 mM+ Vd	150.06±21.39 f-ı	2.90±0.35 f-j	1.69±0.33 e-ı
Fm + Bc +150 mM+ Vd	393.94±25.62 bc	1.06±0.09 j	0.86±0.05 h-ı
p ^{treatment}	p=0.0001	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level

All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

Soil pH and EC values

Table 6 displays how AMF, biochar, and *V. dahliae* applications affected the soil's pH (mS/cm) and EC (dSm-1) levels in peppers grown under salt stress. According to Table 6, the variations in soil pH and EC values across treatments are statistically significant (p<0.05). As the salt concentration increased, the pH values of Bc, Fm, and Vd applications decreased, and EC values increased (Table 6). The highest pH and EC values were in Bc+Vd (7.90 mS/cm) and Fm+150 mM applications, respectively. In our study, the pH values of the applications made with Vd and Fm were found to be higher than in the control group, and the difference was found to be statistically significant (p<0.05) (Table 6). In the EC parameter, an increase in EC values was observed as the salt concentration increased. It was determined that the EC values of Fm or Bc in individual salt interaction applications (Fm+salt or Bc+salt) were higher than for the triple combination (Fm+Bc+salt). Therefore, it was determined that the Fm + Bc interaction decreased the EC value despite the increasing salt doses (Table 6).

Correlation and Principal Components Analysis (PCA)

As a size reduction technique, PCA (Principal Components Analysis) is generally interpreted by considering the first two or three basic components. The connection between parameters and the differences between treatments was examined in our study using PCA analysis. Correlation analysis was conducted for the 17 features that were

the subject of the study's examination. The correlation values of the relationships between these parameters are given in Figure 1. Correlations enclosed in rectangles are statistically significant. Blue indicates a positive correlation, and red indicates a negative correlation. Strong correlations are boxed and circles which indicate weak correlations are not boxed (Figure 1). The correlation coefficient takes values between -1 and +1. $r=-1$ denotes a negative linear relationship, $r=+1$ indicates a positive linear relationship, $r=0$ indicates no relationship between two variables. A value of 0.00 denotes no relationship, 0.01-0.29 indicates a low level of relationship, 0.30-0.70 indicates a medium level of relationship, 0.71-0.99 indicates a high level of relationship, and 1.00 indicates a relationship.

Table 6. The effects of AMF, biochar and *V. dahliae* on EC (dSm⁻¹) and pH (mS/cm) in the soil in pepper grown under salt stress (mean±standard deviation)

Çizelge 6. AMF, biyokömür ve *V. dahliae*'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve *V. dahliae*'nin topraktaki EC (dSm⁻¹) ve pH (mS/cm) üzerine etkileri (ortalama±standart sapma)

Treatments	Soil pH (mS/cm)	EC (dSm ⁻¹)
Control	7.26±0.08 f-k	661.00±103.76 j
Fm	7.64±0.06 bc	618.00±56.69 j
Bc	7.89±0.05 a	535.75±85.72 j
50 mM	7.18±0.05 h-k	2395.00±82.20 h ₁
100 mM	7.19±0.05 hk	4992.50±274.23 ef
150 mM	6.90±0.15 l	7305.00±498.77 c
Vd	7.54±0.05 b-d	358.50±22.48 j
Fm + Bc	7.48±0.10 c-f	1598.75±444.06 ij
Fm +50 mM	7.27±0.11 f-j	3145.75±228.12 gh
Fm + 100 mM	7.05±0.03 kl	4372.50±462.73 fg
Fm +150 mM	7.06±0.02 j-l	12580.00±225.16 a
Bc + 50 mM	7.50±0.06 b-e	2587.50±185.53 h ₁
Bc + 100 mM	7.51±0.02 b-e	4897.50±506.48 ef
Bc + 150 mM	7.32±0.01 d-1	7805.00±879.30 c
Fm + Vd	7.70±0.01 ab	440.75±47.45 j
Bc + Vd	7.90±0.12 a	491.00±65.58 j
Vd +50 mM	7.28±0.01 e-j	2617.50±108.50 h ₁
Vd +100 mM	7.26±0.05 f-k	3492.50±247.63 gh
Vd +150 mM	7.18±0.01 h-k	5170.00±504.71 ef
Fm + Bc +50 mM	7.36±0.03 d-h	2662.50±220.24 h ₁
Fm + Bc +100 mM	7.38±0.03 d-h	5810.00±301.52 de
Fm + Bc +150 mM	7.24±0.01 g-k	6877.50±622.62 cd
Fm + Bc +Vd	7.40±0.12 d-h	930.75±115.84 j
Fm +50 mM+Vd	7.12±0.03 r-k	2812.50±115.92 h ₁
Fm +100 mM+Vd	7.07±0.04 j-l	5537.50±410.637 d-f
Fm +150 mM+Vd	7.14±0.04 r-k	9372.50±663.46 b
Bc + 50 mM+ Vd	7.47±0.04 c-f	2705.00±133.69 h ₁
Bc + 100 mM+ Vd	7.50±0.05 b-e	4342.50±742.61 fg
Bc + 150 mM+ Vd	7.42±0.03 c-g	6730.00±125.63 cd
Fm + Bc+50 mM+ Vd	7.54±0.09 b-d	2401.00±412.83 h ₁
Fm + Bc +100 mM+ Vd	7.33±0.05 d-1	5577.50±590.35 d-f
Fm + Bc +150 mM+ Vd	7.41±0.08 d-h	6845.00±1127.16 cd
p _{treatment}	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F. mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level

All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to $p<0.05$.

According to Figure 1, there is a strong correlation relationship between SD - NL, LA- NL, LA- SD, Ca/Na - K/Na, CHL- NL, CHL - SD, P-NL, P-SD, P-LA, Fe-Mn, Na-SEC, K-NL, K-SD, K-LA, K-P, Ca- Na, Ca-Mg, K/Na-NL, K/Na- SD, K/Na- LA, K/Na- SpH, K/Na- P, K/Na- K, Ca/Na-NL, Ca/Na-SD, Ca/Na-LA, Ca/Na-SpH, Ca/Na-P, Ca/Na-K (Figure 1). SpH - SD, SpH-LA, Cu-SEC, Fe-SpH, K-CHL, Mg-SEC, Mg-Na, Ca-SEC, and Ca/Na-CHL show moderate correlation (Figure 1).

SEC-NL, SEC-SD, SEC-CHL, SEC-LA, SEC-SpH, P-SEC, Na-NL, Na-SD, Na-CHL, Na-LA, Na-SpH, Na-P, K-SEC,

K-Na, Mg-SpH, Mg- Zn, Ca-NL, Ca-SD, Ca-CHL, Ca-LA, Ca- SpH, K/Na-SEC, K/Na-Na, K/Na-Mg, K/Na-Ca, Ca/Na- SEC, Ca/Na-Na, Ca/Na-Mg, Ca/Na-Ca all have a negative linear relationship (Figure 1). For the data showing a strong correlation relationship, shoot diameter, number of leaves, leaf area, Ca/Na - K/Na are effectively linearly related to each other despite salt stress conditions.

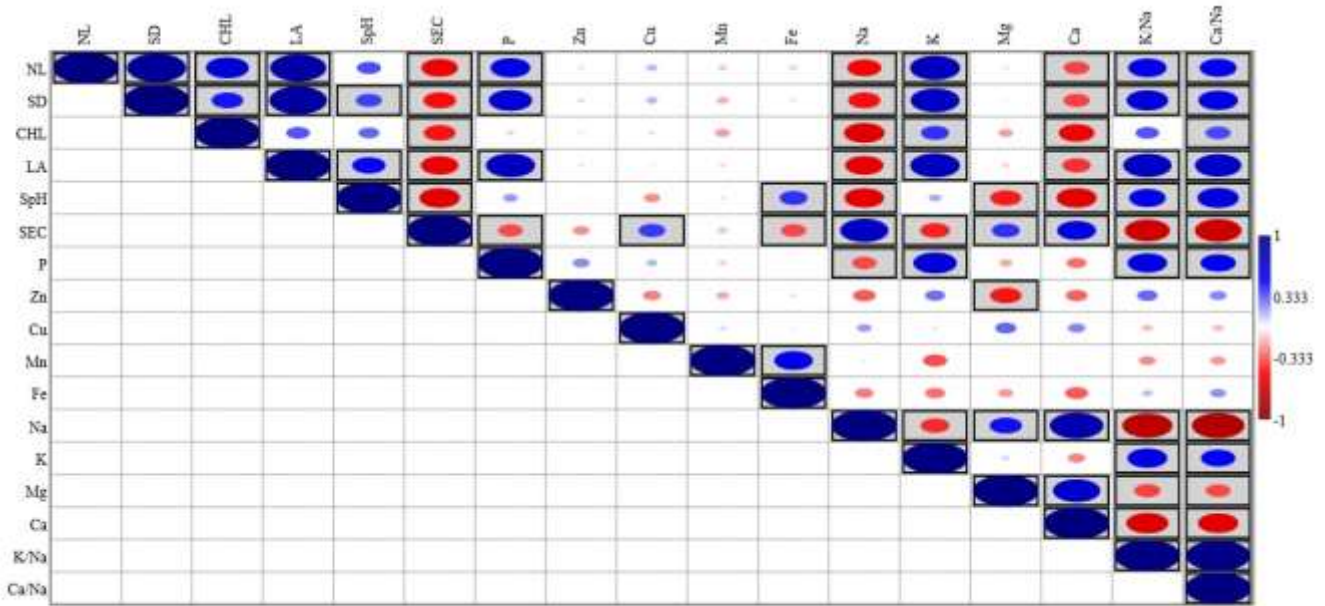


Figure 1. Pearson correlation coefficients between features.

Şekil 1. Özellikler arasındaki Pearson korelasyon katsayıları.

Values in bold frame are different from 0 and the significance level is alpha: 0.05.

NL: Number of Leaves, SD: Shoot Diameter, CHL: Chlorophyll, LA: Leaf Area, SpH: Soil pH, SEC: Soil EC, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese, Fe: Iron, Na: Sodium, K: Potassium, Mg: Magnesium, Ca: Calcium, K/Na: Potassium Sodium Ratio, Ca/Na: Calcium Sodium Ratio

Principal component analysis (PCA) was used to identify the variables that make up the variation. According to the analysis, the contribution of the eigenvalue and variance values to the features differentiating the applications was established (Table 7). The first four components with an eigenvalue larger than 1.00 in the PCA analysis of the 17 different features investigated in the study explained 77.15% of the total variation. To ascertain the suitable quantity of principle components in PCA, one may select components with eigenvalues exceeding 1 or those that account for a minimum of 67% of the total variance (Özdamar, 2010). The first two components in the current study accounted for 60.18% of the general variation (Table 7). The first component (PCA1) accounted for 43.66% of the variation, and the features that best explained the variation were the number of leaves, shoot diameter, leaf area, soil pH, soil EC, Na, K/Na, Ca/Na. Although the second component (PCA2) was responsible for 16.52% of the variation, this component's Cu, K, Mg, and Ca made the most significant contribution to justifying the variation. The third component (PCA3) explained 10.03% of the variation and provided the greatest contribution to variation in all parameters except Zn, Mn, and Fe. In the fourth component (PCA4), the chlorophyll, and P were the most explanatory features (Table 7).

By using a loading plot composed of PCA1 and PCA2 components, the interrelationships between the 17 variables evaluated in the study were determined (Figure 2). In this context, according to Figure 2, there is a positive correlation between Fe, Zn, K/Na, and Ca/Na. There is also a positive relationship between plant growth parameters, chlorophyll, P, and K. However, these parameters had a negative correlation with Ca, Mg, Cu, Mn, Na, and soil EC.

The effect of PCA1 and PCA2 components Fm and Bc on soil EC-pH, leaf mineral content, chlorophyll, and Vd, and growth parameters under salt stress were visualized using a score plot (Figure 3). It was observed that control, Bc, Vd, and 50 salt and Fm+ Bc+ 100+ Vd applications were located close to each other. Interactions with Vd and high salt dose were in the PCA1 negative and PCA2 positive regions, respectively. Fm was determined in the PCA1 and PCA2 positive regions of Fm+Bc, Fm+Vd, and Fm+50+Vd treatments. This was found to be correlated with how biotic and abiotic stress variables interacted (Figure 3). As a result, it was discovered that Fm was better than Bc at mitigating the damage caused by salt stress (Figure 3).

Table 7. PCA results regarding characters used in this study

Çizelge 7. Çalışmada kullanılan karakterlere ilişkin PCA sonuçları

	PCA-1	PCA-2	PCA-3	PCA-4
Eigenvalue	7.423	2.808	1.705	1.179
Variability (%)	43.664	16.520	10.032	6.935
Cumulative (%)	43.664	60.184	70.217	77.152
Factor loadings of parameters				
Number of Leaves	0.290	-0.266	0.142	0.148
Shoot Diameter	0.289	-0.273	0.138	0.005
Chlorophyll	0.212	-0.033	0.008	0.661
Leaf Area	0.310	-0.212	0.159	-0.145
Soil pH	0.237	0.232	0.142	0.139
Soil EC	-0.290	-0.188	-0.091	0.010
P	0.237	-0.217	0.041	-0.465
Zn	0.114	0.104	-0.487	-0.327
Cu	-0.063	-0.326	0.295	0.070
Mn	-0.060	0.218	0.538	-0.255
Fe	0.056	0.379	0.458	-0.147
Na	-0.311	-0.178	0.028	-0.143
K	0.256	-0.336	-0.114	-0.077
Mg	-0.157	-0.369	0.246	0.045
Ca	-0.269	-0.280	0.054	-0.152
K/Na	0.327	0.022	-0.072	-0.157
Ca/Na	0.323	0.062	-0.019	-0.098

* Numbers in bold are the highest values of the attributes.

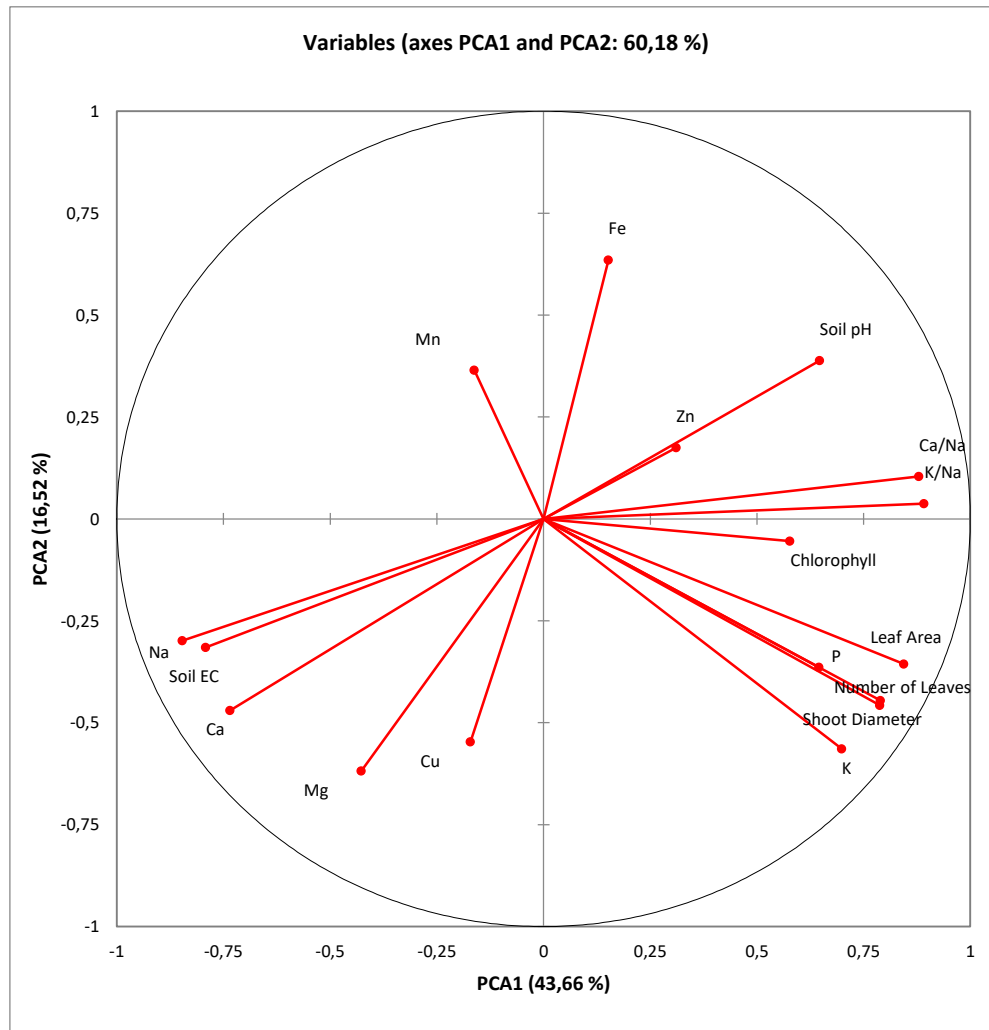


Figure 2. Character-based PCA loading plot for the first two major components
Şekil 2. İlk iki ana bileşen için karakter tabanlı PCA yükleme grafiği

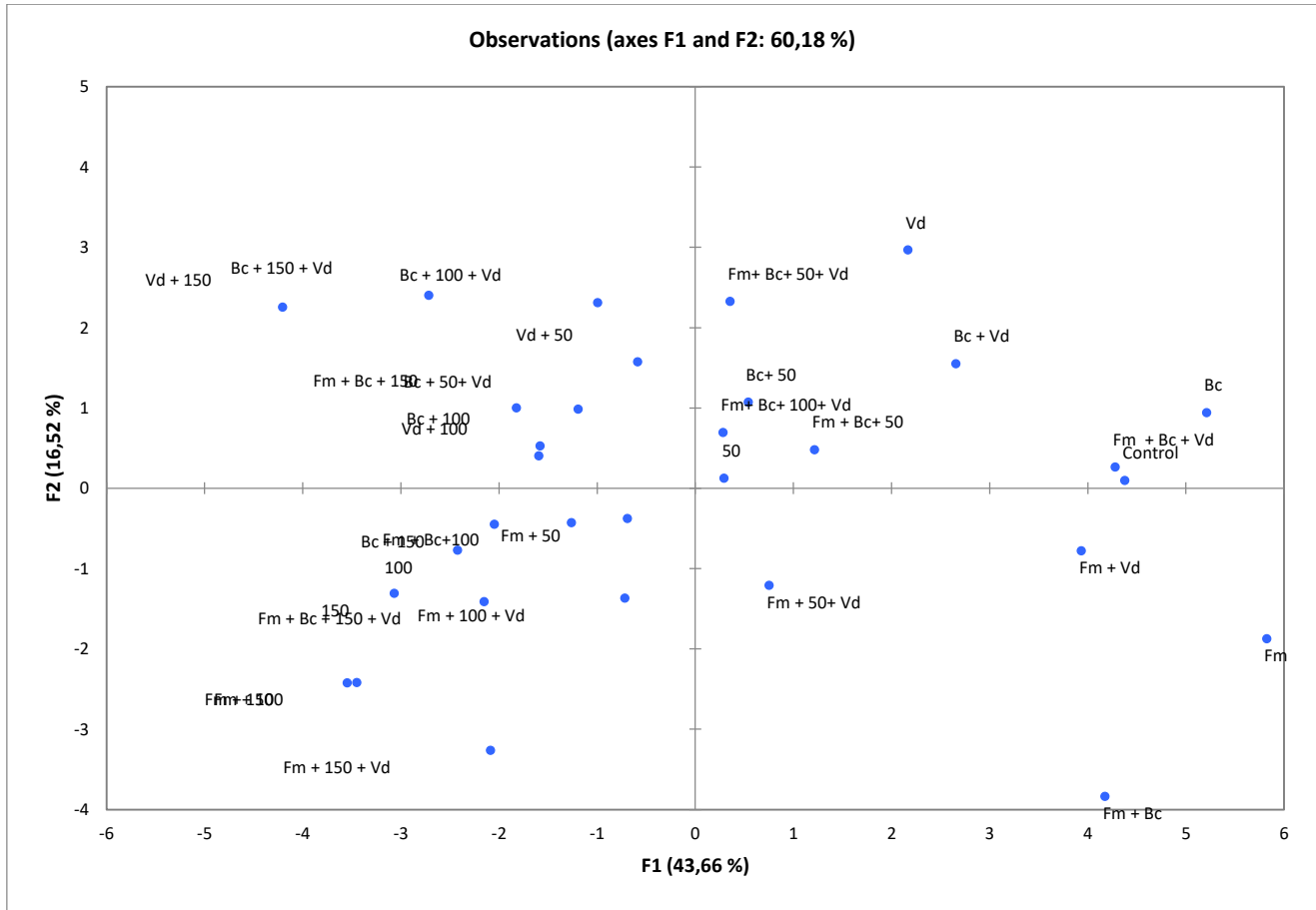


Figure 3. The score plot for the PCA analysis's initial two principal components

Şekil 3. PCA analizinin ilk iki temel bileşeni için skor grafiği

A heatmap graph was created to determine the relationship between 32 applications and 17 parameters (Figure 4). In the graph, the blue color indicates a low value, and the red color indicates a high value. The heatmap for the effects of *F. mosseae* and biochar against salt stress and *V. dahliae* on plant growth and nutrient content in pepper is given in Figure 4. The cluster is divided into two basic groups (top two rows) for parameters and two basic groups (five left rows) for applications. The first is the group from top to bottom between Fm+50 and Bc+50+Vd, and the other is the group between Bc+50 and Bc (Figure 4).

For the parameters in the first group (top right), treatments with 100 mM and 150 mM salt were found to result in colors (red) indicating higher temperatures. Vd also interacts with both Fm and Bc when high salt concentrations (100 and 150 mM) are used (Figure 4). In the first group (red color), there was a high correlation between Mg mineral matter Vd+100 and Fm 150+ Vd as well as between soil EC and Fm+150 (Figure 4). It was also found that the treatments in the first group were positively correlated with all mineral substances (except Zn) and negatively correlated with other parameters (Figure 4).

For the parameters in the second group (bottom right), Control groups (control, Fm, Bc) and low-stress (50 mM) treatments were found to have lower color (blue) temperatures (Figure 4). In the second group (blue color), a high correlation was discovered between Zn and Fm+Bc+100+Vd, Zn and Fm+Bc+50, Zn+Bc+Vd, Soil pH and Bc+Vd, Fe and Vd, K and Fm+50+Vd (bottom row from left) (Figure 4).

In the second group of treatments (bottom row from left), the physiological parameters of pepper (number of leaves, shoot diameter, leaf area, and chlorophyll), soil pH, and Zn values were associated with low-stress conditions or control groups (Figure 4). These parameters were more strongly associated with Vd treatments compared with salt concentrations (Figure 4).

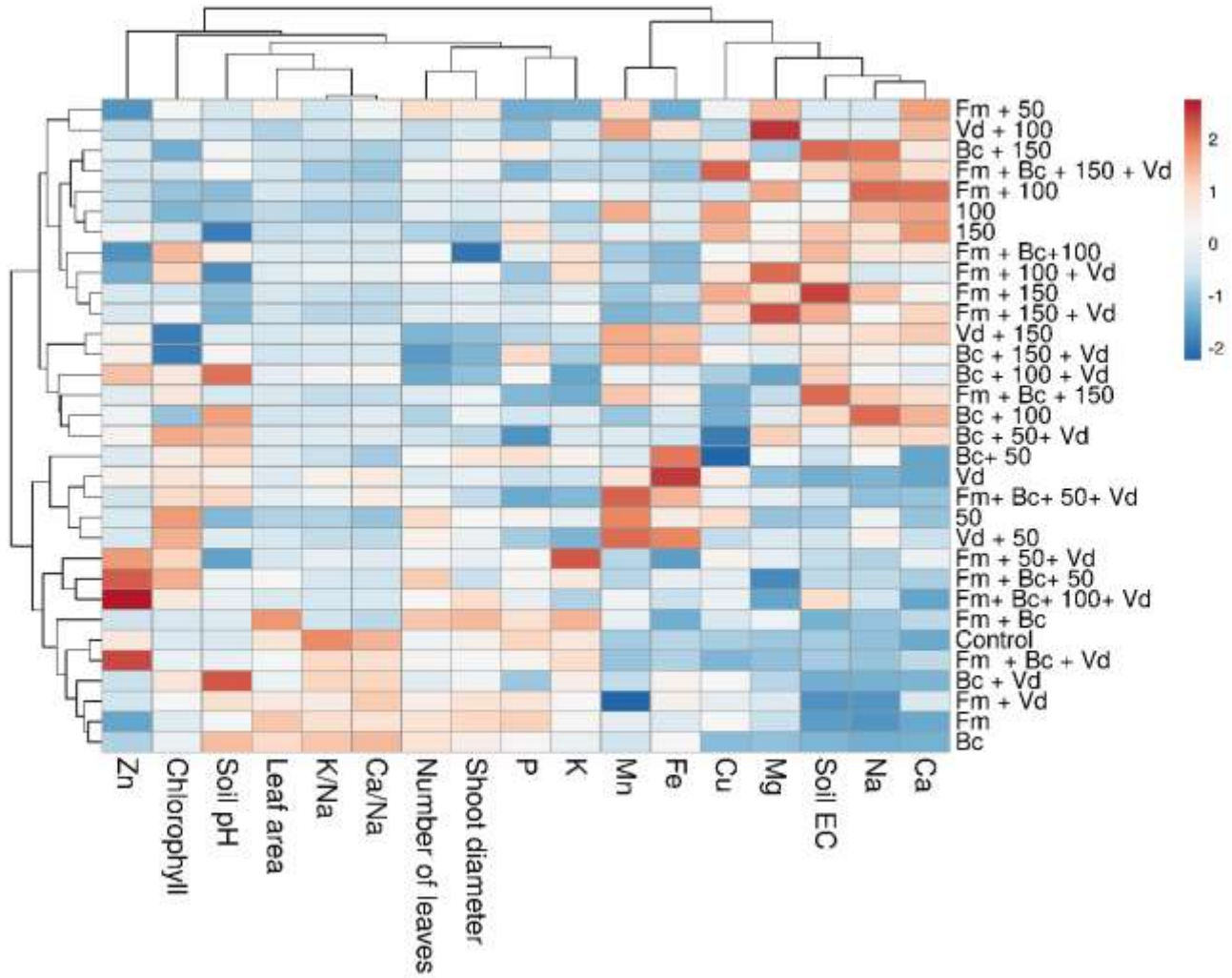


Figure 4. Heatmap of the effects of *V. dahliae* and *F. mosseae* and biochar on plant growth and nutrient content against salt stress in pepper

Şekil 4. Biberde tuz stresine karşı *V. dahliae* ve *F. mosseae* ile biyokömürün bitki büyümesi ve besin içeriği üzerindeki etkilerinin ısı haritası

DISCUSSION

This is the first study to investigate the effects of *F. mosseae* (Fm) and biochar (Bc) on plant growth, nutrient content, soil pH, and soil EC in peppers grown under different concentrations of salt stress and *V. dahliae* (Vd). As salt concentration increased, leaf number, leaf area, and chlorophyll values decreased, indicating that high salt stress concentration negatively affected plant growth (Table 2). In this study, the interaction of *Funneliformis mosseae* with 2% biochar increased the physiological parameters of pepper (leaf number 65%, shoot diameter 46%, leaf area 86%, and chlorophyll 36%). This can be explained by the change in plant cell structure and the inhibition of growth caused by excess salt in the soil (Sagar et al., 2021).

Plants that associate with AMF have been found to have increased resistance or tolerance to biotic and abiotic stresses (Jung et al., 2012; Li et al., 2013). Induced systemic resistance is the alteration of plant hormonal balance by beneficial microorganisms that affect pathogen performance in distant tissues (Van der Ent et al., 2009). Tolerance has been measured as an index that scores the physical difference between damaged and undamaged plants as the plant develops vegetatively despite pathogen infection (Gruntman and Novoplansky, 2011). AMF can stimulate systemic and local resistance against many pathogens or under abiotic stress conditions such as salt stress, as well as intervene or inhibit stress development directly (Köhl et al., 2019). Strategies and mechanisms by which AMF can influence disease development include competition with the pathogen, altering microbial density in the rhizosphere, and inducing resistance in plants (Pozo & Azcón-Aguilar, 2007). AMF, therefore, plays a protective role in plant-pathogen-abiotic stress interaction. AMF is a biotechnological strategy for plant protection optimization (Pozo et al., 2010).

Worldwide, the biochar + AMF combination has attracted attention for its wide range of applications, especially

for nutrient cycling improvement (Castañeda et al., 2020), tolerance to abiotic stress factors (such as salt, drought, heavy metals) (Hashem et al., 2019) and use against plant pathogens (Gujre et al., 2021). In addition to the positive impact of biochar + AMF applications on soil improvement and plant growth, biochar provides a habitat for AMF. As a result, there are both complementary and opposing effects between them (Jaafar, 2014).

The results indicate that the interaction of pepper plants with Vd, whose resistance decreased due to salt stress, negatively affected plant growth criteria. It may be due to the synergistic interaction between biotic and abiotic stress factors. On the other hand, Fm by itself or in combination with Bc increased plant growth in both salt and Vd environments (Table 2). According to Akhter et al. (2015) and Giono et al. (2021), organic wastes not only lessen stress damage to plants but also aid nutrient intake for AMF development and even contribute to the symbiotic relationship. At the same time, it has been determined that the mineral substances absorbed by the biochar are transferred to the host plant via AMF hyphae, and the effect of the combination of AMF and biochar on plant growth is positive (Hammer et al., 2014; Zhuo et al., 2020; Were et al., 2021). Research conducted by Graber et al. (2010) determined that biochar contributes to the development of pepper plants being grown in nutrient-poor soils. Demir et al. (2015) reported that the interaction of AMF with different organic wastes increased plant growth parameters despite *V. dahliae* wilt and that this interaction could be an alternative application to combat biotic stress conditions.

Table 3 shows that phosphorus content, Ca, K, and Mg values varied according to the AMF inoculum and biochar ratio under biotic and abiotic stress conditions. The P value showed that Fm increased the phosphorus value compared to the control groups despite the salt stress, especially in the environment with Vd (Table 3). Beltrano et al. (2013) stated that the effect of AMF on plant growth is largely related to phosphorus uptake, that AMF increases the phosphorus value at all salinity levels, and that the phosphorus value of the plant is low at non-mycorrhizal high salt levels (100 mM and 150 mM). These results, which are compatible with our study, were discovered to be related to one of the mechanisms that increase the plant's tolerance to salinity. Phosphorus averages of three interactive applications (Fm + Bc + 50 mM etc.) were higher than the applications with four interactions (Fm + Bc + 100 mM + Vd etc.), and the difference between these applications was statistically significant ($p < 0.05$) (Table 3). It has been reported that the pyrolysis temperature of the biochar affects the P uptake in the plant, the P value of the biochar pyrolyzed at low temperatures is high, and the P value of the biochar pyrolyzed at high temperatures is low (Xu et al., 2016).

Mycorrhizal fungi are at the heart of terrestrial food webs that support life on Earth. Helping to transport nutrients between ecosystems, mycorrhizal fungi are natural as well as ecologically important organisms (Hawkins et al., 2023). As a biotic component, AMFs obtain carbohydrates necessary for their vital activities from plant roots, while their hyphae act as capillary roots and are effective in the uptake of water and nutrients, especially some nutrients such as phosphorus (P), zinc (Zn), iron (Fe) and copper (Cu) (Fiorilli et al., 2015; Celik, 2023). Although the increase in nutrient content has no direct effect on the plant pathogen, these increases prevent the plant pathogen from further damaging the host plant (Bennett et al., 2006). The tendency of AMF to increase plant vigor may provide biological protection against pathogens. Although research findings on the effects of AMF and organic matter on nutrients under various stress situations differ, our investigation revealed that Fm had a greater impact on the Ca value in the medium with Vd than Bc (Table 3). In other studies, it has been determined that mycorrhiza applications increase the values of Mg, Cu, Mn, N, P, K, and Ca elements in some abiotic stress factors (Zhang et al., 2019a; Zhao et al., 2021). Karagiannidis et al. (2002) determined that under biotic stress conditions (*V. dahliae*), Ca, Mg, and K mineral substance values were similar to the control group, and the difference between them was statistically insignificant. Nzanza et al. (2012) reported that applications made including the interaction of *F. mosseae* and compost did not effect on the nutritional values of Ca, B, Cu, Mn, Na, and Zn.

For the K and Mg parameters, on the other hand, as salt doses increase, a decrease in K values and a change in Mg values are observed (Table 3). Biró et al. (2000) stated that Bc altered Ca, Mg, Fe, Zn, Cu, and S values according to mycorrhizal applications. However, in a different study, it was determined that mycorrhizal applications increased the K concentration in saline soils and the K, Ca, and Mg values of organic wastes (Giri et al., 2007). However, it has been reported in some studies that the opposite is true and that the Zn content of the applications containing Vd is lower than the control (Kesimci et al., 2019; Coşkun, 2021). Abd El-Mageed et al. (2020) reported that biochar increased the Zn value of pepper grown under salt stress and facilitated nutrient uptake. Therefore, in our study, the increase in the Zn in Fm + Bc + Vd interaction is associated with Bc despite Vd.

According to Table 4, Cu content was not statistically different between treatments, but the Manganese (Mn) value increased in Bc and Fm treatments compared to that of the control group. Nzanza et al. (2012) determined that *F. mosseae* and organic waste interaction applications did not affect Ca, B, Cu, Mn, Na, and Zn uptake in leaves, which supports the Cu uptake findings obtained in the study. In this study, the interaction of *Funneliformis*

mosseae with 2% biochar increased the content of some mineral substances of pepper (P 63%, K 126%, Mg 74%, and Mn 110%). Cu values did not differ between treatment groups. Similarly, Abd El-Mageed et al. (2020) reported a decrease in Cu and Mn nutritional values in biochar applications. *V. dahliae* pathogenic treatments applied to different plants showed significant decreases in nutrient mineral values, especially Cu and Mn contents in comparison to the control (Demir et al., 2015).

In both biotic and abiotic conditions, the iron (Fe) element value of binary interactions (such as Fm + salt, Fm + Vd, Bc + salt, Bc + Vd) in the leaf showed an increase (Table 4). In another study by Vahedi et al. (2022), it was stated that the combination of AMF + biochar significantly increased the Fe (2.38) and Zn (1.29) values compared to the control group in terms of mineral substance value, while Biró et al. (2000) reported that the Fe concentration value of the groups that did not receive AMF was higher than the control. In line with these studies, it is believed that the applications made involving Bc increase the value of the Fe element.

In our study, Fm and Bc had a diminishing effect on the uptake mechanism at high salt concentrations. The effect of Bc treatments on the Na value varied according to the treatment groups (Table 5). In different studies, it has been determined that biochar promotes plant growth, increases salt tolerance, improves soil properties, facilitates nutrient uptake, and reduces Na uptake in both salt stress and non-stress conditions (Ali et al., 2017; Farhangi-Abriz and Torabyan, 2018).

In terms of the Na parameter of our study, Na values were lower in the treatments with Fm, Bc, and Vd interactions (Table 5). Giri et al. (2007) determined that the Na concentration value of the plant grown in a salty environment in mycorrhiza applications was lower than in the control group.

In general, the K/Na values in salt concentration applications were lower than those of the control groups. As the salt concentration increased, the K/Na ratio between applications varied (Table 5). Wu et al. (2010) determined that the Na value of the plant grown under salt stress decreased significantly, and the K, Mg, and K/Na ratios increased in applications with *F. mosseae*. Abdel Latef et al. (2014) stated that biochar facilitates plant nutrient uptake (N, P, Ca, and Mg) and increases the K/Na ratio. It was determined that the Ca/Na ratio changed according to the Bc and Fm application groups under stress conditions (Table 5). In similar studies, it has been reported that biochar incorporated into the soil to manage salt stress balances the water content in the soil due to its porous structure, increases the Ca value, and positively changes the Na amount (Zhang et al., 2019a). *F. mosseae* was determined to increase both the Ca/Na ratio and the Mg/Na ratio (Hajiboland et al., 2010; Wu et al., 2010).

As the salt concentration increased, the soil pH values of Bc, Fm, and Vd applications decreased, and soil EC values increased (Table 6). In the study conducted by Zhang et al. (2019b), the pH value did not increase while investigating the effect of biochar against salt stress; It was observed that the difference between other applications was insignificant and that the pH value of pyrolyzed biochar at 300 °C was higher than 600 °C. In our study, the soil pH values of the applications made with Vd and Fm were found to be higher than the control group (Table 6). In parallel with this, it is stated in some studies that the pH value in the soil may affect the microorganism density (Rousk et al., 2009; Dilegge et al., 2019). At the same time, it was determined that biochar, which increases the alkalinity level in the soil, inhibits the growth of fungi that prefer acidic environments (Yao et al., 2017; Shi et al., 2018; Ogundeji et al., 2021). In other studies, it is stated that biochar application can create an antagonistic effect against plant pathogens, change the chemical properties of the soil, and provide a better habitat for fungi (Liu et al., 2015; Dilegge et al., 2019; Ogundeji et al., 2021).

Fm or Bc had higher EC values in applications of individual salt interactions (Fm+salt or Bc+salt) than in the triple combination (Fm+Bc+salt). Thus, it was determined that, despite increased salt doses, the Fm + Bc interaction decreased the soil EC value (Table 6). Moreover, the interaction between *Funneliformis mosseae* and biochar reduced the soil EC value by 78% under severe salt stress conditions (150 mM). Regarding soil pH and Ca/Na ratio, biochar was shown to be more effective than *Funneliformis mosseae*. Azeem et al. (2019) found that high pH and EC values are related to the rate of biochar mixed into the soil. In other studies, conducted within this context, it was stated that mycorrhiza applications increase the salinity tolerance and decrease the soil EC value (Oztekin et al., 2013). Additionally, it has been found that biochar, which has a high cation exchange capacity and strong absorbent characteristics, removes toxic ions from the soil and releases beneficial ions (Novak et al., 2012). Therefore, these study findings support those of our study, and it is thought that the high pH value in applications using Bc is correlated with the pyrolysis temperature of the biochar.

CONCLUSION

In conclusion, biochar and *Funneliformis mosseae* application can minimize the severity of *Verticillium* wilt and enhance plant growth and stress tolerance in pepper plants grown under salt stress. Mycorrhizae in the soil and their interaction with biochar are a tremendous advantage for agricultural biodiversity considering climate change.

It works particularly well against plant diseases that are challenging to treat, such as the soil-borne *Verticillium* pathogen. Alternative approaches to sustainable agriculture are suggested for pepper production, such as the combination of AMF + biochar, which supports plant growth and resistance, is beneficial for the soil ecologically and environmentally friendly, and has a significant economic impact. This approach will impact different pathosystems, and the results will be meaningful for sustainable agriculture. Nonetheless, to completely comprehend the mechanisms underlying these benefits and to make the most effective use of these treatments in agricultural systems, further research is required.

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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.

Research involving human participants and/or animals: Not applicable. The research involved no human participants or animals.

Competing interests There is no conflict of interest, according to the writers.

Data sets generated and analyzed during the current study will be provided by the corresponding author upon request of the editor or reviewers.

REFERENCES

- Abd El-Mageed, T.A., Rady, M.M., Taha, R.S., Abd El Azeem, S., Simpson, C.R., & Semida, W.M. (2020). Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of *Capsicum annuum* under salt stress. *Sci Hortic.* 261, 108930. <https://doi.org/10.1016/j.scienta.2019.108930>.
- Akay Rastgeldi, Z.H. (2010). *The Effects of Different Salt Concentrations in Pepper on Some Physiological Parameters and Mineral Matter Content* (master's thesis, unpublished). HU, Institute of Science and Technology, Sanliurfa (Turkish). [10.1007/s10343-023-00897-2](https://doi.org/10.1007/s10343-023-00897-2).
- Akhter, A., Hage-Ahmed, K., Soja, G., & Steinkellner, S. (2015). Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. sp. *lycopersici*. *Frontiers in Plant Sci.* 6, 529. <https://doi.org/10.3389/fpls.2015.00529>.
- Akköprü, A., & Demir, S. (2005). Biological control of *Fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. *J Phytopathol.* 153, 544-550. <https://doi.org/10.1111/j.1439-0434.2005.01018.x>.
- Ali, S., Rizwan, M., Qayyum, M.F., Ok, Y.S., Ibrahim, M., Riaz, M., & Shahzad, A.N. (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ. Sci Pollut Res.* 24, 12700-12712. <https://doi.org/10.1007/s11356-017-8904-x>.
- Azeem, M., Hayat, R., Hussain, Q., Ahmed, M., Pan, G., Tahir, M.I., & Irfan, M. (2019). Biochar improves soil quality and N₂-fixation and reduces net ecosystem CO₂ exchange in a dryland legume-cereal cropping system. *Soil tillage res.* 186, 172-182. <https://doi.org/10.1016/j.still.2018.10.007>.
- Beltrano, J., Ruscitti, M., Arango, M.C., & Ronco, M. (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. *J Soil Sci Plant Nutr.* 13, 123-141. <https://doi.org/10.4067/S0718-95162013005000012>.
- Bennett, A.E., Alers-Garcia, J., & Bever, J.D. (2006). Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypotheses and synthesis. *The American Naturalist* 167(2), 141-152.
- Biró, B., Köves-Péchy, K., Vörös, I., Takács, T., Eggenberger, P., & Strasser, R.J. (2000). Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. *Appl Soil Ecol.* 15, 159-168. [https://doi.org/10.1016/S0929-1393\(00\)00092-5](https://doi.org/10.1016/S0929-1393(00)00092-5).
- Castañeda, W., Toro, M., Solorzano, A., & Zúñiga-Dávila, D. (2020). Production and nutritional quality of tomatoes

- (*Solanum lycopersicum* var. *Cerasiforme*) are improved in the presence of biochar and inoculation with arbuscular mycorrhizae. *Am. J. Plant Sci.* 11(3): 426-436. [10.4236/ajps.2020.113031](https://doi.org/10.4236/ajps.2020.113031)
- Celik, Y. (2023). The effects of different organic fertilizers and reduced doses of chemical fertilizer applications on yield and quality traits in greenhouse melon cultivation. *Rev. Bras. Frutic.* 45, e-538. <https://doi.org/10.1590/0100-29452023538>
- Coşkun, F., Alptekin, Y., & Demir, S. (2021) Reaction of different pepper (*Capsicum annuum* L.) cultivars to isolates of *Verticillium dahliae* Kleb. from various hosts. *YYU J Agr Sci.* 31, 838-846. <https://doi.org/10.29133/yyutbd.882449>
- Coşkun, F., Alptekin, Y., & Demir, S. (2023). Effects of arbuscular mycorrhizal fungi and salicylic acid on plant growth and the activity of antioxidative enzymes against wilt disease caused by *Verticillium dahliae* in pepper. *Eur J Plant Pathol.* 165, 163-177. <https://doi.org/10.1007/s10658-022-02596-6>
- Demir, S., Şensoy, S., Ocak, E., Tüfenkci, Ş., Demirel Durak, E., Erdinc, C., & Ünsal, H. (2015). Effects of Arbuscular Mycorrhizal Fungus, Humic Acid, and Whey on Wilt Disease caused By *Verticillium dahliae* Kleb. In Three Solanaceous Crops. *Turk J Agric For.* 39, 300-309. <https://doi.org/10.3906/tar-1403-39>
- Demirel, Ö., Güneş, H., & Can, C. (2024). Sustainable and modern bio-based technologies: new approaches to food safety and security. *Environ Dev Sustain.* 1-28. doi: <https://doi.org/10.1007/s10668-024-04683-6>
- Dilegge, M.J., Manter, D.K., Vivanco, J.M. (2019). A novel approach to determine generalist nematophagous microbes reveals *Mortierella globalpina* as a new biocontrol agent against *Meloidogyne* spp. nematodes. *Sci Rep* 9, 1-9. <https://doi.org/10.1038/s41598-019-44010-y>
- Elmer, W.H., & Pignatello, J.J. (2011). Effect of biochar amendments on mycorrhizal associations and *Fusarium* crown and root rot of asparagus in replant soils. *Plant Dis.* 95, 960-966. <https://doi.org/10.1094/PDIS-10-10-0741>
- Etesami, H., & Beattie, G.A. (2018). Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Front Microbiol.* <https://doi.org/10.3389/fmicb.2018.00148>
- Food and Agriculture Organization of the United Nations (2020). FAOSTAT [online]. Website <http://www.fao.org/faostat/en/> [Accessed 11.06.2023].
- Farhangi-Abriz, S., & Torabian, S. (2018). Effect of biochar on growth and ion contents of bean plant under saline condition. *Environ Sci Pollut Res.* 25, 11556-11564. <https://doi.org/10.1007/s11356-018-1446-z>
- Fiorilli, V., Vallino, M., Biselli, C., Faccio, A., Bagnaresi, P., & Bonfante, P. (2015). Host and non-host roots in rice: cellular and molecular approaches reveal differential responses to arbuscular mycorrhizal fungi. *Front. Plant Sci.* 6, 636. <https://doi.org/10.3389/fpls.2015.00636>
- Geleta, L.F., Labuschagne, M.T. (2006). Combining ability and heritability for vitamin C and total soluble solids in pepper (*Capsicum annuum* L.). *J Sci Food Agric.* 86, 1317-1320. <https://doi.org/10.1002/jsfa.2494>
- Giri, B., Kapoor, R., & Mukerji, K.G. (2007). Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. *Microb Ecol* 54, 753-760. <https://doi.org/10.1007/s00248-007-9239-9>
- Giono, B.R.W., Solle, M.S., Idrus, M.I., & Sofyan, S. (2021). Utilization of Biochar and Mycorrhiza to Increase the Absorption of Elemental Nutrients of Cayenne Chili Plant (*Capsicum frutescens* L.). *J Trop Agric.* 26, 75-86. <https://doi.org/10.5400/jts.2021.v26i2.75>
- Gruntman, M., & Novoplansky, A. (2011). Ontogenetic contingency of tolerance mechanisms in response to apical damage. *Annals of Botany* 108(5): 965-973. <https://doi.org/10.1093/aob/mcr204>
- Guevara, L., Domínguez-Anaya, M.Á., Ortigosa, A., González-Gordo, S., Díaz, C., Vicente, F., Corpas, F.J., del Palacio, J.P., & Palma, J.M. (2021). Identification of compounds with potential therapeutic uses from sweet pepper (*Capsicum annuum* L.) fruits and their modulation by nitric oxide (NO). *Int. J Mol Sci.* 22, 4476. <https://doi.org/10.3390/ijms22094476>
- Gujre, N., Soni, A., Rangan, L., Tsang, D.C., & Mitra, S. (2021). Sustainable improvement of soil health utilizing biochar and arbuscular mycorrhizal fungi: A review. *Environmental Pollution* 268, 115549. <https://doi.org/10.1016/j.envpol.2020.115549>
- Güneş, H., Demir, S., & Akköprü, A. (2022). Relationship between some plants species belonging to Brassicaceae, Chenopodiaceae and Urticaceae families, and arbuscular mycorrhizal fungi and rhizobacteria. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 25(6), 1350-1360. <https://doi.org/10.18016/ksutarimdoga.vi.1096156>
- Gunes, H., Demir, S., Erdinc, C., & Furan, M.A. (2023). Effects of Arbuscular Mycorrhizal Fungi (AMF) and Biochar On the Growth of Pepper (*Capsicum annum* L.) Under Salt Stress. *Gesunde Pflanz* 1-13. <https://doi.org/10.1007/s10343-023-00897-2>
- Güneş, H., Demir, S., Demirel Durak, E., & Boyno, G. (2024). The effect of Arbuscular Mycorrhizal fungal species *Funneliformis mosseae* and biochar against *Verticillium dahliae* in pepper plants under salt stress. *Eur. J.*

- Plant Pathol.* 1-18. doi: <https://doi.org/10.1007/s10658-024-02926-w>
- Graber, E.R., Meller Harel, Y., Kolton, M., Cytryn, E., Silber, A., Rav David, D., & Elad, Y. (2010). Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil*. 337, 481-496. <https://doi.org/10.1007/s11104-010-0544-6>.
- Graber, E.R., Frenkel, O., Jaiswal, A.K., & Elad, Y. (2014). How May Biochar Influence Severity of Diseases Caused by Soilborne Pathogens? *Carbon Manag.* 5, 169-183. <https://doi.org/10.1080/17583004.2014.913360>.
- Hajiboland, R., Aliasgharzadeh, N., Laiegh, S.F., & Poschenrieder, C. (2010). Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil*. 331, 313-327. <https://doi.org/10.1007/s11104-009-0255-z>.
- Hammer, E.C., Balogh-Brunstad, Z., Jakobsen, I., Olsson, P.A., Stipp, S.L., & Rillig, M.C. (2014). A mycorrhizal fungus grows on biochar and captures phosphorus from its surfaces. *Soil Biol Biochem.* 77, 252-260. <https://doi.org/10.1016/j.soilbio.2014.06.012>.
- Hashem, A., Kumar, A., Al-Dbass, A.M., Alqarawi, A.A., Al-Arjani, A.B.F., Singh, G., & Abd_Allah, E.F. (2019). Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. *Saudi J. Biol. Sci.* 26(3), 614-624. <https://doi.org/10.1016/j.sjbs.2018.11.005>
- Hawkins, H.J., Cargill, R.I., Van Nuland, M.E., Hagen, S.C., Field, K.J., Sheldrake, M., & Kiers, E.Tç (2023). Mycorrhizal mycelium as a global carbon pool. *Curr. Biol.* 33(11), R560-R573. <https://doi.org/10.1016/j.cub.2023.02.027>
- Ippolito, J.A., Laird, D.A., & Busscher, W.A. (2012). "Environmental Benefits of Biochar". *J Environ Qual.* 41, 967-972. <https://doi.org/10.2134/jeq2012.0151>.
- Jackson, M.L. (1958). *Chemical Composition of Soils*, 71-141. In F.E. Bear (ed.) *Chemistry of the soil*, 2nd edition. Reinhold Publ. Corp., New York.
- Jaafar, N.M. (2014). *Biochar as a habitat for arbuscular mycorrhizal fungi. In Mycorrhizal fungi: use in sustainable agriculture and land restoration* (297-311). Berlin Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-45370-4_19
- Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A., & Pozo, M.J. (2012). Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.* 38, 651-664. <https://doi.org/10.1007/s10886-012-0134-6>
- Kacar, B. (1984). *Practice Guide of Plant Nutrition*. Ankara University, Publications of Agricultural Faculty: Ankara, Turkey.
- Kacjan Maršić, N., Štolfa, P., Vodnik, D., Košmelj, K., Mikulič-Petkovšek, M., Kump, B., & Šircelj, H. (2021). Physiological and Biochemical Responses of Ungrafted and Grafted Bell Pepper Plants (*Capsicum annuum* L. var. *grossum* (L.) Sendtn.) Grown under Moderate Salt Stress. *Plants*, 10, 314. <https://doi.org/10.3390/plants10020314>.
- Karagiannidis, N., Bletsos, F., & Stavropoulos, N. (2002). Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Sci Hort.* 94, 145-156. [https://doi.org/10.1016/S0304-4238\(01\)00336-3](https://doi.org/10.1016/S0304-4238(01)00336-3).
- Kesimci, T.G., Demirci, E., Şimşe, U., Tohumcu, F., & Erdel, E. (2019). The effect of *Verticillium dahliae* on the amount of nutrients in strawberry plants. *J. Instit.Sci. Technol.* 9, 626-635. <https://doi.org/10.21597/jist.556229>.
- Khriebe, M.I., Sharifnabi, B., & Zangeneh, S. (2019). Interaction between Arbuscular Mycorrhiza Fungi (AMF) with *Verticillium dahliae* Kleb. on Olive Tree under Greenhouse Conditions. *Res. J. Agric. Sci.* 6, 185-191. <https://doi.org/10.1128/AEM.00148-11>.
- Kolton, M., Meller Harel, Y., Pasternak, Z., Graber, E.R., Elad, Y., & Cytryn, E. (2011). Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Appl. Environ. Microbiol.* 77, 4924-4930. <https://doi.org/10.1128/AEM.00148-11>.
- Kotuby-Amacher, J., Koenig, R., & Kitchen, B. (2000). *Salinity and Plant Tolerance. Electronic Publication AG-SO-03*, Utah State University Extension, Logan.
- Köhl, J., Kolnaar, R., & Ravensberg, W.J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Front. Plant Sci.* 10, 845. <https://doi.org/10.3389/fpls.2019.00845>
- Lamb, C., Dixon, R.A. (1997). The oxidative burst in plant disease resistance. *Annu Rev Plant Biol.* 48, 251-275. <https://doi.org/10.1146/annurev.arplant.48.1.251>.
- Liu, J., Zheng, Z., Zhou, X., Feng, C., & Zhuang, Y. (2015). Improving the resistance of eggplant (*Solanum melongena*) to *Verticillium* wilt using wild species *Solanum linnaeanum*. *Euphytica*. 201, 463-469. <https://doi.org/10.1007/s10681-014-1234-x>.
- Low, P.S., & Merida, J.R. (1996). The oxidative burst in plant defense: function and signal transduction. *Physiol Plant.* 96, 533-542. <https://doi.org/10.1111/j.1399-3054.1996.tb00469.x>.
- Nguyen, V.T., Edward, C.Y.L., Lester, W.B. (2010). Characterization of *P. capsici* isolates from black pepper in Vietnam. *Fungal Biol.* 114, 160-170. doi.org/10.1016/j.funbio.2009.11.005
- Novak, J.M., Busscher, W.J., Watts, D.W., Amonette, J.E., Ippolito, J.A., Lima, I.M., & Schomberg, H. (2012).

- Biochars impact on soil-moisture storage in an ultisol and two aridisols. *Soil Sci.* 177, 310-320. <https://doi.org/10.1097/SS.0b013e31824e5593>.
- Nzanza, B., Marais, D., & Soundy, P. (2012). Effect of arbuscular mycorrhizal fungal inoculation and biochar amendment on growth and yield of tomato. *Int J Agric Biol.* 14, 965-969.
- Ogundeji, A.O., Li, Y., Liu, X., Meng, L., Sang, P., Mu, Y., & Li, S. (2021). Eggplant by grafting enhanced with biochar recruits specific microbes for disease suppression of *Verticillium* wilt. *Appl Soil Ecol.* 163, 103912. <https://doi.org/10.1016/j.apsoil.2021.103912>.
- Oztekin, G.B., Tuzel, Y., Tuzel, I.H. (2013). Does mycorrhiza improve salinity tolerance in grafted plants? *Sci Hort.* 149, 55-60. <https://doi.org/10.1016/j.scienta.2012.02.033>.
- Özdamar, K. (2010). Paket programlar ile istatistiksel veri analizi II (çok değişkenli analizler) [Statistical data analysis with package programs II (multivariate analysis)], Kaan Kitabevi, 7. Baskı, Eskişehir.
- Palansooriya, K.N., Yang, Y., Tsang, Y.F., Sarkar, B., Hou, D., Cao, X., Meers, E., Rinklebe, J., Kim, K., & Ok, Y.S. (2020). Occurrence of contaminants in drinking water sources and the potential of biochar for water quality improvement: A review. *Crit Rev Environ Sci Technol.* 50, 549-611. <https://doi.org/10.1080/10643389.2019.1629803>.
- Pegg, G.F., & Brady, B.L. (2002). *Verticillium* Wilts. CABI Publishing. CAB International Wallingford, UK.
- Pozo, M.J., Azcón-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.* 10(4), 393-398. <https://doi.org/10.1016/j.pbi.2007.05.004>
- Pozo, M.J., Jung, S.C., López-Ráez, J.A., & Azcón-Aguilar, C. (2010). Impact of arbuscular mycorrhizal symbiosis on plant response to biotic stress: the role of plant defence mechanisms. *Arbuscular mycorrhizas: physiology and function* 193-207.
- Rousk, J., Brookes, P.C., & Baath, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl Environ Microbiol.* 75, 1589-1596. <https://doi.org/10.1128/AEM.02775-08>.
- Sagar, A., Rathore, P., Ramteke, P.W., Ramakrishna, W., Reddy, M.S., & Pecoraro, L. (2021). Plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and their synergistic interactions to counteract the negative effects of saline soil on agriculture: Key macromolecules and mechanisms. *Microorganisms.* 9, 1491. <https://doi.org/10.3390/microorganisms9071491>.
- Schnathorst, W.C. (1981). *Life Cycle and Epidemiology of Verticillium*. *Fungal Wilt Diseases of Plants*. Academic Press, New York, 640. <https://doi.org/10.3390/microorganisms9071491>.
- Schüßler, A., & Walker, C. (2010). *The Glomeromycota: a species list with new families and new genera*. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh; The Royal Botanic Garden Kew; Botanische Staatssammlung Munich, and Oregon State University.
- Shi, R.Y., Hong, Z.N., Li, J.Y., Jiang, J., Kamran, M.A., Xu, R.K., & Qian, W. (2018). Peanut straw biochar increases the resistance of two Ultisols derived from different parent materials to acidification: A mechanism study. *J Environ Manage.* 210, 171-179. <https://doi.org/10.1016/j.jenvman.2018.01.028>.
- Tjamos, E.C., Rowe, R.C., Heale, J.B., & Fravel, D.R. (2000). Advances in *Verticillium* research and disease management: proceedings. In 7. International *Verticillium* Symposium 1971-1997 Silver Jubilee 6-10 Oct 1997 Cape Sounion, Atenas (Grecia) (No. 632.4521 I61 1997). American Phytopathological Society, St. Paul, MN (EUA).
- Tripathi, A., Maurya, S., Pandey, K. K., & Behera, T. K. (2024). Global Scenario of Vegetable Fungal Diseases. *Vegetable Science*, 51, 54-65. <https://doi.org/10.61180/vegsci.2024.v51.spl.06>
- Tyvaert, L., Everaert, E., Lippens, L., Cuijpers, W.J.M., França, S.C., & Höfte, M. (2019). Interaction of *Colletotrichum coccodes* and *Verticillium dahliae* in pepper plants. *Eur J. Plant Pathol.* 155, 1303-1317. <https://doi.org/10.1007/s10658-019-01857-1>.
- U.S. Salinity Laboratory Staff. (1954). Methods for soil characterization. p 83-147. In Diagnosis and improvement of saline and alkali soils. USDA-Agricultural Handbook No. 60. U.S. Government Printing Office, Washington, D.C.
- Vahedi, R., Rasouli-Sadaghiani, M.H., Barin, M., & Vetukuri, R.R. (2022). Effect of Biochar and Microbial Inoculation on P, Fe, and Zn Bioavailability in a Calcareous Soil. *Processes*. <https://doi.org/10.3390/pr10020343>.
- Van der Ent, S., Van Wees, S.C., & Pieterse, C.M. (2009). Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry* 70(13-14): 1581-1588. <https://doi.org/10.1016/j.phytochem.2009.06.009>
- Were, S.A., Narla, R., Mutitu, E.W., Muthomi, J.W., Munyua, L.M., Roobroeck, D., & Valauwe, B. (2021). Biochar and vermicompost soil amendments reduce root rot disease of common bean (*Phaseolous Vulgaris* L.). *Afr J Biol Sci.* 3, 176-196. <https://doi.org/10.33472/AFJBS.3.1.2021.176-196>.
- Wu, Q.S., Zou, Y.N., & He, X.H. (2010). Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. *Acta Physiol Plant.* 32, 297-304.

<https://doi.org/10.1007/s11738-009-0407-z>.

- Xu, G., Zhang, Y., Sun, J., & Shao, H. (2016). Negative interactive effects between biochar and phosphorus fertilization on phosphorus availability and plant yield in saline sodic soil. *Sci Total Environ.* 568, 910-915. <https://doi.org/10.1016/j.scitotenv.2016.06.079>.
- Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., & Wang, G. (2017). Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. *Soil Biol Biochem.* 110, 56-67. <https://doi.org/10.1016/j.soilbio.2017.03.005>.
- Zhang, S., Lehmann, A., Zheng, W., You, Z., & Rillig, M.C. (2019 a). Arbuscular Mycorrhizal Fungi Increase Grain Yields: A Meta-Analysis. *New Phytol.* 222, 543-555. <https://doi.org/10.1111/nph.15570>.
- Zhang, J., Bai, Z., Huang, J., Hussain, S., Zhao, F., Zhu, C., Jin, Q. (2019 b). Biochar alleviated the salt stress of induced saline paddy soil and improved the biochemical characteristics of rice seedlings differing in salt tolerance. *Soil tillage res.* 195. 104372. <https://doi.org/10.1016/j.still.2019.104372>.
- Zhuo, F., Zhang, X.F., Lei, L.L., Yan, T.X., Lu, R.R., Hu, Z.H., & Jing, Y.X. (2020). The effect of arbuscular mycorrhizal fungi and biochar on the growth and Cd/Pb accumulation in *Zea mays*. *Int J Phytoremediation.* 22, 1009-1018. <https://doi.org/10.1080/15226514.2020.1725867>.

Adult emergence course of *Kermania pistaciella* Amsel (Lepidoptera:Tineidae) in Culture Cages and Population Change on Pheromone Traps at Three Different Altitudes of Gaziantep

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ABSTRACT

Pistachio twig borer moth [*Kermania pistaciella* Ams. (Lep: Tineidae)] is the economically damaging species where pistachio is grown. As a result of the feeding of the larvae of the pest, the fruit buds fall off, the damaged shoots remain short. During the fruit formation period, the larvae enter the clusters and feed on the cluster stems, so the fruits fail to develop or fall off. With this work, adult emergence of *K. pistaciella* in culture cages and pheromone traps placed at three different altitudes (705 m, 531 m and 856 m) between 2015 and 2017 has been followed. Between 2015 and 2017, population monitoring in pheromone traps started on average between the last week of March and the first week of April in all locations, peaked in the third week of April, and ended between the last week of April and the last week of May. Adult emergence in culture cages in all locations in 2015 and 2017 started in the second week of April on average, and in the last week of March in all locations in 2016. Adult emergence peaked in the third week of April in all locations between 2015 and 2017 and ended between the last week of April and the first week of May. This work is the first study in which population follow-up of pistachio twig borer in pheromone traps and culture cages was carried out together.

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Population dynamics

Antepfıstığı Dalgüvesi *Kermania pistaciella* Amsel (Lepidoptera:Tinediae) 'nın Gaziantep İlinde Üç Farklı Rakımda Kültür Kafeslerinde Ergin Çıkış Seyri ve Feromon Tuzaklardaki Popülasyon Değişimi

ÖZET

Antepfıstığı dalgüvesi [*Kermania pistaciella* Ams. (Lep: Tineidae)] antepfıstığının yetiştirildiği alanlarda ekonomik anlamda zarar yapan türler arasındadır. Zararlının larvalarının beslenmesi sonucu meyve gözleri dökülmekte, zarar gören ve sürgünler kısa kalmaktadır. Meyve oluştuğu dönemde ise salkımlardan içeri giren larvalar salkım saplarında beslenmekte, bu nedenle meyveler gelişmemekte veya dökülmektedir. Bu çalışma ile; 2015-2017 yılları arasında üç farklı rakımda (705 m, 531 m ve 856 m) yerleştirilen kültür kafeslerinde ve feromon tuzaklarda antepfıstığı dalgüvesi (*K. pistaciella*) 'nın ergin çıkış seyri takip edilmiştir. 2015-2017 yılları arasında feromon tuzaklardaki popülasyon takibi bütün lokasyonlarda ortalama Mart ayının son haftası ile Nisan ayının ilk haftası arasında başlamış, Nisan ayının üçüncü haftası pik yapmış ve Nisan ayının son haftası ile Mayıs ayının son haftası arasında sona ermiştir. 2015 ve 2017 yıllarında tüm lokasyonlarda kültür kafeslerinde ergin çıkışları ortalama Nisan ayının ikinci haftasında, 2016 yılında ise farklı olarak bütün lokasyonlarda Mart ayının son haftasında başlamıştır. Ergin çıkışları 2015-2017 yılları arasında bütün lokasyonlarda Nisan ayının üçüncü haftasından itibaren pik yapmış ve Nisan ayının son haftası ile Mayıs ayının ilk haftası arasında sona ermiştir. Bu çalışma; Antepfıstığı dalgüvesinin feromon tuzak ve kültür kafeslerinde popülasyon takibinin birlikte yapıldığı ilk çalışma özelliğini taşımaktadır.

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Feromon tuzak
Antepfıstığı dalgüvesi
Popülasyon değişimi

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INTRODUCTION

Pistachio fruit, which has been cultivated by the people of the Southeastern Anatolia region since ancient times, provides a significant economic contribution to the producers of the region by increasing the quality and taste of every food item it is used with, together with the richness of protein, antioxidants, aroma, vitamins and minerals it contains. In addition, in this region, pistachio cultivation is a great gain for both the country and the farmer's economy in lands with limited irrigation water, low rainfall (300-500 mm) and which cannot be used economically by many cultivated plants (Eldoğan and Şahin,2015). Pistachio production provides the people living in the region with the opportunity to earn a living and additional income (Arpacı, 2010).

The world's major pistachio producing countries were examined between 2020 and 2023, and it was seen that the USA ranked first with 433,521 tons of production (USDA 2024), Turkey ranked second with 207,755 tons of production (TUIK 2024), and Iran ranked third with 162,500 tons (IPA 2024). There are 4.087.086 da pistachio production is carried out in the area of Türkiye (TUIK 2024c). The majority of pistachio production is obtained from the provinces in the Southeastern Anatolia and Eastern Anatolia regions. Among the provinces in Southeastern Anatolia, where pistachios are grown intensively in Turkey, the number of fruit bearing pistachio trees is 18.681.133 in Gaziantep, 22.446.288 in Şanlıurfa, 5.129.726 in Adıyaman, 943.400 and 7.285.643 in Kahramanmaraş and Siirt, respectively (TUIK, 2024a). Moreover, as of 2022, there are 25.469.721 pistachio trees that have not yet produced fruit (TUIK 2024b), and with the increase in new pistachio plantations, it is expected that fruit yield and economic gain will increase in the future.

In pistachios, which make a significant contribution to the country's economy, there are different types of insects that damage both the crop produced in the same year and the crop of the next year (Karagöz). At the same time, they reduce the yield and quality of the product. Özer (1958) in the wild pistachio fields of Balıkesir and Kütahya provinces, Ulu et al. (1972) in the Aegean Region, Çelik (1975) in Gaziantep; Günaydın (1978), Bolu, (2002) found more than 40 pest and mite species in their studies on pistachio fields in the Southeastern Anatolia Region.

Among these important pests, pistachio moth is concentrated in Gaziantep, Şanlıurfa and Siirt; it has been determined by preliminary studies that fruit clusters and leaves fall as a result of feeding the larvae in the shoot, and that the larvae in the clusters cause damage to the cluster stems, leaving the fruits small and at the same time causing losses such as fruit drop (Şahan and Tunaz 2021).

Mehrnejad (2001, 2003), Van Achterberg and Mehrnejad (2002), Emami et al., (2004), Bassirat (2005), Manickavasagam et al., (2008), Abbaszadeh et al. (2006, 2011), Tezerji, (2011), Izadi et al. (2011) Arbabtafti et al. (2012), Gries et al. (2006, 2007), Mollaei et al. (2017) conducted various studies on the biology, development threshold, damage threshold, population dynamics, some morphological features, parasitoids and control of *K. pistaciella*. In Türkiye, Küçükarslan (1966), Bolu (2002), Mart et al. (1995), Yanık and Yücel (2001), Özgen et al. (2012), Şimşek and Bolu (2016), Yanık and Yıldırım (2016) obtained important data on some biological and morphological characteristics, population dynamics, parasitoids and control of *K. pistaciella*.

In this study, which was carried out to prepare the groundwork for studies on controlling pistachio twig borer (*K. pistaciella*), the pest was monitored both in culture cages and pheromone traps at three different altitudes of Gaziantep which were Alahacı (705 m) and Yağmurlan locations (531 m) in Nizip district and Institute orchard (856 m) in Şahinbey district.

MATERIAL and METHOD

The main material of the study, Pistachio orchards infested with pests in Gaziantep (Nizip district Alahacı and Yağmurlan locations and Şahinbey district Pistachio Research Institute Directorate orchard) one-year pistachio shoots obtained from these orchards, adults and pupae of the pest were formed. Stereo binocular microscope (Leica EZ4D brand), forceps, scalpel, mouth aspirator, ethyl acetate, 70% ethyl alcohol, insect needles (000 and 2 size), camera, vineyard scissors, petri dishes (9x1cm), ice bowl, culture cages with wooden frames and insect net (70x70x100 cm), eppendorf tubes, culture dishes, soft-tipped brushes (number 00, 0, 1, 2 and 3), transparent polyethylene bags, paper bags, plastic jars, culture containers and other laboratory materials, delta type pheromone trap [(2S,12Z)-2-Acetoxy-heptadecene] and climate data logger (Hobo and Metos brand) were used as materials. Additionally, data such as the highest-lowest temperature values and the daily average humidity values were used.

Sampling Method:

Two delta type sexual attractive traps were hung in the orchards of Şahinbey location and Yağmurlan and Alahacı locations of Nizip District selected for the experiment in mid-March between 2015 and 2017 and were checked twice a week. Sexual attractive traps were hung at a height of 1–1.5 m from the ground and in the prevailing wind direction to represent the orchard. The traps were counted daily in the parcel located in the orchard of the Institute Şahinbey location after the first butterfly was caught, and once a week in Alahacı (705 m) and Yağmurlan locations (531 m), and the numbers of the caught butterflies were recorded and removed from the traps. Trap capsules were changed every 4 weeks in line with the company's recommendation, and the sticky tray was changed when it became dirty.

In order to monitor the adult emergence of the pest from the culture cages, two culture cages were left at the same locations at the end of February in the same years. One or two-year-old shoots on which pupae were detected, collected in February-March, were placed in each of the cages. In the study, 100 shoots with pupae were placed in the culture cages in all locations in 2015. However, since the number of adults collected from the experimental area in Şahinbey location in 2015 was found insufficient to be used in other studies, 600 shoots with pupae were placed in cages in Şahinbey location and 100 shoots with pupae were placed in cages in Yağmurlan and Alahacı locations in 2016 and 2017.

RESULTS

Adult population changes of *Kermania pistaciella* in pheromone traps at different altitudes

Adult population changes of *Kermania pistaciella* in pheromone traps and culture cages in Şahinbey Location of Gaziantep Province.

In order to monitor the adult population change, pheromone traps were set up in the orchard of the Institute in Şahinbey location of Gaziantep province.

The adult emergence course of *K. pistaciella* in pheromone traps between 2015 and 2017 at the location Şahinbey is given in Figure 1. According to Figure 1. In 2015, the adult emergence in the orchard in Şahinbey location started on 17 April, reached the highest number on 28 April, and ended on 9 May. The adult emergence took 22 days. When the temperature values started to reach a minimum of 18 °C, a maximum of 22 °C and the average proportional humidity value was about 36%, the first adult emergences were seen. The highest temperature was 26°C, the lowest temperature was 19°C, and the daily average proportional humidity was around 42% at the time when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 1.B. the adult emergence in pheromone traps started on April 4, reached the highest number on April 16, and ended on April 26. The adult emergence took 22 days in traps. When the temperature values started to reach a minimum of 16 °C, a maximum of 22 °C and the average proportional humidity value was about 20%, the first adult emergences were seen. The highest temperature was measured at 25 °C, the lowest temperature was 19 °C and the daily average proportional humidity was around 25% at the time when the adult emergence reached its highest level. When Figure 1.C. of 2017 is examined, the adult emergence in the orchard in Şahinbey location started on 18 April in pheromone traps, reached the highest number on 29 April and ended on 12 May. The adult emergence took 24 days in traps. When the temperature values started to reach a minimum of 7 °C, a maximum of 20 °C and the average proportional humidity value was about 64%, the first adult emergences were seen. The highest temperature was 29 °C, the lowest temperature was 10 °C and the daily average proportional humidity was around 38% on the date when the adult emergence reached its highest level.

Adult population change of *Kermania pistaciella* in pheromone traps in Gaziantep Province Nizip District Yağmurlan Location

In order to monitor the adult population change, pheromone traps were set up in the farmer's orchard in the Yağmurlan location of the Nizip District of Gaziantep.

The data of the study in which the adult emergence course of *K. pistaciella* in pheromone traps was determined between the years 2015-2017 in the farmer's orchard of Gaziantep province Nizip district Yağmurlan location are given in Figure 2 A.B.C. According to Figure 2.A, in 2015, the adult emergence in the orchard in Yağmurlan location started on 7 April, reached the highest number on 28 April and ended on 12 May. The adult emergence took 35 days. When the temperature values started to reach a minimum of 8 °C, a maximum of 25 °C and an average proportional humidity was about 53%, the first adult emergences were observed. The highest temperature was 31°C, the lowest temperature was 11°C, and the daily average proportional humidity was around 38% at the time when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 2.B, the adult emergence in pheromone traps started on March 23, reached the highest number on April 12, and

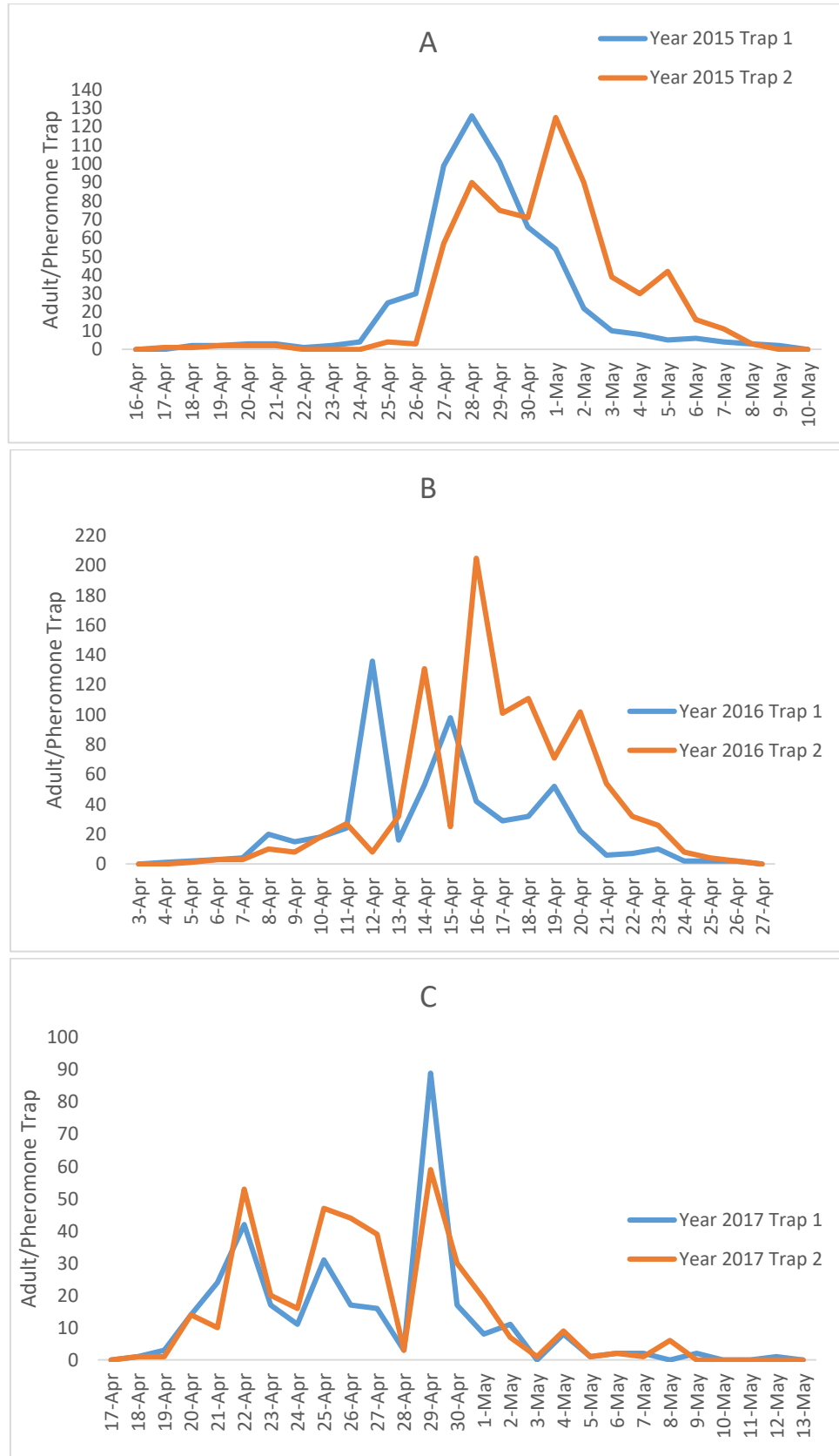


Figure 1. Population change of *Kermania pistaciella* in pheromone traps in Gaziantep Şahinbey Pistachio Research Institute Directorate orchard in 2015 (A), 2016 (B) and 2017 (C)

Şekil 1. *Kermania pistaciella*'nın Gaziantep Şahinbey antepfıstığı Araştırma Enstitüsü Müdürlüğü bahçesindeki feromon tuzaklarda 2015 (A), 2016 (B) ve 2017 (C) yılı populasyon değişimi

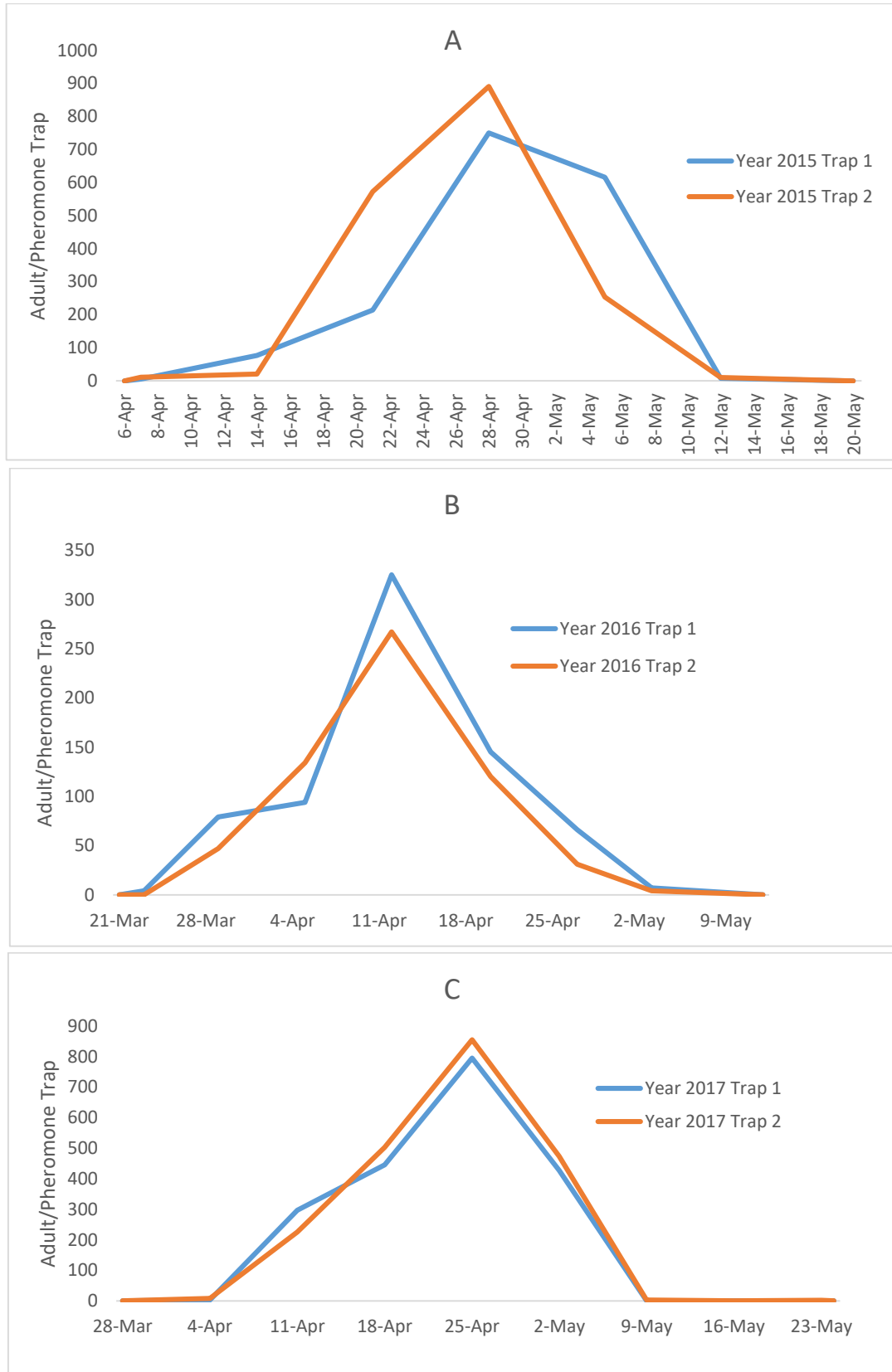


Figure 2. Population change of *Kermania pistaciella* in 2015 (A), 2016 (B) and 2017 (C) years in the farmer's orchard in Gaziantep Nizip district Yağmuralan location

Şekil 2. *Kermania pistaciella*'nın Gaziantep Nizip ilçesi Yağmuralan lokasyonu çiftçi bahçesindeki 2015 (A), 2016 (B) ve 2017 (C) yılı populasyon değişimi

ended on May 3. The adult emergence took 42 days in traps. When the temperature values started to reach a minimum of 9 °C, a maximum of 22 °C and the average proportional humidity value was around 43%, the first adult emergences were seen. The highest temperature was 18 °C, the lowest temperature was 10 °C and the daily average proportional humidity was about 64% on the date when the adult emergence reached its highest level.

When Figure 2.C of 2017 is examined, it is seen that the adult emergence in the orchard in Yağmurlan location started on 4 April in pheromone traps, reached the highest number on 25 April and ended on 23 May. Adult emergence took 50 days in traps. When the temperature values started to reach a minimum of 13 °C, a maximum of 16 °C and the average proportional humidity value was about 83%, the first adult emergences were seen. The highest temperature was 23 °C, the lowest temperature was 12 °C and the daily average proportional humidity was around 25% at the time when the adult emergence reached its highest level.

Adult population change of *Kermania pistaciella* in pheromone traps in Gaziantep Province Nizip District Alahacı Location.

In order to monitor the adult population change, pheromone traps were set up in the farmer's orchard in the Alahacı location of Nizip District of Gaziantep.

The data of the study in which the adult emergence course of *K. pistaciella* in pheromone traps was determined between 2015-2017 in the farmer's orchard of Alahacı location. According to Figure 3.A, in 2015, the adult emergence in the orchard in Alahacı location started on 7 April, reached the highest number on 28 April and ended on 28 May. The adult emergence lasted 51 days. When the temperature values started to reach a minimum of 11 °C, a maximum of 22 °C and the average proportional humidity value was around 55%, the first adult emergences were seen. The highest temperature was 26 °C, the lowest temperature was 14 °C and the daily average proportional humidity was around 39% on the date when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 3.B, the first adult emergence started on March 24, reached the highest number on April 20 and ended on May 12. The adult emergence lasted 50 days. When the temperature values started to reach a minimum of 9°C, a maximum of 22°C and the average proportional humidity value was about 47%, the first adult emergences were seen. The highest temperature was 19 °C, the lowest temperature was 11 °C and the daily average proportional humidity was around 63% on the date when the adult emergence reached its highest level. When Figure 3.C of 2017 is examined, the adult emergence in the orchard in Alahacı location started on 3 April in pheromone traps, reached the highest number on 19 April and ended on 24 May. Adult emergence took 51 days in traps. When the temperature values reached a minimum of 10 °C, a maximum of 18 °C, and the average proportional humidity value was around 39%, the first adult emergences were seen. The highest temperature was 20 °C, the lowest temperature was 11 °C and the daily average proportional humidity was around 80% on the date when the adult emergence reached its highest level.

Adult population variation of *Kermania pistaciella* in culture cages at different altitudes

From the areas where the experiment was established, two culture cages were placed in the orchard in the Şahinbey District of Gaziantep and the Yağmurlan and Alahacı neighborhoods of the Nizip District, and the population of the pest was monitored at three different altitudes between 2015 and 2017.

Adult population change of *Kermania pistaciella* in culture cages in Şahinbey District of Gaziantep Province

In order to monitor the adult population change, two culture cages were placed in the Institute orchard in Şahinbey District of Gaziantep province. In order to monitor adult emergence, shoots with pupae over 100 in 2015 and 600 each in 2016 and 2017 due to insufficient number of adults to be used in other studies were placed in culture cages.

The adult emergence course of *K. pistaciella* in culture cages between 2015-2017 at the Şahinbey location is given in Figure 4.A.B.. According to Figure 4.A, in 2015, the adult emergence in culture cages in the orchard in Şahinbey location started on April 27, reached the highest number on April 29 and ended on May 7. The adult emergence took 10 days. When the temperature values started to reach a minimum of 15 °C, a maximum of 22 °C and the average proportional humidity value was around 46%, the first adult emergences were seen. The highest temperature was 27 °C, the lowest temperature was 21 °C and the daily average proportional humidity was around 36% on the date when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 4.B., the adult emergence in the culture cages started on 25 March, reached the highest number on 15 April and ended on 25 April. Adult emergence took 22 days in culture cages. When the temperature values started to reach a minimum of 12 °C, a maximum of 14 °C and the average proportional humidity value was around 52%, the first adult emergences were seen in the culture cages. The highest temperature was 19 °C, the lowest temperature was 18 °C and the daily average proportional humidity was around 52% when the adult emergence reached the highest level in the culture cages. When Figure 4.C. of 2017 is examined, the adult emergence in the orchard in Şahinbey location started on 18 April in the culture cages, reached the highest number on 22 April and

ended on 9 May. The adult emergence took 22 days in culture cages. When the temperature values started to reach a minimum of 7 °C, a maximum of 20 °C and an average proportional humidity was around 63%, the first adult emergences were observed. The highest temperature was 26 °C, the lowest temperature was 10 °C and the daily average proportional humidity was around 40% at the time when the adult emergence reached its highest level.

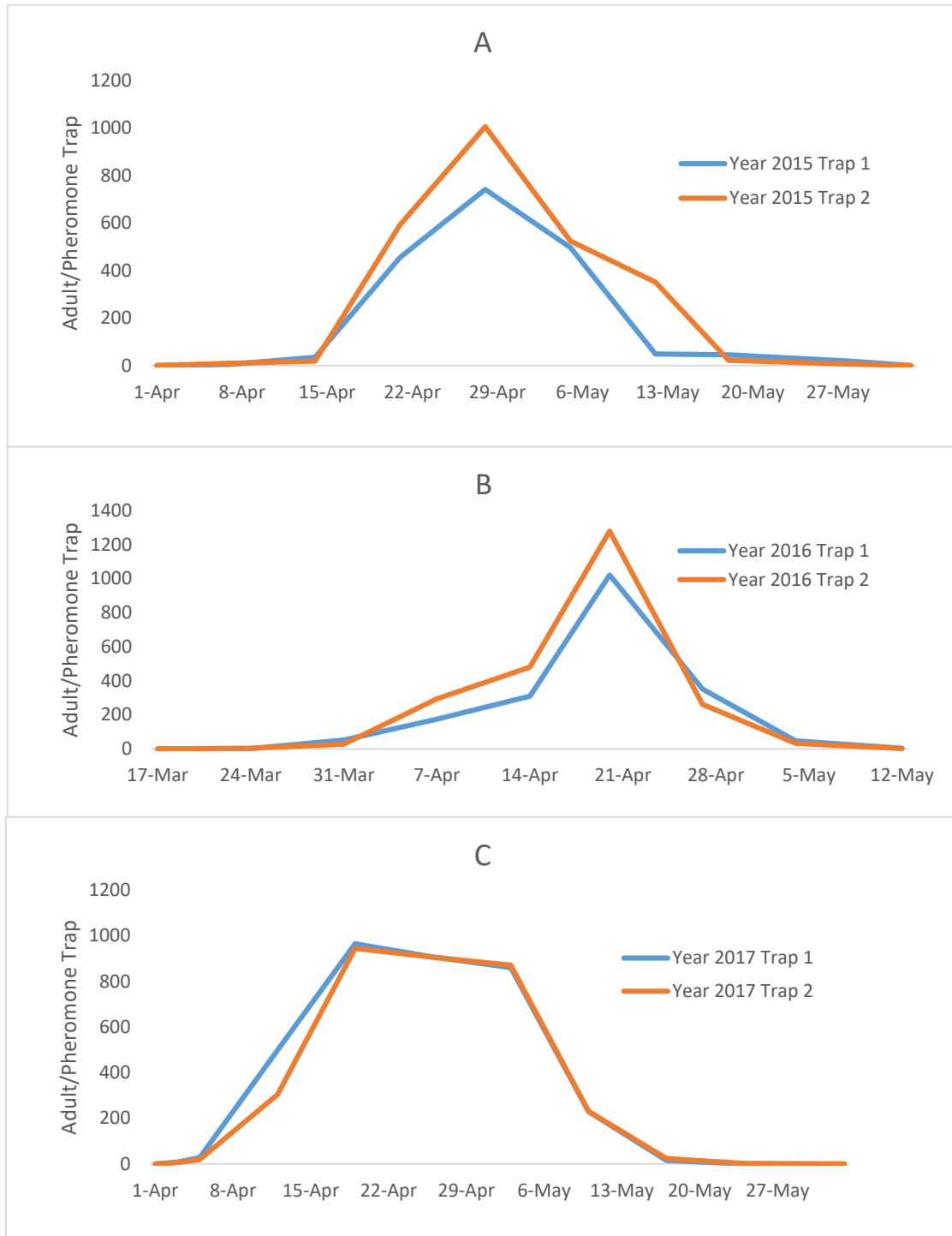


Figure 3. Population change of *Kermania pistaciella* in the farmer's orchard in Gaziantep Nizip district Alahacı location in 2015 (A), 2016 (B) and 2017 (C) years

Şekil 3. *Kermania pistaciella*'nın Gaziantep Nizip ilçesi Alahacı lokasyonu çiftçi bahçesindeki 2015 (A), 2016 (B) ve 2017 (C) yılı popülasyon değişimi

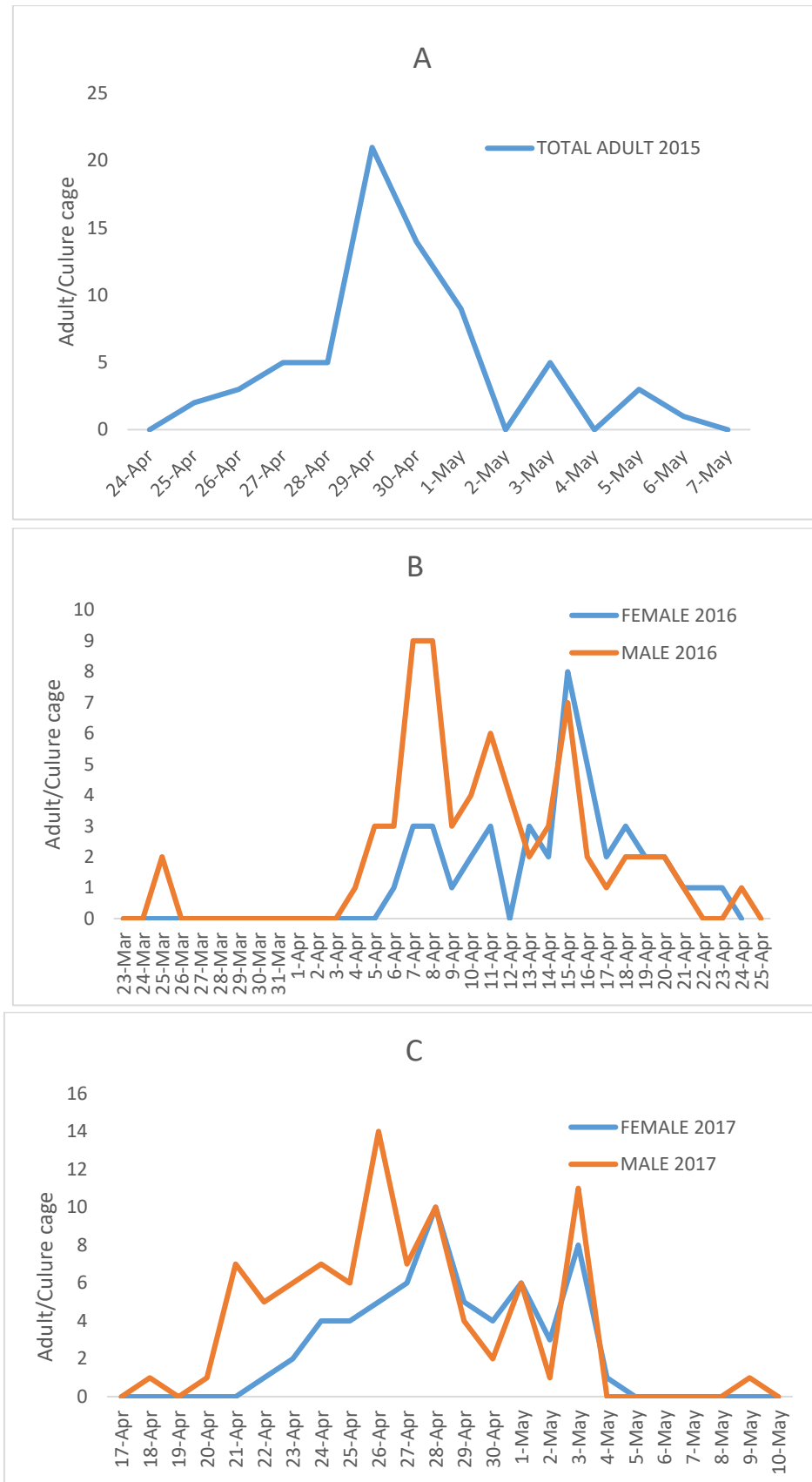


Figure 4. Population change of *Kermania pistaciella* in culture cages in the orchard of Gaziantep Şahinbey Pistachio Research Institute Directorate in 2015 (A), 2016 (B) and 2017 (C)

Şekil 4. *Kermania pistaciella* 'nın Gaziantep Şahinbey Antepfıstığı Araştırma Enstitüsü Müdürlüğü bahçesindeki 2015 (A), 2016 (B) ve 2017 (C) yılı kültür kafeslerinde ergin popülasyon değişimi

Adult population change of *Kermania pistaciella* in culture cages in Gaziantep Province Nizip District Yağmuralan Location

In order to monitor the adult population change, two culture cages were placed in the farmer's orchard in Yağmuralan location. In order to monitor the adult emergence, shoots with more than 100 pupae were placed in culture cages between 2015 and 2017.

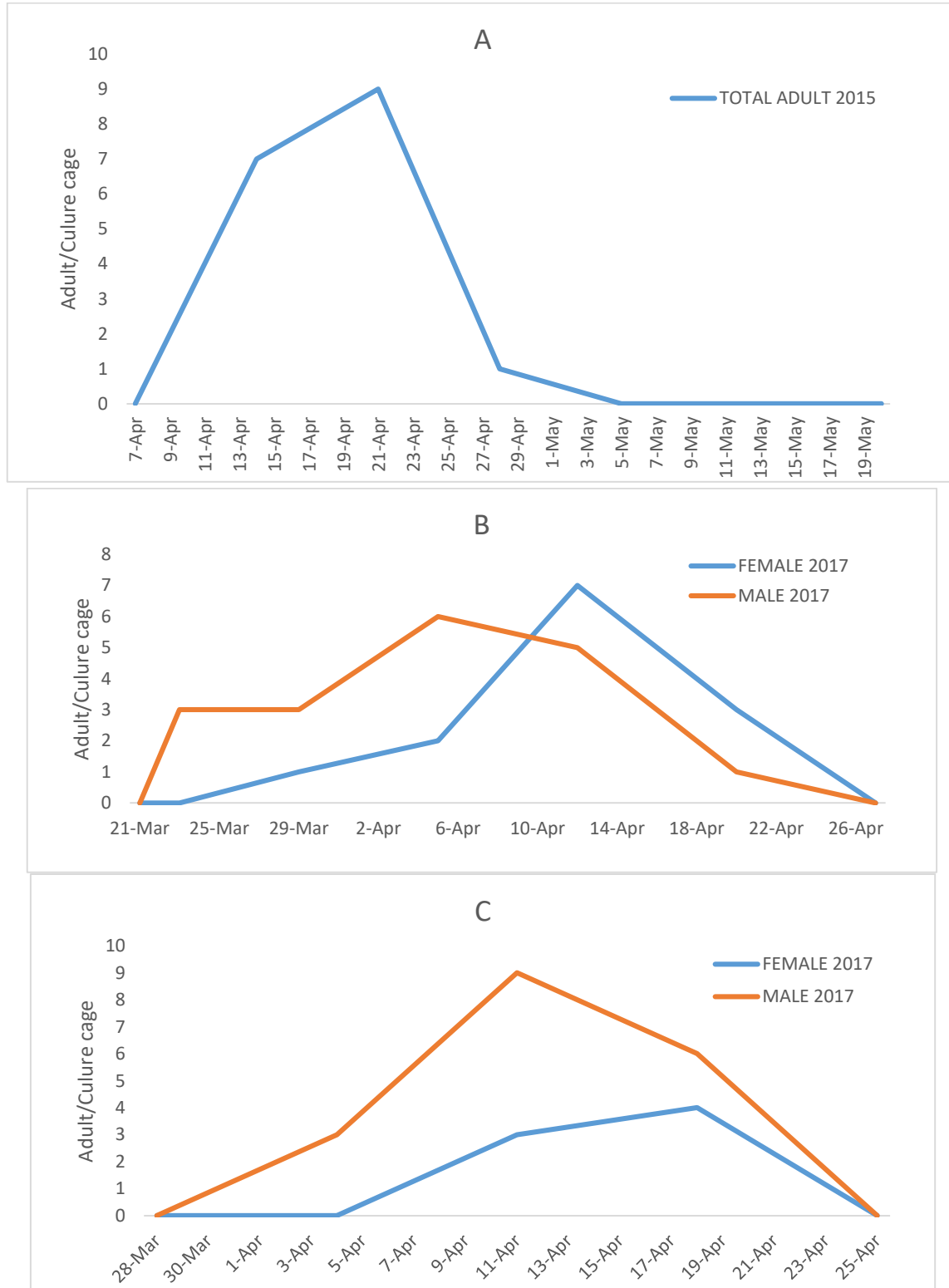


Figure 5. Adult population change of *Kermania pistaciella* in 2015 (A), 2016 (B) and 2017 (C) culture cages in Gaziantep Province Nizip District Yağmuralan neighborhood farmer's orchard

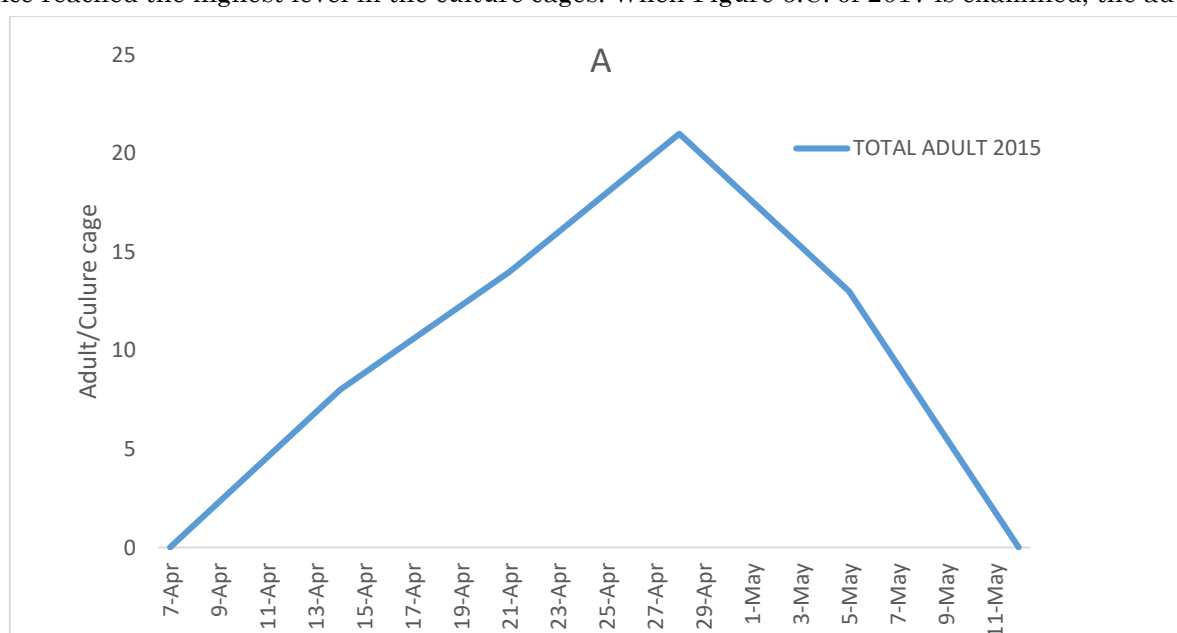
Şekil 5. *Kermania pistaciella*'nın Gaziantep İli Nizip İlçesi Yağmuralan lokasyonu çiftçi bahçesindeki 2015 (A), 2016 (B) ve 2017 (C) yılı kültür kafeslerinde ergin populasyon değişimi

According to Figure 5.A., in 2015, the adult emergence in culture cages in the orchard in Yağmurlan location started on 14 April, reached the highest number on 21 April and ended on 28 April. The adult emergence took 14 days in culture cages. When the temperature values started to reach a minimum of 5 °C, a maximum of 19 °C and the average proportional humidity value was around 46%, the first adult emergences were seen. The highest temperature was 23 °C, the lowest temperature was 7 °C and the daily average proportional humidity was around 59% on the date when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 5.B., the adult emergence in the culture cages started on March 23, reached the highest number on April 12 and ended on April 20. The adult emergence took 27 days in culture cages. When the temperature values reached a minimum of 9 °C, a maximum of 22 °C, and the average proportional humidity value was around 56%, the first adult emergences were seen in the culture cages. At the time when the adult emergence reached the highest level in the culture cages, the highest temperature was 18 °C, the lowest temperature was 10 °C, and the daily average proportional humidity was around 64%. When Figure 5.C of 2017 is examined, the adult emergence in the same orchard started on April 4 in the culture cages, reached the highest number on April 11 and ended on April 25. Adult emergence took 21 days in culture cages. When the temperature values started to reach a minimum of 10 °C, a maximum of 22 °C and the average proportional humidity value was around 46%, the first adult emergences were seen. The highest temperature was 18 °C, the lowest temperature was 8 °C and the daily average proportional humidity was around 41% on the date when the adult emergence reached its highest level.

Adult population change of *Kermania pistaciella* in culture cages in Gaziantep Province Nizip District Alahacı Location

In order to monitor the adult population change, two culture cages were placed in the farmer's orchard in Nizip District of Gaziantep province. In order to monitor the adult emergence, shoots with more than 100 pupae were placed in culture cages between 2015 and 2017.

The adult emergence course of *K. pistaciella* in culture cages between 2015-2017 at the Alahacı location was shown in figure 6. According to figure 6.A., in 2015, adult emergence in the culture cages in the farmer's orchard in Alahacı location started on 14 April, reached the highest number on 28 April, and ended on 12 May. Adult emergence took 28 days in culture cages. When the temperature values start to reach a minimum of 7 °C, a maximum of 16 °C and the average proportional humidity value of around 44%, the first adult emergences were seen. The highest temperature was 26 °C, the lowest temperature was 14 °C and the daily average proportional humidity was around 39% on the date when the adult emergence reached its highest level. In 2016, as can be seen from the examination of Figure 6.B., the adult emergence in the culture cages started on March 24, reached the highest number on April 7 and ended on April 20. Adult emergence took 26 days in culture cages. When the temperature values started to reach a minimum of 9 °C, a maximum of 23 °C and the average proportional humidity value of around 47%, the first adult emergences were seen in the culture cages. The highest temperature was measured at 26 °C, the lowest temperature was 15 °C and the daily average proportional humidity was around 39% at the time when the adult emergence reached the highest level in the culture cages. When Figure 6.C. of 2017 is examined, the adult



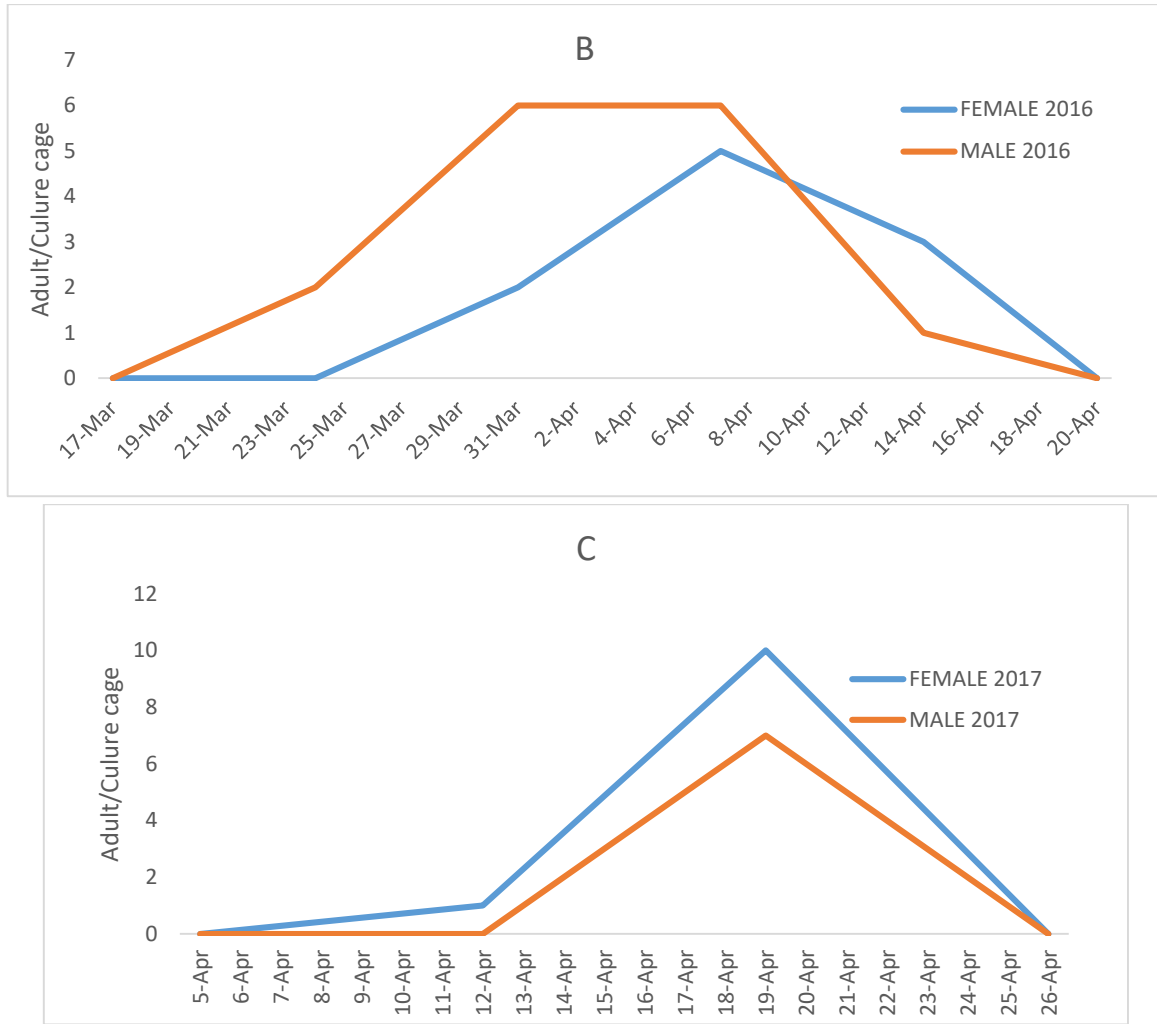


Figure 6. Adult population change of *Kermania pistaciella* in 2015 (A), 2016 (B) and 2017 (C) culture cages in Gaziantep Province Nizip District Alahacı location farmer's orchard

Şekil 6. *Kermania pistaciella*'nın Gaziantep İli Nizip İlçesi Alahacı lokasyonu çiftçi bahçesindeki 2015 (A), 2016 (B) ve 2017 (C) yılı kültür kafeslerinde ergin populasyon değişimi

emergence in the same orchard started on 12 April in the culture cages, reached the highest number on 19 April and ended on 26 April. Adult emergence took 26 days in culture cages. When the temperature values start to reach a minimum of 9 °C, a maximum of 18 °C and the average proportional humidity value of around 50%, the first adult emergences were seen. The highest temperature was 20 °C, the lowest temperature was 11 °C and the daily average proportional humidity was around 80% on the date when the adult emergence reached its highest level.

DISCUSSION

Küçükarslan (1966) states that *K. pistaciella* gives offspring once a year and its butterflies fly in a month between mid-April and mid-May.

Table 1 Date of first adult observation and adult emergence time in pheromone traps in three different locations
Çizelge 1. Üç farklı lokasyonda feromon tuzaklarda ilk ergin görüldüğü tarih ve ergin çıkış süresi

Pheromone Traps	First Adult Monitoring Date/Adult Monitoring Time		
Location-Altitude	2015	2016	2017
Yağmuralan (531 m)	7 April 35 days	23 March 42 days	4 April 50 days
Alahacı (705 m)	7 April 51 days	24 March 50 days	3 April 51 days
Şahinbey (856 m)	17 April 20 days	4 April 33 days	18 April 26 days

Adult emergence of *K. pistaciella* in pheromone traps in Şahinbey district of Gaziantep province between 2015-2017 started between April 4-17, reached the highest number on April 16-29 and ended between April 26 and May 12. Adult emergence lasted between 20-33 days. Also, Zamani et al. (2012) reported that the first adults to be caught, the peak and the last adult to be caught by pheromone traps of *K. pistaciella* in pistachio orchards in the Isfahan region of Iran were on 8 April, 28 April and 19 May, respectively. Similarly, Mart et al. (2003) reported that the first adult was caught on 12 April in the pheromone trap in Gaziantep Center in 2001, it reached its peak on 23 April, and no adults were caught after 15 May.

Adult emergence of *K. pistaciella* in pheromone traps in Gaziantep province Nizip district Yağmuralan location, between 2015-2017, started between 23 March-7 April, reached the highest number on 12-28 April and ended between 3-23 May. Adult emergence lasted between 35-50 days.

Adult emergence of *K. pistaciella* in pheromone traps in Gaziantep province Nizip district Alahacı location, between 2015-2017, started between 24 March-7 April, reached the highest number on 19-28 April, and ended between 12-28 May. Adult emergence lasted an average of 50 days. Similarly, Abbaszadeh et al. (2006), conducted in five different geographical regions of Iran, determined the emergence, peak and last adult capture dates of the adults of *K. pistaciella* as 18 April, 20 April and the second half of May, respectively.

Table 2 Date of first adult observation and adult emergence time in culture cages at three different locations

Çizelge 2. Üç farklı lokasyonda kültür kafeslerinde ilk ergin görüldüğü tarih ve ergin çıkış süresi

Culture Cages	First Adult Monitoring Date/Adult Monitoring Time		
Location-Altitude	2015	2016	2017
Yağmuralan (531 m)	14 April	23 March	11 April
	14 days	27 days	21 days
Alahacı (705 m)	21 April	24 March	12 April
	28 days	26 days	26 days
Şahinbey (856 m)	25 April	25 March	18 April
	10 days	22 days	22 days

For pheromone traps and culture cages, the shortest adult emergence time occurred in the Şahinbey location, which is at the highest altitude. The longest emergence time was observed in the Alahacı location, while the emergence time in the Yağmuralan location, which has a similar altitude, is similar to Alahacı. The shortest adult emergence time occurred in the highest altitude. However, the slowest adult emergence time was observed in the Alahacı location. These data refute the hypothesis that adult emergence time will increase as altitude decreases.

Mart et al. (1995) reported that the first adult of *K. pistaciella* pupae collected in Gaziantep province in 1992 emerged on 24 April. In this study, adult emergence of *K. pistaciella* in culture cages in Şahinbey district of Gaziantep province between 2015-2017 started between 18-25 April, reached the highest number on 15-29 April and ended between 25 April and 9 May. Adult emergence lasted between 10-22 days. Mart et al. (2003), again in Gaziantep Merkez, Oğuzeli and Nizip districts, stated that the first adults of *K. pistaciella* emerged on 17-18 April in culture cages in 2000, reached the peak of the population on 24-27 April, and that the adult emergence started from 1-4 May. They report that they have not been seen since.

Adult emergence of *K. pistaciella* in culture cages in Gaziantep province Nizip district Yağmuralan location, between 2015-2017, started between 23 March-14 April, reached the highest number on 11-21 April and ended between 20-28 April. Adult emergence lasted between 14-27 days.

Adult emergence of *K. pistaciella* in culture cages in Gaziantep province Nizip district Alahacı location between 2015-2017, started between 24 March-14 April, reached the highest number between 7-28 April and ended between 14 April 5 May. Adult emergence lasted an average of 27 days. Mart et al. (2003) found that the first adults of *K. pistaciella* emerged on April 2-9 in culture cages in Gaziantep Center and Nizip districts in 2001, reached the peak on April 17-25, and adult emergence was not seen after April 20-May 6, which In the study, the results obtained between 2015-2017 are similar, but it was determined that the adult emergence in Alahacı location took one week longer in 2015.

CONCLUSION

When the adult emergences in culture cages and pheromone traps were compared, it was concluded that pheromone traps gave clearer information while monitoring the population of the pest. The reason for this is that the shoots placed in the culture cage and the air temperature inside the cage may slightly deviate from the natural ambient temperature. With this, Population follow-up in both pheromone traps and culture cages helped to determine whether male individuals were monitoring earlier. The advantage of using culture cages as an

alternative for population follow-up is to obtain new species of parasitoids and new registered parasitoids for our country, and to follow-up the emergence of female butterflies. In this way, important data on female butterflies of control against this pest has been obtained.

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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 are no conflicts of interest.

REFERENCES

- Abbaszadeh, G., Seiedoleslami, H., Samih, M.A., & Hatami, B., 2006. Bioecology of Pistachio Twig Borer Moth *Kermania pistaciella* Amsel, in Rafsanjan and Isfahan-Iran. *Communications in Agricultural and Applied Biological Sciences*, 71(2), 563-569.
- Abbaszadeh, G., Seiedoleslami, H., & Hatami, B., 2011. Ecological, Cultural and Pheromonal Studies on Pistachio Wood Borer, *Kermania pistaciella* A. *Annals of Plant Protection Sciences*, 19(1), 25-28.
- Arbabsafti, R., Sheikhhigarjan, A., Mahmoudvand, M., & Mohammadipour, A., 2012. Cost-Benefit Analysis of Pistachio Twig Borer, *Kermania pistaciella* Amsel (Lepidoptera: Oinophylidae) Chemical Control. *Archives of Phytopathology and Plant Protection*, 45(16), 1972-1979. <https://doi.org/10.1080/03235408.2012.718690>
- Arpacı S., 2010. Antepfıstığı ve Geleceği, TMMOB Kent Sempozyumu
- Bassirat, M., 2005. Determination of Heat Requirements for Pistachio Twig Borer Moth, *Kermania pistaciella*. IV International Symposium on Pistachios and Almonds, ISHS Acta Horticulturae 726, 519-524. <https://doi.org/10.17660/ActaHortic.2006.726.86>
- Bolu, H., 2002. Güneydoğu Anadolu Bölgesi Antepfıstığı Alanlarındaki Böcek ve Akar Faunasının Saptanması. *Türkiye Entomoloji Dergisi*, 26(3), 197-208. (in Turkish with English summary).
- Çelik, M.Y., 1975. *Gaziantep İli'nde Antepfıstığının zararlıları ve Bunların Faydalı Böcekleri Üzerinde Çalışmalar*. Ziraî Mücadele Araştırma Yıllığı, 9, 43-44 (in Turkish).
- Eldoğan, Ü., & Şahin, A. (2015). Gaziantep İlinde Antepfıstığı Üretim Ekonomisi Araştırması. *KSÜ Doğa Bilimleri Dergisi*, 18(3), 29-36. <https://doi.org/10.18016/ksujns.22332>
- Emami, Y., Aliakbar, A., Farivar Mehini, H., Rajabi, A., Hajabdollahi, M., 2004. Study on Possibility of Extraction and Identification of Pistachio Twig Borer Sexual Pheromone (*Kermania pistaciella*). <https://agris.fao.org/search/en/providers/122649/records/64724a6f53aa8c8963055329> (in Persian with Farsi and English summary)
- Gries, R., Khaskin, G., Darougheh, H., Mart, C., Karadag, S., Er, M. K., 2006. (2S, 12Z)-2-Acetoxy-12-heptadecene: Major Sex Pheromone Component of Pistachio Twig Borer, *Kermania pistaciella*. *Journal of Chemical Ecology*, 32(12), 2667-2677. <https://doi.org/10.1007/s10886-006-9190-0>
- Gries, G., Sasaerila, Y., Darougheh, H., Sheykhi Garjan, A., & Yazdani, A., 2007. Field Testing of A Pheromone-Based Attracticide for Control of Pistachio Twig Borer Moth, *Kermania pistaciella* Amsel (Lep.: Oinophylidae). <https://agris.fao.org/search/en/providers/122649/records/647356d1e17b74d2224ff6cb> (Persian with Farsi and English summary)
- Günaydın, T., 1978. *Güneydoğu Anadolu Bölgesi'nde Antepfıstıklarında Zarar Yapan Böcek Türleri, Tanımları, Yayılışları ve Ekonomik Önemleri Üzerinde Araştırmalar*. Diyarbakır Ziraî Mücadele Araştırma Enstitüsü (Yayınlanmamış Uzmanlık Tezi, 106 s.) (in Turkish)
- IPA., 2024, Iran Pistachio Association, URL: https://iranpistachio.org/en/images/stat/_ARCHIVE/_Analytic_Comparative_Data/4_Iran_US_Annual_Pistachio_Production_Comparison.pdf (erişim tarihi 22.02.2024)
- İzadi, H., Samih, M.A., Behroozy, E., Hadavi, F., Mahdian, K., 2011. Energy Allocation Changes During Diapause in Overwintering Larvae of Pistachio Twig Borer, *Kermania pistaciella* Amsel (Lepidoptera: Tineidae) in Rafsanjan. *Arpn Journal of Agricultural and Biological Science*, 6(5), 12-17.
- Küçükarslan, N., 1966. *Antepfıstıklarında Zarar Yapan Fıstık Dalgüvesi (Kermania pistaciella Amsel, Lep. – Oinophilidae) 'nin Biyoloji ve Savaşı Üzerinde Bazı İncelemeler*, Sabri A.Ş. Basımevi, İstanbul, 64s. (in Turkish)
- Manickavasagam, S., Mehrnejad, M.R., Noyes, J.S., 2008. *Cheiloneurus pistaciae* sp. nov. (Hymenoptera:

- Encyrtidae) A Facultative Hyperparasitoid of *Kermania pistaciella* Amsel (Lepidoptera: Tineidae), A Pest of Pistachio Trees in Iran. *Zootaxa*, 1958, 61–64. <https://doi.org/10.11646/zootaxa.1958.1.6>
- Mart, C., Yigit, A., & Çelik, M.Y., 1995. Biological Observations and Chemical Control of Pistachio Twig Borer, *Kermania pistaciella*, Injurious in Pistachio Orchards in Turkey. *ISHS Acta Horticulturae* 419: I International Symposium on Pistachio; 373-378. <https://doi.org/10.17660/ActaHortic.1995.419.62>
- Mart, C., Karadağ, S., Aslan, M.M. & Eroğlu, N., 2003. *Antepfıstığı Alanlarında Entegre Mücadeleye Veri Tabanı Oluşturacak Bazı Konularda Araştırmalar*, TÜBİTAK Tarp-2076 Nolu proje raporu, 315 s. (in Turkish)
- Mehrnejad, M. R., 2001. The Natural Parasitism Ratio of The Pistachio Twig Borer Moth, *Kermania pistaciella*, in Iran. III International Symposium on Pistachios and Almonds. *ISHS Acta Horticulturae* 591; (541-544). <https://doi.org/10.17660/ActaHortic.2002.591.83>
- Mehrnejad, M. R., 2003. The Influence of Host Species on Some Biological and Behavioural Aspects of *Dibrachys boarmiae* (Hymenoptera: Pteromalidae), Parasitoid of *Kermania pistaciella* (Lepidoptera:Tineidae). *Biocontrol Science and Technology*, 13(2), 219-229. <https://doi.org/10.1080/0958315021000073484>
- Mollaei, M., Izadi, H., Moharramipour, S., Moghadam, E. B., 2017. Physiology of Hibernating Larvae of the Pistachio Twig Borer, *Kermania pistaciella* Amsel (Lepidoptera: Tineidae), Collected From Akbari Cultivar of *Pistacia vera* L. *Neotropical Entomology*, 46(1), 58-65. <https://doi.org/10.1007/s13744-016-0438-2>
- Özer, M., 1958. *Balıkesir ve Kütahya Vilayetlerindeki Yabani Antepfıstıklarında Rastlanan Bazı zararlılar üzerinde incelemeler*. Atatürk Üniversitesi Ziraat Fakültesi Yıllığı, 8(2), 111-120 (in Turkish)
- Özgen, İ., Bolu, H., Beyarslan, A., 2012. *Chelonus flavipalpis* Szépligeti, 1896 and *Mirax rufilabris* Haliday, 1833 (Hymenoptera: Braconidae): Two New Larva-Pupa Parasitoids of Pistachio Twig Borer *Kermania pistaciella* Amsel, 1964 (Lepidoptera: Oinophilidae) With the Parasitization Ratios From Turkey. *Munis Entomology and Zoology*, 7(1), 238-242.
- Şahan, Y. B., & Tunaz, H. (2021). Antepfıstığı Dalgüvesi *Kermania pistaciella* AMSEL (LEPIDOPTERA: TINEIDAE)'nın Braconidae ve Ichneumonidae Familyasından Yeni Kayıt Parazitöitleri. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 24(6), 1230-1236. <https://doi.org/10.18016/ksutarimdoga.vi.803418>
- Şimşek, A., & Bolu, H., 2016. Determination of The Beneficial Insect Fauna in Pistachio (*Pistacia vera* L.) Areas in Diyarbakır Province. *Bitki Koruma Bülteni*, 56(3), 267-282. <https://doi.org/10.16955/bkb.56653>
- Tezerji, Z. S., 2011. Determination of Damages of Pistachio Twig Borer Moth *Kermania pistaciella* Amsel, to Fruit Clusters of Pistachio Trees. V. International Symposium on Pistachios and Almonds, *ISHS Acta Horticulturae*, 912, 701-707. <https://doi.org/10.17660/ActaHortic.2011.912.105>
- TUIK, 2022., Türkiye İstatistik Kurumu URL: <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr> (erişim tarihi: 04.01.2022)
- TUIK, 2024a., Türkiye İstatistik Kurumu, Ağaç sayıları il bazında URL: <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr> (erişim tarihi 20.02.2024)
- TUIK, 2024b., Türkiye İstatistik Kurumu, Meyve vermeyen ağaç sayısı URL: <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr> (erişim tarihi 20.02.2024)
- TUIK, 2024c., Türkiye İstatistik Kurumu, 2022-2023 Yılları Antepfıstığı üretim miktarı URL: <https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2023-49535> (erişim tarihi 22.02.2024)
- Ulu, O., Zümreoğlu, A. ve San, S., 1972. *Ege Bölgesi'nde Antepfıstığı Zararlıları İle Bunların Parazit ve Predatörleri Üzerinde Ön Çalışmalar*. Ziraî Mücadele Araştırma Yıllığı, 6, 55 (in Turkish)
- USDA., 2024, Foreign Agricultural Service, URL: <https://apps.fas.usda.gov/psdonline/circulars/TreeNuts.pdf> (erişim tarihi 22.02.2024)
- Van Achterberg, C., & Mehrnejad, M. R., 2002. The Braconid Parasitoids (Hymenoptera: Braconidae) of *Kermania pistaciella* Amsel (Lepidoptera: Tineidae: Hieroxestinae) in Iran. *Zoologische Mededelingen*, 76; 27-39.
- Yanık, E., & Yücel, A., 2001. The Pistachio (*P. vera* L.) Pests, Their Population Development and Damage State in Şanlıurfa Province. *Cahiers Options Mediterraneenes*, 56, 301-309.
- Yanık, E., & Yildirim, Y., 2016. Effectiveness of Mass Trapping for Control of The Pistachio Twig Borer, *Kermania pistaciella*. *Bulletin of Insectology*, 69(1), 35-40.
- Zamani, Z., Khajehali, J., Sabrazalian, M. R., 2012. Influence of Trap Type, Trapping Location and Cardinal Direction on the Capture of the Pistachio Twig Borer Moth, *Kermania pistaciella* Amsel. (Lepidoptera: Tineidae)'in Isfahan. *Plant Pest Research*, 2(2), 59-61.

Kırşehir İli Aspir Tarlalarında Görülen Yabancı Ot Çeşitliliği, Yaygınlığı ve Yoğunluğunun Belirlenmesi

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ÖZET

Yağışa dayalı, bitkisel üretim yapılan alanlarda sınırlı su kaynağını etkin kullanabilecek veya ürün rotasyonun da yer alabilecek tarla bitkilerine ihtiyaç bulunmaktadır. Bu kapsamda, Kırşehir ilinde aspir (*Carthamus tinctorius* L.) özellikle Mucur ilçesinde rotasyon ürünü olarak yer almaktadır. Bu araştırmanın amacı; Kırşehir ili Merkez ve Boztepe, Çiçekdağı, Kaman ve Mucur ilçeleri aspir tarlalarında bulunan yabancı ot türlerinin, yaygınlık ve yoğunluklarının belirlenmesidir. Kırşehir aspir üretim alanlarında 2023 ve 2024 Haziran-Ağustos ayları içerisinde yürütülen araştırma da üretim alanlarını temsil edebilecek şekilde toplam 62 nokta da 80 tarla tesadüfen seçilmiştir. Yabancı ot sayımları 1m x 1m ebatlarında kare çerçeve kullanılarak yapılmıştır. Sayımlar da çerçeve içerisinde yer alan yabancı otların türleri dikkate alınmış ve sonuçlar değerlendirilmiştir. Çalışma sonucunda, 2023 yılında 7 tür monokotiledon ve 25 tür dikotiledon olmak üzere toplam 14 familyaya dahil 32 yabancı ot türü belirlenmiştir. Poaceae familyası 7 tür ile (%21.8) üretim alanlarında en fazla tür içeren familya olarak belirlenmiştir. *Convolvulus arvensis* L. (%80.0), *Hordeum vulgare* L. (%73.33), *Polygonum aviculare* L. (%73.33), *Salsola kali* (%66.66) en yaygın türlerdir. 2024 yılında 7 tür monokotiledon ve 25 tür dikotiledon olmak üzere 16 familyaya ait 32 tür tespit edilmiştir. Bu vejetasyon döneminde Asteraceae (%26.67), Poaceae (%20) ile en fazla tür içeren familya olmuştur. *Chenopodium album* L. (%75.0), *Salsola kali* L. (%75.0), *Convolvulus arvensis* L. (% 62.5) en yaygın türler olarak saptanmıştır. Çalışma sonucu 2023 ve 2024 vejetasyon dönemlerinde Kırşehir aspir tarlalarında soruna neden olan 43 yabancı ot türü belirlenmiş olup bu türlerin yaygınlığı ve yoğunlukları ortaya konmuştur.

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Determination of Weed Diversity, Prevalence, and Density in Safflower Fields in Kırşehir Province

ABSTRACT

There is a need for field crops that can effectively use limited water resources in areas where rainfall-based crop production is carried out or that can be included in crop rotation. In this context, safflower (*Carthamus tinctorius* L.) is a rotation crop in Kırşehir province, especially in the Mucur district. The aim of this study was to determine the prevalence and density of weed species in safflower fields of Kırşehir province Centre and Boztepe, Çiçekdağı, Kaman, and Mucur districts. In the research carried out in Kırşehir safflower production areas in 2023 and 2024 June-August, 80 fields in a total of 62 points were randomly selected to represent the production areas. Weed counts were made using a 1m x 1m square frame. The types of weeds in the frame were taken into consideration and the results were evaluated. As a result of the study, 32 weed species including 7 monocotyledonous and 25 dicotyledonous weeds belonging to 14 families were identified in 2023. The Poaceae family was determined as the family with the highest number of species in production areas with 7 species (21.8%). *Convolvulus arvensis* L. (80.0%),

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Hordeum vulgare L. (73.33%), *Polygonum aviculare* L. (73.33%), *Salsola kali* (66.66%) were the most common species. In 2024, 32 species belonging to 16 families, including 7 monocotyledonous and 25 dicotyledonous species, were identified. In this vegetation period, Asteraceae (26.67%) was the most abundant family with Poaceae (20%). *Chenopodium album* L. (75.0%), *Salsola kali* L. (75.0%), *Convolvulus arvensis* L. (62.5%) were the most common species. As a result of the study, 43 weed species causing problems in Kırşehir safflower fields in 2023 and 2024 vegetation periods were identified and the prevalence and densities of these species were revealed.

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GİRİŞ

Asteraceae familyasında yer alan aspir (*Carthamus tinctorius* L.) esas olarak yüksek oranda doymamış yağ asidi içeren bitkisel yağ üretmek için, tohumu üretilen geniş yapraklı kserofitik dikenli ve dikensiz formları bulunan tek yıllık bir üründür (Fernandez-Martinez ve ark., 1993). Boya, gıda ve kozmetik ürünlerin renklendirilmesi, hayvan yemi ve tıbbi kullanımlarının yanı sıra biyoyakıt ile endüstriyel yağ üretimi gibi farklı gıda dışı kullanımlarının da olduğu bilinmektedir (Ekin, 2005; İlkılıç ve ark., 2011). Bitkisel yağ sanayisi, hayvan yemi sektörü ile biyodizel üretimi için potansiyel hammadde kaynağının olmasının yanı sıra yağ üretimi sırasında elde edilen küspe, hayvan yemi olarak da önemlidir. Bununla birlikte aspir, soğuğa, kuraklığa, tuzluluğa toleransı ve tarımsal girdilere sınırlı ihtiyacının olması nedeniyle yağışa dayalı üretim yapılan tahıl bazlı ekim sistemlerine kullanılabileceği (Abou Chehade ark., 2022) gibi alternatif bitkisel üretim alanlarında yetiştirilebilmesi, farklı ekim nöbetlerine dahil edilebilmesi ile nadas alanlarının daraltılmasına katkı sağlaması dikkat çeken bir bitkidir.

Aspir, yağışa dayalı üretim yapılan üretim alanlarında 80-100 cm arasında boylanabilmesinin yanı sıra 220 cm kadar uzayabilen derin kazık kök yapısı ile soğuk, sıcaklık, kurak ve tuz stresinin olduğu lokasyonlarda yetiştirilebilen diğer ürünlerle karşılaştırıldığında da toleransı nedeniyle dikkat çekmektedir. Özellikle artan bölgesel ve küresel fiyat rekabeti nedeniyle düşük tarımsal girdileri ile üreticilerinde dikkatini çekmektedir (Pannacci ve ark., 2024). Alternatif ürünü olduğu ayçiçeği yetiştiriciliği ile karşılaştırıldığında, kuş, zararlı ve hastalık streslerine daha toleranslı olduğu bilinmektedir (Abou Chehade ark., 2022).

Ekim alanlarının geliştirilmesi için harcanan çabanın yanı sıra aspir kullanım alanlarının genişletilmesiyle yeterli kadar değerlendirilmeyen bir ürün olan aspir, insan ve hayvan beslenmesinde kullanımın yanı sıra endüstriyel kullanımlarının artırılması ile farklı amaçlara yönelik olarak kurak alanlarda üretimin yapılması potansiyeli bulunmaktadır (Rocchi ve ark., 2022). Aspirin 60'tan fazla ülkede üretimi yapılmakta olup en fazla üretim yapan ülkeler Hindistan, Kazakistan, Çin, Türkiye, Meksika, Rusya ve Tanzanya Birleşik Cumhuriyeti olup Avrupa kıtasında son 20 yılda üretim alanlarının artışına bağlı olarak üretim miktarı da artmıştır (Rocchi ve ark., 2022; FAOSTAT, 2024). Türkiye'de yazlık olarak ekilişi yapılan aspir, kışlık veya yazlık tahıllar ile baklagil bitkileri ile rotasyon programlarına dahil edilerek su eksikliği stresi görülen Kırşehir ili gibi kurak ve yarı kurak üretim alanlarına adapte edilerek üretiminin yapılması mümkün olabilecek bir bitkidir (Koutroubas ve ark., 2009; Coşkun, 2014; Abou Chehade ark., 2022).

Aspir tarımında bitki gelişimini olumsuz etkileyerek verim ve kalitede dikkat çeken kayıplara yol açabilecek hastalık (Kalafat ve ark., 2009), zararlı (Şengonca, 1983) ve yabancı otlarla (Anderson, 1987) mücadele edilmesi gereklidir. Yabancı otlar tarımsal açıdan önemli olabilecek zararlılara da konukçuluk etmektedirler (Gözübenli ve ark., 2024; Sırrı&Özaslan, 2024). Yabancı ot stresi diğer tarımsal ürünler de olduğu gibi aspir üretiminde de önemli bir strestir. Yabancı otlar su ve bitki besin maddelerinin kullanımı için aspir bitkisiyle rekabet etmesi, toprağa bırakabilecekleri toksin maddelerle allelopatik etkileri sonucu aspir üretiminde farklı düzeyde verim ve kalite kayıplarına neden olduğu rapor edilmiştir (Kong ve ark., 2007; Joshi & Joshi, 2016). Aspir bitkisi rozet aşamasında, soğuğa, hatta dona dayanıklı olmasına karşın, rekabet halinde olduğu hızla gelişen yabancı otlara karşı çok hassastır (Li & Mündel, 1996). Bu nedenle, aspir üretiminin hedef verim düzeyine ulaştırılması için iyi bir yabancı ot kontrolü şarttır. Aspir bitkisinin özellikle erken büyüme evrelerinde yabancı otlarla rekabeti sınırlı olup (Uslu ve ark., 1998) verim potansiyelinin üst sınırlarına ulaşılabilmesi için yabancı ot kontrolü gerekli olup kontrol için kimyasal uygulama alternatifleri sınırlıdır. Diğer taraftan aspir bitkisinin büyümesi, tohumların kimyasal içerikleri ve kalitesi genotip, çevre ve tarımsal uygulamalar gibi birçok faktörden az veya çok

etkilenmektedir. Yazlık ekilişlerde çimlenme sonrası fide evresinde bitki gelişimi birkaç haftalık (rozet aşamasında ve sapa kalkma öncesi) süreçte gelişme yavaş olmakla birlikte sapa kalkma evresiyle birlikte hızlı bir büyüme gözlenir. Bu dönemde aspir'in yabancı otlarla rekabet sınırlı olup hızlı büyüme ile birlikte ilerleyen bitki dönemleriyle beraber rekabet ettiği yabancı otlardan daha fazla uzayarak diğer bitkileri etkili bir şekilde gölgelemesi mümkündür (Helm ve ark., 1985). Ancak aspir bitkisinin rozet aşaması gibi erken gelişim dönemlerinde (3-4 haftalık) yabancı ot mücadelesi yürütülmesi gerekmektedir (Babaoğlu, 2024).

Budak ve ark., (2023) tarafından yürütülen bir çalışmada 2017, 2018 ve 2019 üretim sezonun da yabancı otların aspir veriminde sırasıyla %50.61, %65.87 ve %63.34 düzeyinde kayba neden olduğu rapor edilmiştir. Yabancı ot – aspir rekabeti sonucu verim kayıplarının olduğu bilinmekte olup yabancı ot stresinin şiddeti birim alanda yabancı otun türü ve yoğunluğuna bağlı olarak yaşanabilen kayıpların 2.000 kg/ha'ı aşabileceği ve ek olarak hasadın da zorlaştığı (Anderson, 1985), farklı bir çalışma da %39-73 düzeyinin de kayıpların oluşabileceği bildirilmiştir (Blackshaw ve ark., 1990). Aspir üretim alanlarında 16 bitki/m² (*Sinapis arvensis* L.) bulunması durumunda verim kayıplarının %63'e ulaştığı (Budak ve ark., 2023), yine benzer şekilde Helm ve ark., (1985) tarafından Kanada'da yürütülen bir çalışma da ise verim kayıplarının %73'e ulaştığı bildirilmiştir. Aspride yabancı otların verim üzerine etkileri yanında tarım alanlarında yayılış gösteren yabancı ot türlerinin tespiti üzerine de sınırlı sayıda çalışma bulunmaktadır. Ankara aspir üretim alanlarında 16 familyaya ait 35 farklı yabancı ot türü tespit edildiği çalışmada; en fazla tür içeren familyalar Asteraceae (10 tür), Poaceae (5 tür) ve Brassicaceae (3 tür) olarak belirlenmiştir. Aspir alanlarında *Sinapis arvensis* L., *Avena fatua* L., *Convolvulus arvensis* L., *Xanthium strumarium* L., *Triticum aestivum* L. ve *Amaranthus retroflexus* L. rastlanma sıklığı en fazla türler olarak tespit edilmiştir (Serim ve ark., 2015).

Bu çalışmanın amacı, Kırşehir koşullarında ilkbaharda ekilen aspir tarlalarında yabancı otların yaygınlık ve yoğunluklarının belirlenmesidir.

MATERYAL ve METOD

Materyal

Araştırma materyalini 2023 ve 2024 üretim sezonlarında Kırşehir ilinde ekimi yapılan bazı aspir tarlaları ve bu tarlalarda tespit edilen yabancı ot türleri oluşturmaktadır. Bu kapsamda, belirlenen yabancı ot türlerinin incelenen tarlada yaygınlığı ve yoğunluğu ve çeşitliliği incelenmiş, tarlaların genel bitki örtüsü kompozisyonu üzerine değerlendirme yapılmıştır.

Yöntem

Araştırmanın amacına uygun olarak, önceki üretim sezonların üretim verileri göz önünde bulundurularak çalışma tasarlanmış olup Çizelge 1'de belirtildiği şekilde uygulanmıştır. Yabancı ot surveyi, Kırşehir ili Merkez, Boztepe, Çiçekdağı, Kaman ve Mucur ilçelerinde 2023 ve 2024 yılları Haziran-Ağustos aylarında yürütülmüştür. Bu planlama ile mevcut durumun daha iyi anlaşılması ve değerlendirilmesi hedeflenmiştir.

Bir önceki üretim sezonu verileri dikkate alınarak, ekimi yapılan alanların en az %1'lik kısmında surveyler yürütülmüştür. Aspir tarımı Mucur ilçesinde yoğunlaşmış olup, tüm ekim alanları dikkate alındığında 2022 yılında %85.5 düzeyinde, 2023 yılında %89.0 düzeyinde bu ilçede yapılmakta olup Kırşehir ili genelinde homojen bir aspir üretiminden söz etmek mümkün değildir. Tüm survey sürecinde inceleme yapılan tarlaların aynı rota üzerinde olsa bile farklı üretim alanlarında ve yöney olarak farklı olmasına dikkat edilmiş olup Mucur ilçesinde yürütülen çalışmalar da incelenen tarlaların arasında mesafenin en az 3 km olmasına dikkat edilmiştir. Çalışma sürecinde örneklemeler tesadüfi olarak yapılmıştır. Gözlem yapılacak tarlalarda kenar tesiri etkisinin en aza indirilebilmesi için, gözlemler tarlanın en az 15 metre iç kısmından başlanarak gerçekleştirilmiştir (Yılar ve ark., 2022). Bu yöntemle, incelemelerin doğruluğu ve güvenilirliği artırılmıştır. Değerlendirme yapılan tarlalar da yabancı ot sayımları 1m x 1m ebatlarında ki kare çerçeve içerisindeki belirlenen yabancı otların türleri dikkate alınarak sayım yapılmış ve sonuçları kayıt altına alınarak değerlendirilmiştir. Sayımlar, 5.0 dekar kadar olan alanlarda 4 farklı noktadan, 5.1-10.0 dekarlık alanlarda 6, 10.1-20.0 dekarlık alanlarda 8, 20.1-50.0 dekarlık alanlarda 12 ve 50.1 dekar ve üzeri alanlarda 16 farklı noktadan yapılmıştır (Akça & Işık, 2016). Her bir aspir üretim alan büyüklüğüne uygun olarak belirlenen bu sayım noktaları, yabancı otların yaygınlık ve yoğunluklarının doğru bir şekilde belirlenmesini sağlamıştır. 2023 ve 2024 Haziran-Ağustos ayları içerisinde yürütülen sayımlar sonucunda, aspir tarım alanlarında yabancı ot türlerinin çeşitliliği, yaygınlığı ve yoğunluğu ayrıntılı olarak tespit edilmiştir. Yabancı ot türlerinin rastlanma sıklığı ve yoğunluklarının belirlenmesinde aşağıda bildirilen eşitlikler(Eşitlik 1, Eşitlik 2) kullanılmıştır (Odum, 1971; Uygur, 1991). Ayrıca iki yıl arasındaki yabancı ot tür benzerlik indeksi aşağıda verilen Eşitlik 3'e göre belirlenmiştir (Pala ve ark., 2018).

Çizelge 1. Kırşehir ili aspir ekim alanları (TUİK, 2024), survey yapılması planlanan (da) ve survey yapılan üretim alanları (da)

Table 1. Safflower planting areas in Kırşehir province (TUİK, 2024), planned survey areas (da) and surveyed production areas (da)

İlçe (District)	Yıl (Year)	Üretim alanı (da) Production area (da)	% Üretim alanı Production area (%)	İncelenmesi planlanan alan (da) Area planned to be examined (da)	2023, 2024 üretim yılında incelenen üretim alanı (da) The production area examined in the production year 2023, 2024(da)	
					2023	2024
Akpınar	2022	750	7.1	7.5	0	
	2023	650	2.9	6.5		0
Akçakent	2022	20	0.2	0.2	0	
	2023	0	0.0	0		0
Boztepe	2022	304	2.9	3.04	100.060	
	2023	77	0.3	0.77		0
Çiçekdağı	2022	177	1.7	1.77	106.975	
	2023	200	0.9	2		44.547
Kaman	2022	0	0.0	0	0	
	2023	350	1.6	3.5		101.580
Merkez	2022	273	2.6	2.73	196.398	
	2023	1026	5.4	12.06		93.226
Mucur	2022	9.000	85.5	90	796.864	
	2023	20.000	89.0	200		838.932
Toplam	2022	10.524		105.24	1.200,297	
	2023	22.303		223.03		1.078,285

RS= $n \cdot m^{-1} \times 100$ (Eşitlik 1)

(RS: Rastlama Sıklığı, n: Yapılan örneklemenin kaçında aynı tür tespit edildi, m= Toplam örnekleme sayısı)

Y= $b \cdot n^{-1}$ (Eşitlik 2)

(Y: Yoğunluk b= Alınan örnekte toplam birey sayısı, n= Alınan örnek sayısı)

Bİ= $2c \cdot a + b^{-1}$ (Eşitlik 3)

(Bİ = Benzerlik indeksi, a = a habitatındaki tür sayısını, b = b habitatındaki tür sayısını, c = a ve b habitatında bulunan ortak türlerin sayısını ifade etmektedir).

Survey çalışmaları sırasında teşhis edilemeyen yabancı ot türü/türlerinin örnekleri, tekniğe uygun olarak toplanmıştır. Toplanan örnekler, kâğıt zarfa konularak Kırşehir Ahi Evran Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Herboloji Laboratuvarı'na en kısa sürede ulaştırılmıştır. Örneklerin teşhisi, Dr. Melih YILAR tarafından literatür bilgisine dayalı olarak yapılmıştır. Yürütülen survey çalışmalarının sonucu, teşhis edilen yabancı ot türlerinin bilimsel adlandırmaları, *Flora of Turkey* (Davis, 1965-1989) ve yaygın olarak bilinen Türkçe adlandırmaları Uluğ ve ark., (1993) tarafından hazırlanan kaynaklara göre gerçekleştirilmiştir. Survey çalışmaları sonucu tarlalarda teşhis edilmiş yabancı otların çeşitlilik, yaygınlık ve yoğunluğunun değerlendirmelerine göre sınıflandırılması ve önemli türlerin belirlenmesi ile skala değerleri (Çizelge 2) Arslan (2018)'e göre yapılmıştır. Teşhisleri tamamlanan yabancı ot türleri Raunkiaer (1934) tarafından geliştirilen "bitki yaşam formu kategorilerine" göre de ayrıca değerlendirilmiştir.

Çizelge 2. Belirlenen yabancı otların yoğunlukları ve yaygınlıklarının derecelendirilmesi (Arslan, 2018)

Table 2. Grading of the density and prevalence of the identified weeds (Arslan, 2018)

Yaygınlık (Frequency)			Yoğunluk (Density)		
Simge (Symbol)	Anlamı (Meaning)	Oran (Ratio)	Simge (Symbol)	Anlamı (Meaning)	Oran (Ratio)
Ç	Çok yaygın	≥ 50	A	Çok yoğun	≥ 10 adet/m ²
Y	Yaygın	%25-49	B	Yoğun	5.00 - 9.99 adet/m ²
O	Orta yaygın	%13-24	C	Orta yoğun	1.00 - 4.99 adet/m ²
N	Düşük yaygın	≤ 12	D	Düşük yoğun	0.10 - 0.99 adet/m ²
			E	Çok düşük yoğun	0.01 - 0.09 adet/m ²
			F	Nadir	<0.01 adet/m ²

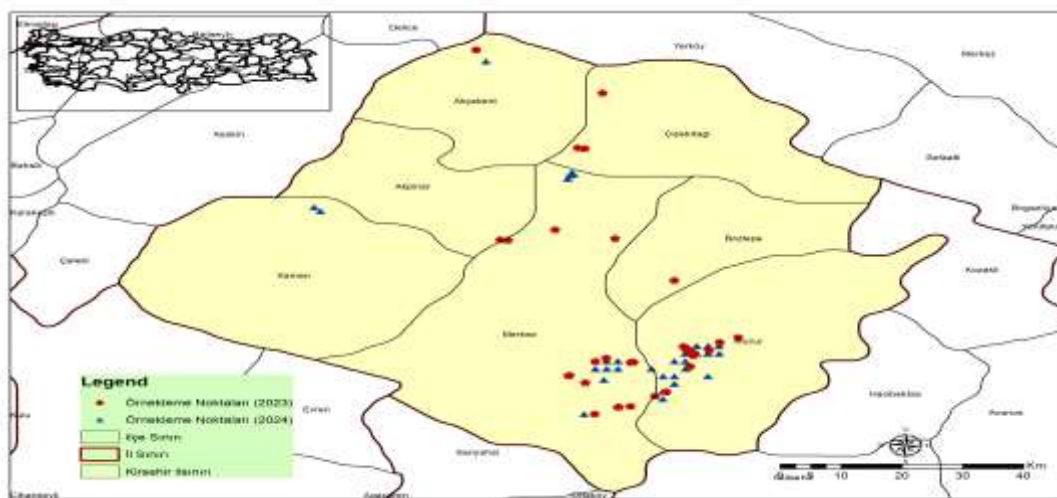
BULGULAR ve TARTIŞMA

Sürvey çalışmaları 2023 yılında 1.200,297 da alan, 2024 yılında ise 1.078,285 da aspir ekili alan incelenmiştir (Şekil 1). Sürvey çalışması sonuçlarına göre “Bitki Yaşam Formu Kategorileri Sistemi” dikkate alınarak yapılan değerlendirmeye göre; 2023 yılında 7 tür monokotiledon ve 25 tür dikotiledon olmak üzere toplam 14 familyaya dahil 32 yabancı ot türü belirlenmiştir. Bu yabancı otların 20’si tek yıllık, 7’si çok yıllık ve 5’i tek/çok yıllık, iki/çok yıllık, tek/iki yıllık türler oluşturmuştur. Bu vejetasyon döneminde Poaceae familyası 7 tür ile (%21.8) üretim alanlarında en fazla tür içeren familya olarak belirlenmiştir. 2024 yılında ise 7 tür monokotiledon ve 25 tür dikotiledon olmak üzere 16 familyaya ait 32 tür tespit edilmiştir. Bu türlerin 19’u tek yıllık, 7’si çok yıllık geri kalan 6 türü ise tek/çok yıllık, iki/çok yıllık, tek/iki yıllıklar oluşturmuştur. Bu vejetasyon döneminde Asteraceae (%26.67), Poaceae (%20) ile en fazla tür içeren familya olmuştur. Sonuçta 2023 yılında 14, 2024 yılında 16 familyaya dahil olan toplam 43 yabancı ot türü tespit edilmiştir. Belirlenen yabancı otlardan, 25 tanesi tek yıllık, 11 adeti çok yıllık, 3 adeti tek veya iki yıllık, 1 adet tek veya çok yıllık, 1 adet iki yıllık veya monokarpik, 2 adet de iki veya çok yıllık olarak belirlenmiştir (Çizelge 3). 2023 ve 2024 yıllarında tespit edilen yabancı ot türlerinin benzerlik indeksi 0.69 olarak bulunmuştur. Benzerlik indeksi farklı habitatlardaki farklılıkları göstermektedir. Kırşehir İlinde aspir tarlalarındaki yabancı ot türlerindeki benzerlik veya farklılık kendinden önce ekilen kültür bitkisi, yürütülen kültürel, kimyasal mücadeledeki farklılık, toprak yapısı gibi faktörlerden kaynaklandığı düşünülmektedir. Ankara aspir alanında görülen yabancı ot türleri ile kıyaslandığında benzerlik indeksi 0.46 olarak bulunmuştur (Serim ark.,2015).

Tespit edilen yabancı otların “Raunkiaer Hayat Formu Kategorileri Sistemi dikkate alınarak yapılan değerlendirmeye göre”; 25 tür Therophyte, 13 tür Hemicryptophyte, 2 tür Geophyte, 2 tür Therophyte/Hemicryptophyte, 1 tür Hemicryptophyte/Geophyte olarak belirlenmiştir (Çizelge 3). Yapılan değerlendirme sonucu tespit edilen yabancı otların %23.26’sı aspir türünün de yer aldığı Asteraceae, %23.26’sı Poaceae, familyasına dahil olduğu tespit edilmiştir (Çizelge 3).

İklim faktörlerinin yanı sıra tarlanın topografyası ile farklı biyotik stres faktörlerin etkisine de bağlı olarak Raunkiaer Hayat Formu Kategorileri Sistemi (Raunkiaer, 1934) göre, Therophyte formlarının dikkati çeken düzeyde baskın olduğu belirlenmiştir. Karasal iklimin yaşandığı benzer coğrafik bölgelerde farklı ürünlerde yürütülen survey çalışmalarında, baskın formların “Therophyte formlar” olduğu araştırmalarda rapor edilmiştir (Heneidy & Bidak, 2001; Önen ve ark., 2018; Yılar ve ark., 2021; Yılar ve ark., 2022).

“Büyüme Bitki Yaşam Formu Kategorileri Sistemi dikkate alınarak yapılan değerlendirmeye göre”; Tek yıllık yabancı ot türlerinin dikkati çeken düzeyde baskındır. Bunun başlıca nedeninin bu türlerinin kısa ömürlü ve stres koşullarının olduğu yetiştiricilik alanlarında bile dikkat çeken düzeyde çoğalma materyal sayısı oluşturmalarından kaynaklanabileceği öngörülmektedir (Sans & Masalles, 1995; Önen ve ark., 2018). Benzer topografya ve iklim şartlarına sahip Kayseri ili’nde bulunan şeker pancarı tarlalarında “tek yıllık türlerin” (Akça & Işık, 2016), benzer topografya ve iklim şartlarına sahip Kırşehir ilinde bulunan nohut ve şeker pancarı tarlalarında “tek yıllık türlerin” baskın olduğu rapor edilmiştir (Yılar ve ark., 2021; Yılar ve ark., 2022).



Şekil 1. 2023 ve 2024 vejetasyon döneminde gerçekleştirilen survey noktaları
Figure1. Survey points carried out in the 2023 and 2024 vegetation period

Çizelge 3. Teşhis edilen yabancı ot türlerinin Bayer kodları, yaşam formları, rastlanma sıklığı, yoğunluğu, yaygınlık ve yoğunluk skala değeri
Table 3. Bayer codes, life forms, frequency of occurrence, density, prevalence and density scale value of identified weed species

Familya	Bilimsel İsmi Scientific Name	Bilinen Yaygın Adı Commonly Known Name	Bayer Kodu Bayer Code	Bitki Yaşam Formu Plant Life Form	Büyüme Raunkiaer (Hayat Formu)	2023		2024		YaSD*	YoSD**	Rastlanma Sıklığı (%) Frequency of occurrence (%)	Yoğunluk (bitki/m ²) Density (plants/m ²)	YaSD*	YoSD**
						Rastlanma Sıklığı (%) Frequency of occurrence (%)	Yoğunluk (bitki/m ²) Density (plants/m ²)	Rastlanma Sıklığı (%) Frequency of occurrence (%)	Yoğunluk (bitki/m ²) Density (plants/m ²)						
Amaranthaceae	<i>Amaranthus retroflexus</i> L.	Kırmızı köklü tilki kuyruğu	AMARE	Tek yıllık	Therophyte	13.33	0.02	O	E			12.5	0.02	O	E
	<i>Amaranthus albus</i> L.	Yatık horoziböğü	AMAAL	Tek yıllık	Therophyte	13.33	0.02	O	E			12.5	0.02	O	E
Apiaceae	<i>Echinophora sibthorpiana</i>	Tarhana otu	EHPTS	İki veya çok yıllık	Hemicryptophyte	6.66	0.033	N	E			12.5	0.063	O	E
	<i>Caucalis platycarpus</i> L.	Kavkal	CUCLA	Tek yıllık	Therophyte	13.33	0.044	O	E						
Asteraceae	<i>Acroptilon repens</i> (L.) DC	Kekre	CENRI	Çok yıllık	Hemicryptophyte							12.5	0.4	O	D
	<i>Centaurea solstitialis</i>	Güneş dikenli	CENSO	Tek yıllık	Therophyte	33.3	0.20	Y	D			12.5	0.025	O	E
	<i>Centaurea depressa</i> M.Bieb.	Peygamber çiçeği	CENDE	Tek yıllık	Therophyte	33.33	0.064	Y	E						
	<i>Chondrilla juncea</i> L.	Karakavuk	CHOJU	İki veya çok yıllık	Hemicryptophyte, Geophyte	53.33	0.23	Ç	D			25	0.08	Y	E
	<i>Cirsium arvense</i> (L.) Scop	Köy göçüren	CIRAR	Çok yıllık	Geophyte							12.5	0.18	O	D
	<i>Lactuca serriola</i> L.	Dikenli yabani marul	LACSE	Tek/İki yıllık	Hemicryptophyte	20	0.09	O	E						
	<i>Helianthus annuus</i>	Ayçiçeği	HELAN	Tek yıllık	Therophyte							12.5	0.02	O	E
	<i>Sonchus arvensis</i> L.	Tarla eşek marulu	SONOL	Tek /İki yıllık	Hemicryptophyte	13.33	0.02	O	E			12.5	0.02	O	E
	<i>Xanthium spinosum</i> L.	Zincir pıtrağı	XANSP	Tek yıllık	Therophyte	13.3	0.188	O	D			12.5	0.75	O	E
	<i>Xanthium strumarium</i> L.	Domuz Pıtrağı	XANST	Tek yıllık	Therophyte							12.5	0.04	O	E
Boraginaceae	<i>Anchusa officinalis</i> L.	Sığırdili	ANCOF	Çok yıllık	Hemicryptophyte										
	<i>Heliotropium europaeum</i> L.	Beyaz bambul	HEOEU	Tek yıllık	Therophyte	13.33	0.032	O	E			37.5	0.30	Y	E
	<i>Echium vulgare</i> L.	Adi engerek otu	EHIVU	İki yıllık/ Monokarpic	Hemicryptophyte							25	0.045	Y	E
Brassicaceae	<i>Boreava orientalis</i> Jaub and Spach	Sarı ot	BOAOR	Tek yıllık	Therophyte	6.66	0.01	N	E						
	<i>Descurainia sophia</i> (L.) Webb ex Prantl	Sadır otu	DESSO	Tek yıllık	Therophyte	6.66	0.007	N	F						
	<i>Crambe orientalis</i> L.	Akyumak	CRMOR	Çok yıllık	Hemicryptophyte	13.33	0.02	O	E						
	<i>Sinapis arvensis</i> L.	Yabani hardal	SINAR	Tek yıllık	Therophyte	33.33	0.31	O	D			12.5	0.02	O	E
Caryophyllaceae	<i>Gypsophila pilosa</i> Hudson	Tarla çöven otu	GYPPI	Tek yıllık	Therophyte	13.33	0.02	O	E						
Chenopodiaceae	<i>Chenopodium album</i> L.	Siriken	CHEAL	Tek yıllık	Therophyte	60	3.12	Ç	C			75	0.55	Ç	D
	<i>Salsola kali</i>	Adi soda otu	SASKA	Tek yıllık	Therophyte	66.66	1.456	Ç	C			75	0.46	Ç	D
Convolvulaceae	<i>Convolvulus arvensis</i> L.	Tarla sarmaşığı	CONAR	Çok yıllık	Hemicryptophyte	80	1.326	Ç	C			62.5	0.70	Ç	D

Çizelge 3'ün devamı

Euphorbiaceae	<i>Euphorbia esula</i> L.	Sütleğen	EPHES	Çok yıllık	Hemicryptophyte	13.33	0.044	O	E	12.5	0.02	O	E
Lamiaceae	<i>Marrubium vulgare</i> L.	Boz ot	MAQVU	Çok yıllık	Hemicryptophyte	26.66	0.039	Y	E	12.5	0.02	O	E
Papaveraceae	<i>Fumaria officinalis</i> L.	Hakiki şahtere	FUMOF	Tek yıllık	Therophyte	6.66	0.007	N	F	12.5	0.02	O	E
Poaceae	<i>Secale cereale</i> L.	Kendi gelen Çavdar	SECCE	Tek yıllık	Therophyte	13.33	0.177	O	D	12.5	0.063	O	E
	<i>Triticum aestivum</i> L.	Kendi gelen Buğday	TRZAS	Tek yıllık	Therophyte					12.5	0.02	O	E
	<i>Avena sativa</i> L.	Kendi gelen yulaf	AVESA	Tek yıllık	Therophyte	40	1.40	Y	C	25	0.04	Y	E
	<i>Bromus tectorum</i>	Püsküllü çayır	BROTE	Tek yıllık	Therophyte	6.66	0.01	N	E				
	<i>Cynodon dactylon</i> (L.) Pers.	Köpek dişi ayrığı	CYNDA	Çok yıllık	Therophyte Hemicryptophyte,					12.5	0.02	O	E
	<i>Agropyron repens</i> (L.) P. Beauv.	Adi ayrık	AGRRE	Çok yıllık	Hemicryptophyte	13.33	0.042	O	E				
	<i>Triticosecale</i>	Tritikale	TTLWI	Tek/İki yıllık	Therophyte					12.5	0.02	O	E
	<i>Aegilops columnaris</i> Zhukovsky	Buğday anası	AEGCO	Tek yıllık	Therophyte	40	0.294	Y	D	12.5	0.1	O	E
	<i>Hordeum vulgare</i> L.	Kendi gelen Arpa	HORVX	Tek yıllık	Therophyte	73.33	0.393	Ç	D	12.5	0.025	O	E
	<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Kamış	PHRCO	Çok yıllık	Geophyte	6.66	0.022	N	E				
Polygonaceae	<i>Polygonum aviculare</i> L.	Yabani madımak	POLAV	Tek yıllık	Therophyte	73.33	2.326	Ç	C	37.5	0.468	Y	D
	<i>Polygonum convolvulus</i> L.	Sarmaşık çoban değneği	POLCO	Çok yıllık	Hemicryptophyte	13.33	0.021	O	E	12.5	0.25	O	D
Resedaceae	<i>Reseda lutea</i> L.	Muhabbet çiçeği	RESLU	Tek veya çok yıllık	Therophyte, Hemicryptophyte	40	0.15	Y	E	37.5	0.16	Y	D
Solanaceae	<i>Solanum nigrum</i> L.	Siyah itüzümü	SOLNI	Tek yıllık	Therophyte					12.5	0.29	O	D
Zygophyllaceae	<i>Tribulus terrestris</i> L.	Demir diken	TRBTE	Tek yıllık	Hemicryptophyte					12.5	0.02	O	E

*YaSD: Yaygınlık skala değeri, **YoSD: Yoğunluk skala değeri

*YaSD: Prevalence scale value, **YoSD: Intensity scale value

Türkiye aspir üretim alanlarında görülen yabancı ot çeşitlilik, yaygınlık ve yoğunluğunun belirlenmesi üzerinde sınırlı çalışmaya rastlanmıştır. 2014 ve 2015 yıllarında Ankara'nın Bala, Gölbaşı, Haymana, Polatlı ve Şereflikoçhisar ilçelerinde aspir tarlalarında yürütülen benzer bir çalışmada; toplam ekiliş alanın %2'si tesadüfi olarak incelenmiştir. Araştırmada, 16 familyaya dahil 35 farklı yabancı ot türünün belirlendiği raporlanmıştır. Tespit edilen familyaların ise 10 tür ile Asteraceae, 5 tür ile Poaceae ve 3 tür ile Brassicaceae en fazla tür içerenler olduğu ortaya konmuştur. Çalışma sonucu, *Convolvulus arvensis* L., *Sinapis arvensis* L., *Xanthium strumarium* L., *Avena fatua* L., *Triticum aestivum* L. ve *Amaranthus retroflexus* L. 'un yaygın türler olduğu bildirilmiştir (Serim ve ark., 2015). Benzer iklim coğrafik koşullarının yaşandığı Ankara ve Kırşehir aspir tarlalarında benzer bulguların olduğu değerlendirilmiştir. Dikkat çeken farklılıkların, yetiştiriciliği yapılan ürün farklılığı ve yabancı ot kontrolü için farklı uygulama pratiklerinin veya herbisitlerin tercih edilmesinin olduğu düşünülmektedir. Diğer bir çalışmada ise; Uslu ve ark., (1998) tarafından Ankara'da 1995 yılında yürütülen bir çalışmada yazlık olarak ekilen aspir bitkisinin çeşit ile bazı agronomik karakterlerin yabancı otlar üzerine olan etkisini incelemişlerdir. Araştırmanın yürütüldüğü tarlada; *Cirsium arvense* L., *Centaurea diffusa* Lam., *Salsola kali* L., *Convolvulus arvensis* L., *Ononis spinosa* L. ve *Sinapsis arvensis* L. türlerinin daha yaygın olduğu rapor edilmiştir. Her iki çalışma da tespit edilen yabancı ot türlerin benzerliği dikkat çekmektedir.

Kırşehir aspir alanlarında; *Convolvulus arvensis* (%80.0-%62.5), *Salsola kali* (%66.66-%75.0), *Chenopodium album* (%60-%75.0) her iki yılda da çok yaygın (YaSD:Ç) türler olduğu belirlenmiştir. 2023 vejetasyon döneminde *Chenopodium album* L. (3.12 bitki/m²), *Polygonum aviculare* L. (2.326 bitki/m²), *Salsola kali* (1.456 bitki/m²), *Convolvulus arvensis* (1.326 bitki/m²) orta yoğunlukta; 2024 vejetasyon döneminde ise *Solanum nigrum* L. (0.29 bitki/m²), *Reseda lutea* L. (0.16 bitki/m²), *Polygonum convolvulus* L. (0.25 bitki/m²), *Polygonum aviculare* (0.468 bitki/m²), *Convolvulus arvensis* L. (0.70 bitki/m²), *Salsola kali* (0.46 bitki/m²), *Chenopodium album* L. (0.55 bitki/m²) düşük yoğunlukta olduğu tespit edilmiştir (Çizelge 3). Kırşehir İli aspir üretimi yapan çiftçilere uygulanan bir anket çalışmasında üreticiler tarafından *Sinapis arvensis* (%44.9), *Centaurea depressa* M.Bieb. (%20.2), *Chenopodium album* L. (%12.4) en sorunlu yabancı otlar olarak beyan edilmiştir. Bu sorunlu yabancı otlardan *Chenopodium album* L. mevcut çalışmada rastlanma sıklığı ve yoğunluk bakımından önemli türlerden birisi olduğu ortaya konmuştur (Eroğlu ve Demir, 2021). Kırşehir de farklı ürünler üzerinde yürütülen çalışmalarda da benzer sonuçlar rapor edilmiştir. Yılar ve ark. (2021) tarafından yürütülen bir çalışmada; Kırşehir ili nohut üretimi alanında, *Convolvulus arvensis* L. (1.29 bitki/m²), *Chenopodium album* L. (0.56 bitki/m²), *Salsola kali* (0.53 bitki/m²), *Polygonum aviculare* (0.44 bitki/m²)'nin yoğunluk bakımından öne çıkan türler olduğunu bildirmişlerdir. Bu iki çalışma bu yönüyle kısmen örtüşmektedir.

SONUÇ ve ÖNERİLER

Kırşehir koşullarında aspir yetiştiriciliği, nadas alanları veya tahıl ekimi yapılan sezon sonrası münavebe bitkisi olarak ekilişinin yapıldığı değerlendirilmiştir. Bu nedenle değişen düzeyde önceki sezon ekilişinin yapıldığı ürün (buğday, arpa, Tritikale vb.) yabancı ot olarak belirlenmiş olup önceki sezon kullanılan tarımsal makine ve ekipman ile kullanılan herbisitler ile uygulama dozlarının farklı tarlalarda yabancı ot çeşitliliğini, yaygınlığı ve yoğunluğunu değişen düzeylerde etkilediği değerlendirilmiştir. Farklı tarlalarda değişen yabancı ot çeşitliliği yaygınlığı ve yoğunluğunun bu şekilde açıklanabileceği düşünülmektedir. Konu önceki sezon buğday veya arpa ekilişi yapılan tarlalarda buğday ve arpanın yabancı ot varlığının, yoğunluğu ve yaygınlığı örneği ile açıklanabilir.

Aspir özellikle rozet döneminde yabancı otlanmaya karşı hassas bir bitkidir. Aspir üretiminde sürdürülebilir yabancı ot kontrolü veya entegre mücadele programının oluşturulması veya revize edilmesi için öncelikle yürütülecek çalışmalar arasında yabancı ot türlerinin belirlenmesi ile birim alan yoğunluk ve yaygınlıklarının tespit edilmesi bir gerekliliktir. Sürvey yapılan alanların neredeyse tamamında dikenli formların/çeşitlerin yetiştirildiği belirlenmiştir. Bu durum insan işgücü kullanılarak yabancı ot kontrolünün mümkün olamayacağını ve herbisit kullanımının bir tercihten çok bir zorunluluk olduğunu söylemek yanlış olmaz. Diğer taraftan Bitki koruma ürünleri veri tabanı incelendiğinde, *Capsella bursa-pastoris* ve *Amaranthus retroflexus* türleri 450 g/l Pendimethalin ile *Avena* spp. türlerinin kontrolü için 116.2 g/l Clethodim aktif maddesi "İl/İlçe Bazında Geçici Tavsiyeleri 10.7.2024 tarihine" kadar önerilmektedir (BKU, 2024). Yabancı ot stresinin azaltılması veya kontrolü için öncelikle aspir tarlalarında yabancı ot çeşitliliği, yaygınlığı ve yoğunluklarının belirlenerek ürün de fitotoksisteye neden olmayan farklı gruplarda yer alan herbisitlerin içerisinden biyolojik etkinlik denemelerinin yapılarak belirlenmesi gereklidir. Çalışma sonucu elde edilen veriler bu konuda yürütülecek benzer yabancı ot kontrol çalışmalarına değişen düzeylerde katkı sağlayabileceği açıktır.

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KAYNAKLAR

- Abou Chehade, L., Angelini, L. G., & Tavarini, S. (2022). Genotype and seasonal variation affect yield and oil quality of safflower (*Carthamus tinctorius* L.) under Mediterranean conditions. *Agronomy*, 12(1), 122. <https://doi.org/10.3390/agronomy12010122>
- Akça, A. & Işık, D. (2016). Kayseri ili şeker pancarı (*Beta vulgaris* L.) ekiliş alanlarında bulunan yabancı otların tespiti. *Bitki Koruma Bülteni*, 56(1), 115-124.
- Anderson, R.L. (1985). Chlorsulfuron for weed control in safflower (*Carthamus tinctorius*). *Weed Science*, 33(6), 840-842. <https://doi.org/10.1017/S0043174500083466>
- Anderson, R.L. (1987). Broadleaf weed control in safflower (*Carthamus tinctorius*) with sulfonylurea herbicides. *Weed Technology*, 1(3), 242-246. <https://doi.org/10.1017/S0890037X00029614>
- Arslan, Z.F. (2018). Şanlıurfa ili mısır tarlalarında bulunan yabancı otların yaygınlık ve yoğunlukları ile mücadele sorunlarına çözüm önerileri. *Turkish Journal of Agriculture-Food Science and Technology*, 6(10), 1322-1328. <https://doi.org/10.24925/turjaf.v6i10.1322-1328.1796>
- Babaoğlu, M. (2024). Aspir Bitkisi ve Tarımı. <https://arastirma.tarimorman.gov.tr/ttae> (Alınma Tarihi: 01.08.2024)
- BKU, (2024). Bitki Koruma Ürünleri Veri Tabanı <https://bku.tarimorman.gov.tr/> (Alınma Tarihi 01.08.2024)
- Blackshaw, R.E., Mündel, H.H. & Derksen, D.A. (1990). Herbicides for weed control in safflower (*Carthamus tinctorius*). *Canadian Journal of Plant Science*, 70(1), 237-245. <https://doi.org/10.4141/cjps90-026>
- Budak, I., Serim, A. & Asav, Ü. (2023). The Critical Period for Weed Control in Safflower Fields of Turkey. *In Proceedings of the Bulgarian Academy of Sciences* 76(12), 1935-1942. <https://doi.org/10.7546/CRABS.2023.12.17>
- Coşkun, Y. (2014). Aspir (*Carthamus tinctorius* L.)'in Kışlık ve Yazlık Ekim Olanakları. *Türk Tarım ve Doğa Bilimleri Dergisi* 1(4): 462-468
- Davis P.H. (1965-1989). *Flora of Turkey and The East Aegean Islands*, Vol. 1-10, University of Edinburg, England
- Ekin, Z. (2005). Resurgence of safflower (*Carthamus tinctorius* L.) utilization: A global view. *Journal of Agronomy*, 4(2), 83-87. <https://doi.org/10.3923/ja.2005.83.87>
- Eroğlu, K. & Demir, İ. (2021). Aspir (*Carthamus tinctorious* L.) tarımında çiftçi eğiliminin belirlenmesi Mucur ilçesi örneği. *Kırşehir Ahi Evran Üniversitesi Ziraat Fakültesi Dergisi*, 1(2), 67-80. <https://dergipark.org.tr/tr/download>
- FAOSTAT, (2024). Food and Agriculture Organization (FAO) of United Nations. The Food and Agriculture Organization Corporate Statistical Database Production Quantities of Safflower Seed and Plantains. 2022. <http://www.fao.org/faostat/en/#data/QC> (Alınma Tarihi: 01.08.2024)
- Fernandez-Martinez, J., Del Rio, M., & De Haro, A. (1993). Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica*, 69, 115-122. <https://doi.org/10.1007/BF00021734>
- Gözübenli, Z.S., Aslan, M.M. & Sabancı, K. (2024). Kahramanmaraş İlindeki Bazı Yabancı Otlar Üzerinde Saptanan Curculionoidea (Insecta: Coleoptera) Türleri Kısım II. *KSÜ Tarım ve Doğa Derg* 27 (Ek Sayı 1), 416-433. <https://doi.org/10.18016/ksutarimdoga.vi.1432756>.
- Helm, J.L., Riveland, N., Schneiter, A.A. & Sobolik, F. (1985). Safflower production. *NDSU Extension Service [publication]-North Dakota State University (USA)*.
- Heneidy, S.Z. & Bidak, L. M. (2001). Biodiversity of the plant species in Bisha, Asir region, southwestern Saudi Arabia. *Pakistan Journal of Biological Sciences*, 4(11), 1323-1330. <https://doi.org/10.3923/pjbs.2001.1323.1330>
- İlkılıç, C., Aydın, S., Behcet, R. & Aydın, H. (2011). Biodiesel from safflower oil and its application in a diesel engine. *Fuel processing technology*, 92(3), 356-362. <https://doi.org/10.1016/j.fuproc.2010.09.028>
- Joshi N., & Joshi, A. (2016). Allelopathic effects of weed extracts on germination of wheat. *Annals of Plant Sciences*, 5(5), 1330-1334. <https://doi.org/10.21746/aps.2016.05.001>
- Kalafat, S., Karakaya, A., Kaya, M.D. & Bayramin, S. (2009). Bazı aspir genotiplerinin pas hastalığına karşı reaksiyonları hakkında ön çalışma. *Bitki Koruma Bülteni*, 49(4), 183-187. <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20103184142>
- Kong C.H., Zhao H., Xu X.H., Wang P. & Gu Y. (2007). Activity and allelopathy of soil of flavone O-Glycosides from rice. *Journal Agric. Food Chem.*, 55, 6007-6012. <https://doi.org/10.1021/jf0703912>
- Koutroubas, S.D., Papakosta, D.K. & Doitsinis, A. (2009). Phenotypic variation in physiological determinants of yield in spring sown safflower under Mediterranean conditions. *Field Crops Research*, 112(2-3), 199-204. <https://doi.org/10.1016/j.fcr.2009.03.002>

- Li D. & Hans-Henning M. (1996). Safflower. *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. 7. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
- Odum, E.P. (1971). Fundamentals of Ecology, 3rd (ed.) WB Saunders. *Philadelphia, PA*.
- Önen, H., Akdeniz, M., Farooq, S., Hussain, M., & Ozaslan, C. (2018). Weed flora of citrus orchards and factors affecting its distribution in western Mediterranean region of Turkey. *Planta Daninha*, 36, e018172126. <https://doi.org/10.1590/S0100-83582018360100036>
- Pala F., Mennan H. & Demir A. (2018). Diyarbakır İli Mercimek Ekim Alanlarında Bulunan Yabancı Ot Türlerinin, Yaygınlıklarının ve Yoğunluklarının Belirlenmesi. *Türk J Weed Sci*, 21(1), 33-42 <https://dergipark.org.tr/tr/pub/tjws/issue/42558/513086>
- Pannacci, E., Farneselli, M., Monni, V. & Tei, F. (2024). Effects of Pre-Emergence Herbicides on Weed Control and Yield of Safflower (*Carthamus tinctorius* L.) in Central Italy. *Agronomy*, 14(3), 482. <https://doi.org/10.3390/agronomy14030482>
- Raunkiaer, C. (1934). The life forms of plants and statistical plant geography; Being the collected papers of C. Raunkiaer. Oxford: University Press
- Rocchi, L., Farneselli, M., Paolotti, L., Falcinelli, B., Guiducci, M. & Boggia, A. (2022). Life Cycle Inventory for Safflower Production in Southern Europe. *Environmental and Climate Technologies*, 26(1), 862-870. <https://doi.org/10.2478/rtuect-2022-0065>
- Sans, F. X. & Masalles, R. M. (1995). Phenological patterns in an arable land weed community related to disturbance. *Weed Research*, 35(5), 321-332. <https://doi.org/10.1111/j.1365-3180.1995.tb01627.x>
- Şengonca, Ç. (1983). Çukurova Bölgesindeki aspir zararlıları üzerinde bir araştırma. *Türk. Bit. Kor. Derg.* 7, 117-127.
- Serim, A. T., Asav, Ü. & Türkseven, S. (2015). Ankara ili aspir (*Carthamus tinctorius* L.) ekiliş alanlarında bulunan yabancı otların tespiti. *Turkish Journal of Weed Science*, 18(1), 19-23. <https://dergipark.org.tr/tr/pub/tjws/issue/42240/508191>
- Sırrı, M., & Özaslan, C. (2024). Hakkari/Yüksekova havzasında yabancı otlar üzerinde saptanan Coleoptera türleri. *KSÜ Tarım ve Doğa Derg* 27(3), 610-622. DOI: 10.18016/ksutarimdoga.vi.1317489.
- TUİK, 2024. Aspir istatistikleri. <https://biruni.tuik.gov.tr/medas/?locale=tr> (Alınma Tarihi: 15.09.2024)
- Uluğ, E., Kadioğlu, İ., & Üremiş, İ. (1993). Türkiye'nin yabancı otları ve bazı özellikleri. T.C. Tarım ve Köyişleri Bakanlığı, Zirai Mücadele Araştırma Enstitüsü Müdürlüğü, Yayın No:78. 513 sayfa. Adana
- Uslu, N., Akin, A. & Halitligil, M. B. (1998). Cultivar, weed and row spacing effects on some agronomic characters of safflower (*Carthamus tinctorius* L.) in spring planting. *Turkish Journal of Agriculture and Forestry*, 22(6), 533-536. <https://journals.tubitak.gov.tr/agriculture/vol22/iss6/2>
- Uygur, F.N. (1991). Herboloji Araştırma Yöntemleri. *ÇÜ Ziraat Fakültesi Bitki Koruma Bölümü, Yardımcı Ders Notu, Adana*.
- Yılar, M., Bayar, Y. & Akan, K. (2021). Kırşehir İli Nohut Üretim Alanlarında Görülen Yabancı Otların Yaygınlık ve Yoğunluklarının Belirlenmesi. *Turkish Journal of Weed Science*, 24(2), 83-90. <https://dergipark.org.tr/tr/pub/tjws/issue/67412/1009636>
- Yılar, M., Bayar, Y., Akan, K. & Türkay, İ. (2022). Kırşehir ili şeker pancarı (*Beta vulgaris* L.) üretim alanlarında yayılış gösteren yabancı otların flora, yaygınlık ve yoğunluklarının belirlenmesi. *Harran Tarım ve Gıda Bilimleri Dergisi*, 26(3), 268-279. <https://doi.org/10.29050/harranziraat.1071821>

Effects of Aqueous Plant Extracts on Adult Females *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

The Two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), reduces quality and yield in cultivated plants. The most widely employed method for controlling this mite is the application of chemical acaricides. However, over time, researchers have focused on the adverse effects such as resistance, side effects on beneficial organisms, and environmental issues. In this laboratory study, the acaricidal effects of aqueous extracts of *Juglans regia*, *Dieffenbachia amoena*, *Lantana camara*, *Eucalyptus globulus*, and *Nerium oleander* against *T. urticae* were investigated. Plant extracts at concentrations of 1%, 3%, 6%, and 12% (v/v) were applied to *T. urticae* adult females using leaf dipping and spraying methods. The numbers of dead and live individuals were evaluated 24, 72, and 144 hours after the applications. In the dipping method, the highest contact effect was obtained in *J. regia* and *D. amoena* at 12% concentration at 99.6 and 94.5%, respectively, 144 hours after treatments. Conversely, at the same time and at 1% concentration, *L. camara* extract exhibited a low contact effect of 20.68%. *Juglans regia* extract at 12% concentration showed the highest effect and reached a 71% mortality rate at the end of 24 hours in the dipping method. The lowest effect (10.8% mortality rate) was obtained with *L. camara* at 1% concentration, and the same duration. *Dieffenbachia amoena* showed the highest toxicity with an LC₅₀ value of 2561 mg L⁻¹ in the spraying method, while the lowest toxicity was found in the *E. globulus* application (4388 mg L⁻¹). This study revealed that aqueous plant extracts showed toxic effects on *T. urticae*.

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Sulu Bitkisel Ekstraktların *Tetranychus urticae* Koch (Acari: Tetranychidae) Ergin Dişileri Üzerine Etkileri

ÖZET

İki noktalı Kırmızıörümcek, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) kültür bitkilerinde kalite ve verimi düşürmektedir. Bu akarlar mücadelede yaygın olarak kullanılan yöntem kimyasal akarisitlerin uygulanmasıdır. Ancak zaman içerisinde araştırmacılar direnç, faydalı organizmalar üzerindeki yan etkiler ve çevresel sorunlar gibi olumsuz etkiler üzerine odaklanmıştır. Bu laboratuvar çalışmasında *Juglans regia*, *Dieffenbachia amoena*, *Lantana camara*, *Eucalyptus globulus* ve *Nerium oleander* sulu ekstraktlarının *T. urticae*'ye karşı akarisit etkileri araştırılmıştır. %1, 3, 6 ve 12 (v/v) konsantrasyonlardaki bitki ekstraktları yaprak daldırma ve püskürtme yöntemleri ile *T. urticae* ergin dişilerine uygulanmıştır. Uygulamalardan, 24, 72 ve 144 saat sonra elde edilen ölü-canlı birey sayıları değerlendirilmiştir. Daldırma yönteminde en yüksek değme etkisi, %12 konsantrasyonda *J. regia* ve *D. amoena*'da uygulamalardan 144 saat sonra sırasıyla %99.6 ve 94.5 olarak elde edilmiştir. Buna karşılık, aynı süre ve %1'lik konsantrasyonda *L. camara* ekstraktında %20.68'lik düşük değme etkinliği belirlenmiştir. Daldırma yönteminde %12 konsantrasyonda *J. regia* ekstraktı en

Pestisitler ve Toksikoloji

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Anahtar Kelimeler

Akar
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Mortalite

yüksek etkiyi göstermiş ve 24 saatlik sürenin sonunda %71 ölüm oranına ulaşmıştır. En düşük etki (%10.8'lik ölüm oranı) ise %1 konsantrasyonda ve aynı sürede *L. camara* ile elde edilmiştir. Püskürtme metodunda en yüksek toksisiteyi 2561 mg L⁻¹ LC₅₀ değeri ile *Dieffenbachia amoena* gösterirken , en düşük toksisite *E. globulus* uygulamasında (4388 mg L⁻¹) saptanmıştır. Bu çalışma, sulu bitki ekstraktların *T. urticae*'ye toksik etki gösterdiğini ortaya koymuştur.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (TSSM) is a harmful organism that highly prevalent pest in agricultural fields in favorable climate conditions (Helle & Sabelis, 1985; Maniania et al., 2009; Alpkent & Ferizli, 2024). This polyphagous mite feeds on approximately 3877 host plants, encompassing more than 150 plant species (Zhang, 2003; Attia et al., 2013). Ineffective control strategies of crop plants often lead to inevitable economic losses (Gore et al., 2013; İnak et al., 2022; Alpkent & Çobanoğlu, 2024). Acaricides are commonly used for their rapid effectiveness in controlling this mite (Van Leeuwen et al., 2006). Among arthropods, TSSM is notable for developing resistance at a rapid rate exhibiting the highest resistance coefficients (Van Leeuwen et al., 2010; Sugimoto & Osakabe, 2014). Factors such as parthenogenesis reproduction type in mite species, short life cycle, and high reproductive capacity under optimal conditions contribute to the accelerated development of resistance to acaricides (Van Leeuwen et al., 2009, Döker & Kazak, 2012; Döker et al., 2021). Cross-resistance can between acaricides with different modes of action but similar detoxification mechanisms, leading to rapid resistance development to other pesticides with different modes of action. *Tetranychus urticae* has developed resistance to a total of 92 active ingredients including various organophosphates, carbamates, pyrethroids, pyrazoles, pyridazines, and ketones (Van Leeuwen et al., 2010). The increased application of multiple pesticides including acaricides, has accelerated this resistance development in *T. urticae*. Consequently, researchers have begun to explore the impacts of these pesticides on non-target organisms, humans, and ecosystems. Studies indicate that pesticides from various groups can affect even phylogenetically distant non-target organisms (Wan et al., 2023). The intensive use of chemical pesticides has led to significant resistance problems for various pests (Ulusoy, 2020; Ulusoy, 2024). As a result, researchers are investigating alternative control methods, including biological control, bio-pesticides, and pesticide rotation with environmentally friendly active ingredients (Kahya et al., 2021; Hazir et al., 2022; Ulusoy et al., 2022). The focus is increasingly on finding or producing natural, non-harmful alternative acaricides that do not negatively affect humans or the environment. Under the European Commission's Green Deal, sustainable practices have gained prominence. The European Commission's Environmental initiative aims to significantly reduce agricultural pesticide use. The Farm to Table strategy within the Green Deal promotes sustainable farming practices by reducing reliance on pesticides and fertilizers (Wang et al., 2022). These efforts align with the Green Deal's overarching goals of achieving a climate-neutral economy and ensuring a healthy environment (Orgiazzi et al., 2022). Current research shifting towards natural materials that harmonize with both nature and human health. Among them, phytochemicals are a major focus. Natural acaricides, such as plant extracts are non-permanent on the environment, environmentally benign, and rapidly degradable; they also tend to have lower mammalian toxicity compared to synthetic acaricides (Regnault-Roger et al., 2012; Sararit & Auamcharoen, 2020). Numerous studies investigate the efficacy of plant extract compounds as alternatives to synthetic pesticides, with some demonstrating insecticidal, acaricidal, repellent, and residual effects (Krue Wong & Auamcharoen, 2023; Li et al., 2023; Piffer et al., 2023; De Souza et al., 2023). Consequently, *T. urticae* remains a significant pest in production areas due to several reasons explained earlier (Zhang, 2003).

In this study, aqueous extracts obtained from *Nerium oleander* Linnaeus,1753 (Gentianales: Apocynaceae), *Lantana camara* Linnaeus,1753 (Lamiales: Verbenaceae), *Dieffenbachia amoena* Bull. (Alismatales: Araceae), *Juglans regia* Linnaeus,1753 (Juglandales: Juglandaceae), and *Eucalyptus globulus* Labillardière, 1800 (Myrtales: Myrtaceae) plants collected from Ankara and Adana provinces. No damage caused by mites has been reported for these plant species included in the study. Hence, to evaluate their control potential, the acaricidal properties of aqueous plant extracts from *J. regia*, *D. amoena*, and *N. oleander*, were tested against this mite species for the first time. Additionally, the study supports the broader goals of sustainable agriculture by investigating natural, environmentally friendly alternatives to synthetic pesticides with a focus on plant-based acaricides.

MATERIAL and METHOD

Mass-rearing of Spidermites

A sensitive population of spider mite (German Susceptible Strain, GSS) *Tetranychus urticae* Koch (Acari: Tetranychidae) was obtained from Isparta University of Applied Sciences (Türkiye) in 2015 and reared under pesticide-free conditions. These mites were cultivated on uncontaminated bean plants (*Phaseolus vulgaris* L. (Fabaceae)) in a climate chamber at 26±1°C, 60±5% humidity, and a 16h Light: 8 h Dark photoperiod.

Source of Plants

Leaves of *Juglans regia* L. (Juglandales: Juglandaceae) were gained from the campus area of the Directorate of Plant Protection Central Research Institute, Ankara while other species were collected from the Adana province and garden of the Biological Control Research Institute, Adana, Türkiye (Table 1). Leaves and stems of the plants were collected. Plants were collected in the period after the fresh leaves had emerged.

Table 1. Plants collection sites and date

Çizelge 1. Bitkilerin toplandığı yerler ve tarihi

Heading	Locations (district/province)	Collection date
<i>Juglans regia</i>	39° 57' 17.6" N 32° 48' 19.6" E (Yenimahalle/ Ankara)	2022/6
<i>Dieffenbachia amoena</i>	37° 00' 38.0" N 35° 20' 23.5" E (Yüreğir/ Adana)	2022/6
<i>Lantana camara</i>	37° 00' 38.1" N 35° 20' 23.3" E (Yüreğir/ Adana)	2022/6
<i>Eucalyptus globulus</i>	37° 01' 37.5" N 35° 20' 02.7" E (Yüreğir/ Adana)	2022/6
<i>Nerium oleander</i>	37° 00' 38.0" N 35° 20' 23.8" E (Yüreğir/ Adana)	2022/6

Extraction of Aqueous Plant Extracts

Plant leaves and stems were gathered together and transferred to the laboratory in paper bags. The plant material was rinsed with tap water and then placed on filter paper (Wh 1) to dry. The foliage was separated from the stems and dried at 30°C in an oven (Memmert UM200, Germany) for 72 hours. Dried leaves were crushed in a grinder until the particle size was below 1 mm (Ultra-turra t8 IKA, Germany) (Castillo et al., 2010). Obtained powdered foliage was transferred into 50 ml Falcon tubes (Spinwin PP) containing pure sterile water at a concentration of 200 g L⁻¹. The mixture was kept at 350 rpm speed for 24 hours in a magnetic shaker (Thermo Mixer F1.5, Germany). The resulting solution was centrifuged at 10000 g (NF 1200 R) for 5 minutes in a refrigerated centrifuge to allow particles to settle at the bottom. Subsequently, the solution was filtered with filter paper (Whatman no 41). The solution was passed via sterile cellulose filter membranes (0.22 µm Millex) using an injector. The filtrate was transferred into opaque (amber) 50 mL glass tubes and placed in the refrigerator at +4°C. Each extract was prepared fresh before the experiment.

Biological Assays

Leaf-dip method

Clean bean leaves were grown in a plant growth chamber under conditions of 55±5% moisture and 26±1°C temperature, with a 16-hour light-8-hour dark cycle. Plant leaves with a diameter of 3 cm were cut, and 0.01% triton x-100 purified water was used to prepare plant extract concentrations of 1, 3, 6, and 12% (v/v) (Mironova & Khorkhordin, 1997). The cut bean leaves were dipped into these solutions for fifteen seconds. Subsequently, they were placed with the upper sides of the leaves facing up on glass Petri dishes (9 cm in diameter) containing 1.5% agar gel. The leaves were left in laminar-flow cabinets for thirty minutes to dry. Adult female spider mites (2 to 3 days old) were then transferred to the bean leaves. This process was repeated four times for each concentration. A minimum of six concentrations were established, with 20 adult female individuals per replication. The Petri dishes were placed in a climate-controlled cabinet (Sanyo MIR552, Japan) set at 25±1°C, with a humidity level ranging from 55 to 65%, and a 16/8-hours light/dark photoperiod. Alive and dead evaluations were conducted 24 hours after application to assess acute effects. Counts of dead and alive individuals continued after 72 and 144 hours to assess persistent toxicity. Preparations containing azadirachtin A (Econeem10 g active ingredient L⁻¹), and formulated pyridaben (Sanmite 200 g a.i L⁻¹) were used as positive controls. The negative control group was pure water. All contact activity experiments were set up following the modified IRAC 004 method (IRAC, 2022). Trials were arranged using a randomized block design.

Spraying method by Potter Tower

Cotton pads placed inside Petri dishes (9 cm) were moistened. Circular leaves (3 cm in diameter) were transferred

on the moistened cotton pads. The edges of bean leaves were covered with paper napkins to prevent the mites from escaping. Approximately 25-30 adult female *T. urticae* mites (2 to 3 days old) individuals were transferred to the leaves. The prepared Petri dishes were positioned within a Potter Tower (Burkard Scientific-BS00281, England), and a 2 ml extract solution ($1.83 \pm 0.05 \text{ mg cm}^{-2}$) was evenly sprayed (1 bar pressure) onto them. Preparations containing azadirachtin A (EcoNeem10 g a.i. L⁻¹) and pyridine (Sanmite 200 g a.i. L⁻¹) served as controls. The negative control group received only distilled water. Petri dishes were air-dried for thirty minutes in a fume hood (Telstar, Japan). The Petri dishes were then transferred to incubators set at $25 \pm 1^\circ\text{C}$, with a humidity level between 55% to 65%, and a 16-h light/ 8-h dark photoperiod (Sanyo MIR552, Japan). The cotton pads in the Petri dishes were soaked daily to maintain moisture. At the end of the 72-hour application period, both dead and surviving individuals were counted to assess the effects of the application. Each extract was tested across a minimum of six concentrations to establish lethal concentrations, with each concentration tested in three replicates. Experiments were conducted using a randomized block design. Individuals who did not react to fine brush stimulation were considered dead. Experiments were performed by modifying according to the Irac 004 method.

Statistical Analyses Assessment of Data

Data obtained from biological assays were initially subjected to arcsine transformation. Subsequently, One-way ANOVA was performed, followed by Tukey's test ($\alpha = 0.05$). Statistical analyses were performed using the MINITAB 18 (Release 18.1) software. The efficacy of each extract was evaluated by comparing it with itself in terms of time (hours) and concentration. Additionally, accordingly, data from the bioassays underwent probit analysis according to Finney (1971), and LC₅₀ and LC₉₀ values were determined using Polo-Plus software (LeOra, 1994).

FINDINGS and DISCUSSION

Leaf-dip method

The leaf-dip method was used to assess the contact effects of various extracts on the adult females of the susceptible GSS population of *T. urticae*. Mortality rates increased proportionally with dosage escalation. These increases continued up to the 72nd hour in terms of contact toxicity. Mortality rates did not significantly increase after the 72nd hour (up to the 144th hour) and began to level off (Table 2). The highest contact effect was observed with *J. regia* collected from Ankara, where a 12% concentration resulted in 99.6% mortality after 144h of application ($F=58.02$; $df= 4, 19$; $P<0.05$). At a 12% concentration, the mortality rate was 71.5% at the end of 24 hours, and 99% at the end of 72 hours. Mortality rates continued to increase at a slower rate. The least pronounced effect was observed in a 1% concentration after 24 hours, with a mortality rate of 34.6% ($F=39.03$; $df= 4, 19$; $P<0.05$). The second-highest mortality rate was observed with *D. amoena*, reaching 94.5% after 144 hours of application ($F=53.97$; $df= 4, 19$; $P<0.05$). The contact effect after 72 hours resulted in a 93.4% mortality rate, while the 24-hour contact effect resulted in a 60.0% mortality rate.

For another efficient extract, *E. globulus*, the highest mortality rate was 88.5% at the end of 144 hours of application at a 12% concentration ($F=20.58$; $df= 4, 19$; $P<0.05$). The last contact effect was detected at a 1% concentration after 24 hours, with a mortality rate of 33.5% ($F=41.26$; $df= 4, 19$; $P<0.05$). The highest concentrations and residual effects in *L. camara* and *N. oleander* extracts were observed at 144 hours of application, with mortalities of 81.2 and 77.5%, respectively. Throughout the study, the lowest contact effect was in *L. camara* extract at a 1% concentration after 24-hours, with a mortality rate of 10.8% ($F=2.76$; $df= 4, 19$; $P>0.05$). In general, the impact of almost all extracts diminished after 72 hours. Results from the 144-hour trial showed slight changes compared to the 72-hour test results. This indicates that the efficiency of the extracts decreased after 72 hours. It is believed that the extracts were decomposed quickly in the incubator ($25 \pm 1^\circ\text{C}$), resulting in a reduced residual effect. Azadirachtin A and the formulated synthetic acaricide pyridazine at a 1% concentration caused 100.0% mortality in all exposure times (Table 2).

Spraying method by Potter Tower

The susceptibility of *T. urticae* varied in response to the spray application of five different plant extracts (Table 3). The LC₅₀ value for *D. amoena* on *T. urticae* was determined to be 2561 mg L⁻¹, representing the highest toxicity compared to the other aqueous extracts. This is followed by *N. oleander*, *J. regia*, *L. camara*, and *E. globulus*, respectively. The LC₅₀ and LC₉₀ for azadirachtin A were 73.2 and 250 mg L⁻¹, respectively. The toxicity levels of pyridazine, a synthetic chemical, were found to be 74.8 and 316 mg L⁻¹, respectively.

Water-based extracts obtained from Adana and Ankara, along with two formulated acaricides (pyridine and azadrahtin A), were evaluated for their potential acaricidal effects against adult females of *T. urticae*. Numerous studies have assessed the efficacy of various extracts in controlling insects and mites (Kim et al., 2005; Adenubi et

al., 2019; Piffer et al., 2023; Ellafi et al., 2023; Muhammad et al., 2023; Zhu et al., 2023). The lethal efficacy of plant extracts against various insects and mites has been well-documented. Studies indicate that many plants possess insecticidal and acaricidal properties, extracts demonstrating insecticidal, ovicidal, acute toxicity, larvicidal, reproductive-inhibitory, adulticidal, repellent, and fumigant effects (Kim et al., 2005; Cetin & Yanikoglu, 2006; Pavela et al., 2016; Fang et al., 2020; Assemie & Gameda, 2023). Plant extracts are generally less toxic to parasitoids, predators, and biological agents, making them suitable for sustainable agricultural pest management. They are effective and harmless to non-target organisms (Kilani-Morakchi et al., 2021). Results of the current study indicate that *J. regia* extracts caused significant mortality in adult females of *T. urticae* after 6-days (Table 2). Moreover, the LC₅₀ for *J. regia* (3257 mg L⁻¹) was found to be the second most toxic against *T. urticae*, following *D. amoena* (2561 mg L⁻¹) after a 3-day application (Table 3).

Table 2. Mortality rates of Tetranychus urticae adult females after exposure to aqueous plant extracts using the leaf-dip method, at 24, 72, and 144 hours of post-application

Cizelge 2. Sulu bitki ekstratlarının yaprak daldırma yöntemiyle uygulamadan 24, 72 and 144 saat sonra Tetranychus urticae ergin dişilerinde neden oldukları ölüm oranları

Plant extract	Concentration (%)	Time (h)		
		Mortality (%) ± SE*		
		24	72	144
<i>Juglans regia</i>	1	34.61 ± 2.15A**b	56.53 ± 1.87Ab	59.24 ± 2.36Ab
	3	48.71 ± 1.75Bab	70.06 ± 0.20Ab	72.54 ± 0.10Ab
	6	49.99 ± 0.50Bab	71.42 ± 0.51Ab	76.92 ± 1.49Ab
	12	71.52 ± 0.88Ba	99.01 ± 3.90Aa	99.60 ± 2.57Aa
	Control	1.27 ± 0.32Ac	2.57 ± 0.65Ac	2.57 ± 0.65Ac
	F	39.03	55.75	58.02
<i>Dieffenbachia amoena</i>	1	25.72 ± 1.43Bb	55.06 ± 0.85Ab	58.80 ± 0.41Ab
	3	36.13 ± 0.61Bb	63.91 ± 0.65Ab	66.37 ± 0.46Ab
	6	58.85 ± 0.60Ba	92.40 ± 3.55Aa	93.38 ± 3.11Aa
	12	60.03 ± 0.17Ba	93.38 ± 3.11Aa	94.52 ± 2.95Aa
	Control	1.27 ± 0.32Ac	1.69 ± 1.27Ac	2.83 ± 1.27Ac
	F	56.05	47.64	53.97
<i>Lantana camara</i>	1	10.84 ± 0.66Ab	19.48 ± 1.10Ab	20.68 ± 1.22Ab
	3	43.73 ± 0.23Ba	62.72 ± 1.02Aa	65.21 ± 0.93Aa
	6	52.52 ± 0.42Ba	71.34 ± 0.28Aa	75.23 ± 0.64Aa
	12	55.01 ± 0.17Ba	75.30 ± 0.73Aa	81.23 ± 2.41Aa
	Control	1.69 ± 1.27Ac	1.69 ± 1.27Ac	1.69 ± 1.27Ac
	F	2.76	11.53	8.41
<i>Eucalyptus globulus</i>	1	33.56 ± 0.81Ab	46.24 ± 0.40Ac	50.04 ± 1.19Ab
	3	44.97 ± 0.50Aab	58.87 ± 0.77Abc	62.96 ± 2.00Aab
	6	48.73 ± 0.57Aab	69.48 ± 2.36Aab	76.10 ± 5.72Aab
	12	62.72 ± 1.02Aa	80.44 ± 0.90Aa	88.48 ± 5.49Aa
	Control	1.69 ± 1.27Ac	2.83 ± 1.27Ad	2.83 ± 1.27Ad
	F	41.26	46.77	20.58
<i>Nerium oleander</i>	1	32.37 ± 0.48Ba	56.27 ± 0.23Aa	58.76 ± 0.06Aa
	3	37.27 ± 1.18Ba	67.59 ± 0.30Aa	70.06 ± 0.20Aa
	6	46.24 ± 0.40Ba	67.69 ± 0.83Aa	71.34 ± 0.28Aa
	12	51.26 ± 0.57Ba	76.29 ± 0.09Aa	77.55 ± 0.12Aa
	Control	1.27 ± 0.32Ab	1.27 ± 0.32Ab	1.69 ± 1.27Ab
	F	26.32	68.96	90.61
Econeem (Azadirachtin A)	1	100.0 ± 0.0Aa	100 ± 0.0Aa	100 ± 0.0Aa
	3	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	6	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	12	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	Control	0.0±0.0Bb	0.0±0.0Bb	0.0±0.0Bb
	F	-	-	-
Sanmite (Pyridaben)	1	100.0 ± 0.0Aa	100 ± 0.0Aa	100 ± 0.0Aa
	3	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	6	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	12	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	Control	0.0±0.0Bb	0.0±0.0Bb	0.0±0.0Bb
	F	-	-	-

* Standart error, ** Different uppercase letters in the same row and different lowercase letters in the same column indicate a significant difference according to extracts and application concentrations, respectively (p≤0.05).

Table 3. Toxicity of aqueous plant extracts to *Tetranychus urticae* adult females at 72-h of post-application
Çizelge 3. Sulu bitki ekstraktlarının Tetranychus urticae ergin dişilerine uygulamadan 72 saat sonra toksisiteleri (72 s maruziyet)

Plants	n^a	Slope+SE ^b	LC ₅₀ (mg L ⁻¹) (0.95 CL ^c)	LC ₉₀ (mg L ⁻¹) (0.95 CL)	$\chi^2(df)^d$	P-values
<i>Juglans regia</i>	480	1.29 ± 0.18	3257(2393–4158)	32077 (20481–66962)	12.70(17)	0.76
<i>Dieffenbachia amoena</i>	480	1.50 ± 0.19	2561(1894–3223)	18352 (13096–30736)	14.10(17)	0.66
<i>Nerium oleander</i>	560	1.32 ± 0.13	3170(2517–3937)	29833 (19977–53100)	10.94(21)	0.96
<i>Eucalyptus globulus</i>	480	1.29 ± 0.17	4388(3367–5559)	42823 (26548–93249)	13.00(17)	0.73
<i>Lantana camara</i>	560	1.55 ± 0.14	3770(3110–4563)	25310 (18119–40019)	12.87(21)	0.91
Azadirachtin A	480	2.40 ± 0.19	73.2 (65.4–82.7)	249.9 (202.1–330.1)	11.47(17)	0.83
Pyridaben	480	2.05 ± 0.24	74.8 (63.8–90.89)	316.3 (220.9–552.8)	10.53(17)	0.88

^an: Total number of mites; ^bSE: Standart error; ^cCL: Confidence limits; ^dChi-square value and degrees of freedom

Few studies have documented the acaricidal efficacy of *J. regia*. Wang et al. (2007) investigated the contact and systemic effects of *J. regia* leaves on *Tetranychus viennensis* Zacher, 1920 (Acari: Tetranychidae) and *Tetranychus cinnabarinus* Boisduval, 1867 (Acari: Tetranychidae). They found that the mean lethal concentration (LC₅₀) for adult *Tetranychus* species ranged between 60.5 - 87.6 mg mL⁻¹ after 24 hours, respectively, based on the three different bioassay methods. In addition, the LC₅₀ for systemic activity ranged between 95 - 97 mg mL⁻¹ after 48 hours, respectively. In another study, the authors reported that using various extraction methods on *J. regia* samples at a dosage of 1 mg mL⁻¹ resulted in an 83.4% mortality rate for *T. cinnabarinus* after 24 hours (Wang et al., 2012). Erdogan and Yilmaz (2017) demonstrated that a 12% ethanolic leaf extract of *J. regia* was 83% toxic to adult females of *T. urticae* following a 24-hour using the leaf-dipping method. In the spraying method, at the same concentration, a 100% mortality rate was observed for adult females of *T. urticae*. Mahla et al. (2013) showed that an ethanolic leaf extract of *J. regia* affected the immature stages of TSSM, with mortality rates recorded between 13 and 30% after daily observations. Wang et al. (2009) revealed that using different extraction methods such as methanol, chloroform, and petroleum ether on *J. regia* and applying a concentration of 1 mg mL⁻¹ resulted in mortality rates for *T. cinnabarinus* ranging from 23% to 80% after 24 hours. These variations among studies highlight that phytochemical content in the plants can vary. The choice of extraction method is crucial for determining biological activity because it affects the phytochemical composition and the ratio of main chemical components (Kouninki et al., 2005).

Literature on *Dieffenbachia amoena* is limited. Hence, the present study represents the initial investigation into the acaricidal efficacy of *D. amoena* against adult females of *T. urticae*. *Dieffenbachia amoena* contains alkaloids, flavonoids, glycosides, phytic acid, tannins, oxalates, and saponin which is responsible for sudden and severe reactions (Dvorack et al., 1999; Eno & Ubi, 2021). It has been reported that extracts from leaves and stems of *D. amoena* are used as a poison in South America (Paris & Moyse, 1967; Altschul, 1973). Additionally, it is associated with inflammation, vomiting, nausea irritation of the tongue and throat. Plants from this family have been shown to possess high degrees of chronic and acute toxicity, with potential genotoxic, carcinogenic, and mutagenic effects (Eno & Ubi, 2021). Ganiyat et al. (2011) revealed that essential oils (EO's) from the leaves and stems of *Dieffenbachia picta* (Lodd.) Schott (1852) (Arecidae: Arales) exhibited antimicrobial and antioxidant activities. Ulusoy et al. (2019) assessed the impact of inhibiting acetylcholinesterase and carboxylesterase in *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) using *D. amoena*. The results indicated that *D. amoena* extracts at a concentration of 10% exhibited an inhibitory effect of 21% inhibitory effect on acetylcholinesterase (AChE) and a 36% inhibitory effect on carboxylesterase (CarE) with the lowest efficacy observed in the experiment. *Dieffenbachia amoena* extract was found to be the most effective in the current study through the spray method. Many studies report that some essential components of the plant extract being examined are not consistently found, even when sourced from the same plant (Djenane et al., 2011; Akolade et al., 2012; Shala & Gururani, 2021). Conversely, some researchers have noted significant variations in the content of the same product depending on the region where it is grown (Djenane et al., 2011; Hafsa et al., 2016). This variation may explain the differences in the acaricidal effectiveness among plants.

Various phytochemical components of *Nerium oleander* include amino acids, alkaloids, terpenoids, cholesterol, glycosides, flavonoids, proteins, tannins, tannins and floatiness (Kiran & Prasad, 2014; Çelebioğlu et al., 2020). According to the authors, *N. oleander* exhibits insecticidal, antibacterial, and cytotoxic properties (Mijatovic et al., 2007; Zaid et al., 2022; Saeed et al., 2023). Some researchers have mentioned its toxic effects on the nervous, reproductive, and digestive tracts of harmful organisms (Kumar et al., 2013). There is limited research on its acaricidal activity against *Tetranychus* species. The toxic activity of *N. oleander* has been studied by various researchers against different mites. In a study by Tawfik and Mahmoud (2009), ethanol and hexane extracts from

the leaves of *N. oleander* showed contact LC_{50} values of 32.8 mg mL⁻¹ and 44.3 mg mL⁻¹, respectively, against *Rhizoglyphus echinus* Fumouze and Robin, 1868 (Acari: Acaridae). Salim et al. (2016) reported that at a 5% concentration, the mortality increased to 78%. When the concentration was raised to 10%, mortality ranged from 65 to 100% in the second stages of root-knot nematodes. Gholami et al. (2018) found that *N. oleander* hexane and alcohol extracts exhibited LC_{50} values of 3500 and 14000 ppm, respectively, after 24 hours of contact toxicity using the leaf-dip method against *T. urticae* individuals. Furthermore, another study observed a positive impact on mortality with increased application duration and concentration of the aqueous extract. Hasnawi and Shlash (2020) found that *Sarcoptes scabiei* Linnaeus, 1758 (Acariformes: Sarcoptidae) adults exhibited mortality rates of 50%, 80%, and 90% after 24, 48, and 72-h of treatment with EO obtained from *N. oleander* leaves at a concentration of 18 mg mL⁻¹, respectively. The median lethal concentration was (LC_{50}) 13.99 mg mL⁻¹.

Eucalyptus globulus contains phenolic compounds, tannins, cardiac glycosides, saponins, steroids, terpenoids, glycosides, terpenes, alkaloids, resins, acidic compounds, and flavonoids (Obiorah et al., 2012; Ishnava et al., 2013; Jamil et al., 2017; Ajilore et al., 2021; Shala & Gururani, 2021). Numerous studies have previously shown the miticide activity of EOs or extracts of the *E. globulus* or *Eucalyptus* sp. against *T. urticae* and other mites. Afify et al. (2012) reported that 1% and 3% concentrations of *Eucalyptus* sp. essential oil resulted in 27.5% and 70% mortality of adult spider mites after 1-day application, respectively. Hamed et al. (2021) reported an LC_{50} value of 876.15 ppm after 24 hours of exposure against adult female *T. urticae* for *E. globulus* oil. A study conducted by Madreseh-Ghahfarokhi et al. (2019) showed that water-distilled leaf extract from *E. globulus* had the highest acaricidal activity with a mortality of 53.8% on *Rhipicephalus bursa* Canestrini and Fanzago, 1877 (Acari: Ixodidae) adults at 1 (pure) concentration and at the end of a 2-h monitoring time. Aissaoui et al. (2019) noted that the acaricidal impact of *E. globulus* EO, obtained from the stem by the distillation method, was 61%, 76%, and 85% mortality on adult *T. urticae* at concentrations of 1%, 4%, and 8%. Choi et al. (2004) state that they did not include the essential oil experiment they conducted on adult *T. urticae* in their evaluation because the mortality was less than 60%. In another study, the acaricidal activity of *E. globulus* ethanol plant extract against female *T. urticae* was found at an LC_{50} value of 4600 ppm using the dipping method (Eldoksch et al., 2009). Likewise, Monika and Rachna (2008) showed that the miticidal activity of aqueous *E. globulus* leaf extract on various developmental stages of *T. urticae* was 81-93% at a concentration of 10%.

Many constituents have been identified in *Lantana Camara*. Depending on the plant organ (stem, leaf, flower, fruit), there are between 52 and 71 ingredients. The plant is especially rich in terpenes. The main constituents are caryophyllene, germacrene-D, elemene, humulene, sabinene, bicyclogermacrene, palmitic acid, stearic acid, davanone, curcumene, caryophyllene (Ngassoum et al., 1999; Khan et al., 2002; Khan et al., 2003). Caryophyllene is the most common constituent of the compounds studied so far (Saikia & Sahoo, 2011). It has been reported to have insecticidal, nematocidal, larvicidal, attractant, anti-juvenile repellent, feeding deterrent, growth inhibitor, growth regulator, oviposition inhibitor, antibacterial, anticancer, antiproliferative, hemolytic, antifungal, antimutagenic, antiulcerogenic, antioxidant, antihyperglycaemic, anti-inflammatory, antimotility, anti-urolithiasis, anti filarial and antifertility activity (Saikia & Sahoo, 2011; Reddy, 2013; Thanavendanand & Kennedy, 2015; Murugesan et al., 2016). Premalatha et al. (2018) stated that the acaricidal impact of the *L. camara* water extract was the least effective among the plants tested, with 30% efficacy at a 10% concentration against adult female *T. urticae*. Radhakrishnan and Prabhakaran (2014) also reported almost similar results. According to their study, *L. camara* was extracted from leaves using the hydro distillation method. After an observation exposure time of 24-96 hours, a concentration of 2.5% and 5% resulted in 18% and 30% mortality against *Oligonychus coffee* Nietner, 1861 (Acari: Tetranychidae). Laya et al. (2022) found that 5%, 7.5%, and 10% concentrations of aqueous leaf extracts of *L. camara* were toxic to *Tetranychus truncatus* Ehara, 1956 (Acari: Tetranychidae) with 41%, 50% and 72% mortality after 120-h exposure, respectively. Mandal et al. (2013) indicated that the miticidal efficacy of chloroform and hexane extracts from mature *L. camara* leaves on *T. urticae* was low. However, the *L. camara* extract in their study yielded a maximum mortality rate of 8.72% after 3-days of exposure for both extracts. When the study activity was observed on the 2nd and 3rd days, it was noted that the residual and mortality effects diminished over time. This and other studies suggest that the residual and mortality effect diminished over time (Chowdhury et al., 2008; Mandal et al., 2013; Kaoutar et al., 2019).

CONCLUSION and RECOMMENDATIONS

The toxic effects of aqueous plant extracts on spider mites have been demonstrated. Further investigation is recommended to determine which components contribute to the efficacy of extracts, particularly when their phytochemical properties are identified and extracted using different distillation methods. Additionally, there is a need to assess the activity of these extracts against different insect species. The high degradation rate of herbal extracts and their rapid toxic effects on *T. urticae* indicate that they could serve as an alternative to synthetic miticides.

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Statement of Conflict of Interest

The author has declared no conflict of interest.

REFERENCES

- Adenubi, O.T., Leboho, T., Eloff, J.N., Fouche, G., Naidoo, V., Wellington, K.W., & McGaw, L.J. (2019). Acaricidal activity of the aqueous and hydroethanolic extracts of 15 South African plants against *Rhipicephalus turanicus* and their toxicity on human liver and kidney cells. *Onderstepoort Journal of Veterinary Research*, 86(1), 1-7.
- Afify, A.E.-M.M., Ali, F.S., & Turkey, A.F. (2012). Control of *Tetranychus urticae* Koch by extracts of three essential oils of chamomile, marjoram, and Eucalyptus. *Asian Pacific Journal of Tropical Biomedicine*, 2(1), 24-30. [https://doi.org/10.1016/s2221-1691\(11\)60184-6](https://doi.org/10.1016/s2221-1691(11)60184-6)
- Aissaoui, A.B., Zantar, S., & Elamrani, A. (2019). Chemical composition and potential acaricide of *Salvia officinalis* and *Eucalyptus globulus* on *Tetranychus urticae* Koch (Acarina: Tetranychidae). *Journal of Applied Chemistry and Environment Protection*, 4(1), 1-15.
- Ajilore, B.S., Oluwadairo, T.O., Olorunnisola, O.S., Fadahunsi, O.S., & Adegbola, P.I. (2021). GC–MS analysis, toxicological and oral glucose tolerance assessments of methanolic leaf extract of *Eucalyptus globulus*. *Future Journal of Pharmaceutical Sciences*, 7(1), 1-9. <https://doi.org/10.1186/s43094-021-00312-5>
- Akolade, J.O., Olajide, O.O., Afolayan, M.O., Akande, S.A., Idowu, D.I., & Orishadipe, A.T. (2012). Chemical composition, antioxidant and cytotoxic effects of *Eucalyptus globulus* grown in north-central Nigeria. *Journal of Natural Product and Plant Resources*, 2(1), 1-8.
- Alpkent, Y.N., & Çobanoğlu, S. (2024). Determination of target site mutations and detoxification enzymes in the abamectin resistance of spider mite *Tetranychus urticae* collected from cotton fields of Türkiye. *Systematic and Applied Acarology*, 29(7), 794-810.
- Alpkent, Y.N., & Ferizli, A.G. (2024). Monitoring detoxification enzyme levels and resistance of *Tetranychus urticae* against some METI-group chemicals in Türkiye cotton fields. *Experimental and Applied Acarology*, 1-17.
- Altschul, S.R. (1973). *Drugs and Food from Little-known Plants*. Harvard University, Cambridge Press, Massachusetts, USA, 366 pp.
- Assemie, A., & Gemedat, T. (2023). Larvicidal activities of *Allium sativum* L. and *Zingiber officinale* Rosc. Extracts against filariasis vectors in Hadiya Zone, Ethiopia. *BioMed Research International*, 6636837. <https://doi.org/10.1155/2023/6636837>
- Attia, S., Grissa, K.L., Lognay, G., Bitume, E., Hance, T., & Mailleux, A.C. (2013). A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae) with special reference to natural pesticides: Biological approaches to control *Tetranychus urticae*. *Journal of Pest Science*, 86, 361-386. <https://doi.org/10.1007/s10340-013-0503-0>
- Castillo, F., Hernández, D., Gallegos, G., Mendez, M., Rodríguez, R., Reyes, A., & Aguilar, C.N. (2010). In vitro antifungal activity of plant extracts obtained with alternative organic solvents against *Rhizoctonia solani* Kuhn. *Industrial Crops and Products*, 32(3), 324-328. <https://doi.org/10.1016/j.indcrop.2010.05.013>
- Çelebioğlu, H.U., Kesici, A., & Recep, T.A.Ş. (2020). Investigation of possibilities of using *Nerium oleander* L. Extract as Prebiotic for *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *Erzincan University Journal of Science and Technology*, 13(3), 1147-1157. <https://doi.org/10.18185/erzifbed.749317>
- Cetin, H., & Yanikoglu, A. (2006). A study of the larvicidal activity of Origanum (Labiatae) species from Southwest Turkey. *Journal of Vector Ecology*, 31(1), 118-122. [https://doi.org/10.3376/1081-1710\(2006\)31\[118:ASOTLA\]2.0.CO;2](https://doi.org/10.3376/1081-1710(2006)31[118:ASOTLA]2.0.CO;2)
- Choi, W.I., Lee, S.G., Park, H.M., & Ahn, Y.J. (2004). Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Economic Entomology*, 97(2), 553-558. <https://doi.org/10.1093/jee/97.2.553>
- Chowdhury, N., Ghosh, A., & Chandra, G. (2008). Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *BMC Complementary and Alternative Medicine*, 8(1), 1-8. <https://doi.org/10.1186/1472-6882-8-10>

- De Souza, S.A., Padial, I.M.P.M., Domingues, A., Mauad, J.R.C., Formagio, A.S.N., Campos, J.F., Malaquias, J.B., & Mussury, R.M. (2023). An Interesting relationship between the insecticidal potential of *Simarouba* sp. in the biology of Diamondback Moth. *Sustainability*, 15(10), 7759. <https://doi.org/10.3390/su15107759>
- Djenane, D., Yangüela, J., Amrouche, T., Boubrit, S., Boussad, N., & Roncalés, P. (2011). Chemical composition and antimicrobial effects of essential oils of *Eucalyptus globulus*, *Myrtus communis* and *Satureja hortensis* against *Escherichia coli* O157:H7 and *Staphylococcus aureus* in minced beef. *Food Science and Technology International*, 17(6), 505–515. <https://doi.org/10.1177/1082013211398803>
- Döker, I., & Kazak, C., (2012). Detecting acaricide resistance in Turkish populations of *Panonychus citri* McGregor (Acari: Tetranychidae). *Systematic Applied and Acarology*, 17, 368–377. <https://doi.org/10.11158/saa.17.4.4>
- Döker, İ., Kazak, C., & Ay, R. (2021). Resistance status and detoxification enzyme activity in ten populations of *Panonychus citri* (Acari: Tetranychidae) from Turkey. *Crop Protection*, 141, 105488.
- Dvorack, P., Sucman, E., Cervenakona, D., Korinnek, K., & Blechova, R. (1999). Utilization of Bioassay with *Artemia salina* in Pharmacology. Collection of abstracts from 9th Conference on toxicity biodegradability of wastes and chemicals significant in aquatic environment, Solan, Czech Republic, pp 9-15.
- Eldoksch, H.A., Ayad, F.A., & El-Sebae, A.H. (2009). Acaricidal activity of plant extracts and their main terpenoids on the Two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Alexandria Science Exchange Journal*, 30(3), 344-349. <https://doi.org/10.21608/asejaigjsae.2009.3248>
- Ellafi, A., Farhat, R., Snoussi, M., Noumi, E., Anouar, E.H., Ben Ali, R., El May, M.V., Sayadi, S., Aouadi, K., Kadri, A. & Ben Younes, S. (2023). Phytochemical profiling, antimicrobial, antibiofilm, insecticidal, and anti-leishmanial properties of aqueous extract from *Juglans regia* L. root bark: In vitro and in silico approaches. *International Journal of Food Properties*, 26(1), 1079-1097. <https://doi.org/10.1080/10942912.2023.2200561>
- Eno, I.P., & Ubi, G.M. (2021). Toxic effect of the floristic Dumb Cane (*Dieffenbachia amoena*) phytochemicals and anti Nutritional Factors on household. *Journal of Agriculture and Food Sciences*, 5(1), 13-24.
- Erdogan, P., & Yilmaz, B.S. (2017). Acaricidal activity of extracts of *Juglans regia* L. on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Food Science and Engineering*, 7, 202-208. <https://doi.org/10.17265/2159-5828/2017.04.004>
- Fang, F., Li, M., Jiang, Z., Lu, X., Guillot, J., & Si, H. (2020). Comparing acaricidal and ovicidal activity of five terpenes from essential oils against *Psoroptes cuniculi*. *Parasitology Research*, 119, 4219-4223. <https://doi.org/10.1007/s00436-020-06823-z>
- Finney, D.J. (1971). *Probit Analysis*. 3rd Edition, Cambridge University Press, Cambridge.p 333
- Ganiyat, K.O., Patricia, A.O., & Sunday, F.A. (2011). Chemical composition, toxicity, antimicrobial and antioxidant activities of leaf and stem essential oils of *Dieffenbachia picta* (Araceae). *European Journal of Scientific Research*, 49(4), 567-580.
- Gholami, M., Goldasteh, S., Rafiei Karahroudi, Z., & Hosseinzadeh, M.R. (2018). Study on the effect of normal hexane and Alcohol in plant extracts of *Proboscidea fragrans*, *Nerium oleander*, *Datura innoxia* and *Hyoscyamus niger* on *Tetranychus urticae* Koch (Acari: Tetranychidae) in laboratory conditions. *IAU Entomological Research Journal*, 10(3), 177-189.
- Gore, J., Cook, D.R., Catchot, A.L., Musser, F.R., Stewart, S.D., Leonard, B.R., Lorenz, G., Studebaker, G., Akin, D.S., Tindall, K.V. & Jackson, R.E. (2013). Impact of twospotted spider mite (Acari: Tetranychidae) infestation timing on cotton yields. *Journal of Cotton Science*, 17, 34–39.
- Hafsa, J., Ali Smach, M., Khedher, M.R.B., Charfeddine, B., Limem, K., Majdoub, H., & Rouatbi, S. (2016). Physical, antioxidant and antimicrobial properties of chitosan films containing *Eucalyptus globulus* essential oil. *LWT - Food Science and Technology*, 68(2016), 356-364. <https://doi.org/10.1016/j.lwt.2015.12.050>
- Hamed, S.A., El-Rahman, A., Nasseem, H.A., & Anis, M.K. (2021). Efficacy of Some Pesticides and Plant Extracts on Two Spotted Spider Mite *Tetranychus urticae*, (Koch) on Cotton Plant. *Journal of Plant Protection and Pathology*, 12(9), 585-591. <https://doi.org/10.21608/jppp.2021.207635>
- Hasnawi, N.M., & Shlash, S.A. (2020). Competence of *Nerium oleander* oil against *Sarcoptes scabiei* in vitro. *International Journal of Pharmaceutical Research*, 2, 1383-1386.
- Hazir, A., Yayla, M., Kahya, D., & Atakan, E. (2022). Effectiveness of various insecticides and predatory bug, *Orius laevigatus* (Fieber, 1860) (Hemiptera: Anthocoridae) releases on *Thrips hawaiiensis* (Morgan, 1913)(Thysanoptera: Thripidae) in lemon, *Citrus limon* (L.)(Rutales: Rutaceae), orchard in Mersin (Türkiye). *Türkiye Entomoloji Dergisi*, 46(4), 373-383. <https://doi.org/10.16970/entoted.1102841>
- Helle, H., & Sabelis, M.W. (1985). Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, p 353.
- İnak, E., Alpkent, Y.N., Saalwaechter, C., Albayrak, T., İnak, A., Dermauw, W., Geibel, S., & Van Leeuwen, T. (2022). Long-term survey and characterization of cyflumetofen resistance in *Tetranychus urticae* populations from Turkey. *Pesticide biochemistry and physiology*, 188, 105235. <https://doi.org/10.1016/j.pestbp.2022.105235>
- IRAC, (2022). *The IRAC library of susceptibility test methods*. (Web page: <https://irac-online.org/test>)

- methods/)(Date accessed: January, 2023)
- Ishnava, K.B., Chauhan, J.B., & Barad, M.B. (2013). Anticariogenic and phytochemical evaluation of *Eucalyptus globules* Labill. *Saudi Journal of Biological Sciences*, 20(1), 69–74. <https://doi.org/10.1016/j.sjbs.2012.11.003>
- Jamil, K., Asmuddin, M., Ranawat, B., & Rao, C. (2017). Estimation of antibacterial activity of plants extracts from *Phyllanthus emblica*, *Terminalia chebula* and *Eucalyptus globulus* against oral pathogens. *International Journal of Dentistry and Oral Health*, 3(9), 100–104. <https://doi.org/10.25141/2471-657X-2017-9.0087>
- Kaoutar, F., Najiba, B., Rabea, Z., El Hassane, B., & Najib, G. (2019). Evaluation of the impact of five bio-insecticides of plant origin and a chemical insecticide on the survival of imago of the parasitoid *Aphidius colemani* under laboratory conditions. *Journal of Experimental Agriculture International*, 41(6), 1-10. <https://doi.org/10.9734/JEAI/2019/v41i630428>
- Kahya, D., Çalışkan-Keçe, A., & Ulusoy, M.R. (2021). Determining some biological parameters of *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) on cotton mealybug and the rate of parasitism in field conditions. *Turkish Journal of Zoology*, 45(8), 416-424. <https://doi.org/10.3906/zoo-2102-2>
- Khan, M., Srivastava, S.K., Syamasundar, K.V., Singh, M., & Naqvi, A.A. (2002). Chemical composition of leaf and flower essential oil of *Lantana camara* from India. *Flavour and Fragrance Journal*, 17(1), 75-77. <https://doi.org/10.1002/ffj.1047>
- Khan, M., Srivastava, S.K., Jain, N., Syamasundar, K.V., & Yadav, A.K. (2003). Chemical composition of fruit and stem essential oils of *Lantana camara* from northern India. *Flavour and Fragrance Journal*, 18(5), 376-379. <https://doi.org/10.1002/ffj.1197>
- Kilani-Morakchi, S., Morakchi-Goudjil, H., & Sifi, K. (2021). Azadirachtin-based insecticide: Overview, risk assessments, and future directions. *Frontiers in Agronomy*, 3, 676208. <https://doi.org/10.3389/fagro.2021.676208>
- Kim, D.I., Park, J.D., Kim, S.G., Kuk, H., Jang, M.S., & Kim, S.S. (2005). Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. *Journal of Asia-Pacific Entomology*, 8(1), 93-100. [https://doi.org/10.1016/S1226-8615\(08\)60076-X](https://doi.org/10.1016/S1226-8615(08)60076-X)
- Kiran, C., & Prasad, D.N., (2014). A Review on: *Nerium Oleander* Linn. (Kaner). *International Journal of Pharmacognosy and Phytochemical Research*, 6(3), 593–597.
- Kouninki, H., Haubruge, E., Noudjou, F.E., Lognay, G., Malaisse, F., Ngassoum, M.B., Goudoum, A., Mapongmetsem, P.M., & Hance, T. (2005). Potential use of essential oils from Cameroon applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *Communications in agricultural and applied biological sciences*, 70(4), 787-792.
- Kumar, A., De, T., Mishra, A., & Mishra, A.K. (2013). Oleandrin: A cardiac glycosides with potent cytotoxicity. *Pharmacognosy Reviews*, 7(14), 131–139. <https://doi.org/10.4103/0973-7847.120512>
- Krue Wong, W., & Auamcharoen, W. (2023). Acaricidal and repellent activity of *Zanthoxylum myriacanthum* (Rutaceae) fruit extracts against *Tetranychus urticae* and *Tetranychus truncatus* (Acari: Tetranychidae). *Journal of Entomological Science*, 58(2), 119-134. <https://doi.org/10.18474/JES22-29>
- Laya, A.C., Bhaskar, H., Joseph, A., & Deepthy, K.B. (2022). Acaricidal activity of botanical extracts against *Tetranychus Truncatus* Ehara. *Indian Journal of Entomology*, 84(4), 951-954. <https://doi.org/10.55446/IJE.2021.78>
- LeOra, (1994). POLO-PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.
- Li, J., Trinh, H.K., Mirmajlessi, S.M., Haesaert, G., Xhaferi, R., Delaere, I., Höfte, M., Raymaekers, K., Cammue, B.P.A., Jonckheere, W., Van Leeuwen, T., Smet Kyndt, L.D.T., Bernaert, N., Van Poucke, C., Van Droogenbroeck, B., Pequeur, E., De Schamphelaere, K., Callebaut, G., De Cleyn, T., & Geelen, D. (2023). Biopesticide and plant growth-promoting activity in maize distillers' dried grains with solubles. *Industrial Crops and Products*, 193(2023), 116175. <https://doi.org/10.1016/j.indcrop.2022.116175>
- Madreseh-Ghahfarokhi, S., Dehghani-Samani, A., Pirali, Y., & Dehghani-Samani, A. (2019). *Zingiber officinalis* and *Eucalyptus globulus*, potent lethal/repellent agents against *Rhipicephalus bursa*, probable carrier for zoonosis. *Journal of Arthropod-Borne Diseases*, 13(2), 214-223.
- Mahla, A., Kamal, A., Amin, P., & Mandana, M. (2013). Effect of six ethanolic plant derived products on the pre-imago two-spotted spider mite. *Pharmacognosy Communications*, 3(2), 24-27. <https://doi.org/10.5530/pc.2013.2.6>
- Mandal, P., Karmakar, K., & Kole, R.K. (2013). Evaluation of plant extracts against two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) Infesting Okra. *Annals of Entomology*, 31(1), 121-127.
- Maniania, N.K., Bugeme, D.M., Wekesa, V.W., Delalibera, I. & Knapp, M. (2009). Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. *Diseases of Mites and Ticks*, 46(1-4), 259-274. https://doi.org/10.1007/978-1-4020-9695-2_21
- Mijatovic, T., Van Quaquebeke, E., Delest, B., Debeir, O., Darro, F., & Kiss, R. (2007). Cardiotonic steroids on the road to anti-cancer therapy. *Biochimica et Biophysica Acta*, 1776(1), 32–57.

- <https://doi.org/10.1016/j.bbcan.2007.06.002>
- Mironova, M.K., Khorkhordin, E.G., (1997). Effect of NeemAzal-TS on *Tetranychus urticae* Koch. in Proceedings at the 5th Workshop, Wetzlar, Germany, pp. 22–25.
- Monika, G., & Rachna, G. (2008). Evaluation of leaf extract of *Eucalyptus globulus* L. against *Tetranychus urticae* Koch, pest of okra. *Haryana journal of horticultural sciences*, 37(1/2), 104-107.
- Muhammad, K., Idrees, A., Iqbal, J., Arif, U., Baloch, M.S., Shahzad, M.F., Abid, I. & AbdelGawwad, M.R. (2023). Prevalence of cattle ticks in various agro-ecological zones of Khyber Pakhtunkhwa, Pakistan and evaluation of botanical extracts against *Hyalomma detritum*. *Journal of King Saud University - Science*, 102732. <https://doi.org/10.1016/j.jksus.2023.102732>
- Murugesan, S., Senthilkumar, N., Suresh, B.D., & Rajasugunasekar, D. (2016). Chemical constituents and toxicity assessment of the leaf oil of *Lantana camara* Linn from Tamilnadu regions. *Asian Journal of Plant Science and Research*, 6(3), 32-42.
- Ngassoum, M.B., Yonkeu, S., Jirovetz, L., Buchbauer, G., Schmaus, G., & Hammerschmidt, F.J. (1999). Chemical composition of essential oils of *Lantana camara* leaves and flowers from Cameroon and Madagascar. *Flavour and Fragrance Journal*, 14(4), 245-250. [https://doi.org/10.1002/\(SICI\)1099-1026\(199907/08\)14:4<245::AID-FFJ819>3.0.CO;2-X](https://doi.org/10.1002/(SICI)1099-1026(199907/08)14:4<245::AID-FFJ819>3.0.CO;2-X)
- Obiorah, S., Eze, E., Obiorah, D., Orji, N., & Umedum, C. (2012). Phytochemical and antimicrobial studies on the extracts from leaves of *Cajanus cajan* and *Eucalyptus globulus*. In Proceedings of the International Conference on Environment, Chemistry and Biology, Singapore, Volume 49, pp 192-197.
- Orgiazzi, A., Panagos, P., Fernández-Ugalde, O., Wojda, P., Labouyrie, M., Ballabio, C., Franco, A., Pistocchi, A., Montanarella, L., & Jones, A. (2022). Lucas soil biodiversity and lucas soil pesticides, new tools for research and policy development. *European Journal of Soil Science*, 73(5), e13299. <https://doi.org/10.1111/ejss.13299>
- Paris, R.R., & Moysse, H. (1967) Matière Médicale, Vol. 2. Masson & Cie, Paris, France, p. 301
- Pavela, R., Stepanycheva, E., Shchenikova, A., Chermenskaya, T., & Petrova, M. (2016). Essential oils as prospective fumigants against *Tetranychus urticae* Koch. *Industrial Crops and Products*, 94, 755-761. <https://doi.org/10.1016/j.indcrop.2016.09.050>
- Piffer, A.B.M., Fornaciari, I.M., Zuqui, E., Bolsoni, A.M.H., De Paula, Marchiori, J.J., Alves, A.G., Carvalho, J.R., Aguiar, R.L., Fontes, P.S.F.F., & De Paula Gomes, M. (2023). Extracts of plant residues from soursop peels in the management of palm red mite. *Journal of Entomology and Zoology Studies*, 11(2), 19-24. <https://doi.org/10.22271/j.ento.2023.v11.i2a.9168>
- Premalatha, K., Nelson, S.J., Vishnupriya, R., Balakrishnan, S., & Krishnan, V.P. (2018). Efficacy of plant extracts against *Tetranychus urticae* under in-vitro conditions. *Pest Management in Horticultural Ecosystems*, 24(1), 44-49.
- Radhakrishnan, B., & Prabhakaran, P. (2014) Biocidal activity of certain indigenous plant extracts against red spider mite, *Oligonychus coffeae* (Nietner) infesting tea. *Journal of Biopesticides*, 7(1), 29-34.
- Reddy, N.M. (2013). *Lantana camara* Linn. chemical constituents and medicinal properties: a review. *Scholars Academic Journal of Pharmacy*, 2(6), 445-448.
- Regnault-Roger, C., Vincent, C. & Arnason, J.T. (2012). Essential oils in insect control: low-risk products in a high-stakes world. *Annual Review of Entomology*, 57, 405–425. <https://doi.org/10.1146/annurev-ento-120710-100554>
- Saeed, H.M., Ferdosi, M.F., Khan, I.H., Javaid, A., & Sultan, M.W. (2023). Antibacterial activity and gc-ms analysis of white flowers extract of *Nerium oleander* L. *International Journal of Biology and Biotechnology*, 20(1), 163-168.
- Saikia, A.K., & Sahoo, R.K. (2011). Chemical composition and antibacterial activity of essential oil of *Lantana camara* L. *The Middle East Journal*, 8(3), 599-602.
- Salim, H.A., Salman, I.S., Majeed, I.I., & Hussein, H.H. (2016). Evaluation of some plant extracts for their nematocidal properties against root-knot nematode, *Meloidogyne* sp. *Journal of Genetic and Environmental Resources Conservation*, 4(3), 241-244.
- Sararit, P., & Auamcharoen, W. (2020). Biological activities of essential oils from *Anethum graveolens* L. and *Allium sativum* L. for controlling *Tetranychus truncatus* Ehara and *Tetranychus urticae* Koch. *Journal of Biopesticides*, 13(1), 1-12.
- Shala, A.Y., & Gururani, M.A. (2021). Phytochemical properties and diverse beneficial roles of *Eucalyptus globulus* Labill.: A review. *Horticulturae*, 7(11), 450. <https://doi.org/10.3390/horticulturae7110450>
- Sugimoto, N., & Osakabe, M. (2014). Cross-resistance between cyenopyrafen and pyridaben in the twospotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Pest Management Science*, 70(7), 1090-1096. <https://doi.org/10.1002/ps.3652>
- Tawfik, A.A., & Mahmoud, M.A. (2009). Evaluation the efficacy of some plant extracts against the bulb mite, *Rhizoglyphus echinopus* (Fumouze & Rabin)(Acari: Acaridae: Astigmata). *Journal of Plant Protection and Pathology*, 34(2), 1275-1282.

- Thanavendanand, G., & Kennedy, J.S. (2015). Biochemical characterization and insecticidal activity of different solvent crude extracts of *Lantana camara* L. on diamondback moth, *Plutella xylostella* (Linn.). Proceeding of the 2nd International Conference on Agriculture and Forestry, Vol. 1, 2015. Colombo, Srilanka, 10th - 12th June 2015, pp. 117-138
- Ulusoy, S., Özgür, O., & AlpKent, Y.N. (2019). Toxic and in vitro anti-acetylcholinesterase and anti-carboxylesterase effects of various plant extracts on *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae). *Türkiye Entomoloji Dergisi*, 43(3), 339-346. <https://doi.org/10.16970/entoted.506727>
- Ulusoy, S. (2020). Overexpression of cytochrome P450 CYP6CM1 gene in Bemisia tabaci (Gennadius) in the cotton fields of the Çukurova Region, Turkey. *Journal of Agriculture and Nature*, 23(3), 634-640. <https://doi.org/10.18016/ksutarimdoga.vi.1174646>
- Ulusoy, S., Kahya, D., Bilgin, M.G., & Apalak, A. (2022). The effectiveness of wax secretion on chemical control in some mealybug species. *Journal of Asia-Pacific Entomology*, 25(3), 101954. <https://doi.org/10.1016/j.aspen.2022.101954>
- Ulusoy, S. (2024). Rapid Bioassay for Detection of Insecticide Resistance in Aphis gossypii Glover, 1877 (Hemiptera: Aphididae). *Journal of Agriculture and Nature*, 27(2), 344-350. <https://doi.org/10.18016/ksutarimdoga.vi.1174646>
- Van Leeuwen, T., Van Pottelberge, S. & Tirry, L. (2006). Biochemical analysis of a chlorfenapyr-selected resistant strain of *Tetranychus urticae* Koch. *Pest Management Science*, 62,425–433. <https://doi.org/10.1002/ps.1183>
- Van Leeuwen, T., Vontas, J., & Tsagkarakou, A. (2009). Mechanisms of acaricide resistance in the two spotted spider mite *Tetranychus urticae*. In: Sahaaya, I. & Horowitz, A.R. (Eds.). Biorational Control of Arthropod Pests. Springer, The Netherlands, pp 347-393.
- Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., & Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. *Insect Biochemistry and Molecular Biology*, 40(8), 563–572. <https://doi.org/10.1016/j.ibmb.2010.05.008>
- Wan, N., Fu, L., Dainese, M., Kiær, L.P., Hu, Y., Goulson, D., Woodcock, B., Vanbergen, A., Spurgeon, D., & Scherber, C. (2023). The Impact of pesticides on non-target organisms. *European Journal of Soil Science*, 73, e13299.
- Wang, Y.N., Shi, G.L., Zhao, L.L., Liu, S.Q., Yu, T.Q., Clarke, S.R., & Sun, J.H. (2007). Acaricidal activity of *Juglans regia* leaf extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae). *Journal of Economic Entomology*, 100(4), 1298-1303. <https://doi.org/10.1093/jee/100.4.1298>
- Wang, Y.N., Wang, H.X., Shen, Z.J., Zhao, L.L., Clarke, S.R., Sun, J.H., Du, Y.Y. & Shi, G.L. (2009). Methyl palmitate, an acaricidal compound occurring in green walnut husks. *Journal of Economic Entomology*, 102(1), 196-202. <https://doi.org/10.1603/029.102.0128>
- Wang, N., Wang, H.X., Liu, Y., & Shi, G. (2012). Insecticidal activity of *Juglans regia* extracts against *Tetranychus cinnabarinus* and their effects on relative enzymes activity in *Tetranychus cinnabarinus*. *Information Technology and Agricultural Engineering*, 134, 611-620. https://doi.org/10.1007/978-3-642-27537-1_75
- Wang, S.L., Rada, N.M., Williams, R., & Newton, D.J. (2022). Accounting for climatic effects in measuring U.S. field crop farm productivity. *Applied Economic Perspectives and Policy*, 4(44), 1975-1994. <https://doi.org/10.1002/aepp.13289>
- Zaid, R., Canela-Garayoa, R., Ortega-Chacón, N.M., & Mouhouche, F. (2022). Phytochemical analyses and toxicity of *Nerium oleander* (Apocynaceae) leaf extracts against *Chaitophorus leucomelas* Koch, 1854 (Homoptera: Aphididae). *Journal of the Saudi Society of Agricultural Sciences*, 21(5), 310-317. <https://doi.org/10.1016/j.jssas.2021.10.011>
- Zhang, Z.Q. (2003). *Mites of Greenhouses: Identification, Biology and Control*. CAB International Publishing, UK, 244 pp.
- Zhu, Y., Wu, T., Xie, Y., Wu, Y., Deng, J., Cao, L., Zhang, Z., & Rao, Q. (2023). Screening of the acaricidal activity of essential oils against *Panonychus citri* (McGregor)(Acari: Tetranychidae). *Agronomy*, 13(2), 397. <https://doi.org/10.3390/agronomy13020397>

Utilizing Plackett–Burman and Box–Behnken Designs for Plant Extract–Based AgNP Synthesis Optimization: Unveiling Antifungal Potential Against *Phytophthora* Species

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ABSTRACT

This study optimized a green synthesis method for silver nanoparticles (AgNPs) using aqueous extracts of black tea, linden, cherry laurel, kale, and melocan, employing a statistical design of experiments. The plant extracts acted as bio-reducing agents. Total and individual phenolic compounds in the extracts were quantified using ultraviolet-visible (UV–Vis) spectroscopy and ultra-high-performance liquid chromatography (UHPLC). AgNP yields were maximized through a combination of Plackett–Burman and Box–Behnken designs. The synthesized AgNPs were characterized by UV–Vis spectroscopy, Fourier transform infrared (FT–IR) spectroscopy, scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDS), and transmission electron microscopy (TEM). Optimal AgNP production was achieved under the following conditions (determined by response surface methodology, RSM): 9.6 g of plant material, extraction heating at 80°C for 20 minutes, 10 mM AgNO₃, 2.5 mL of extract, 800 W microwave irradiation, and a 90-second reaction time. FT–IR analysis confirmed the role of phenolic compounds in reducing and stabilizing AgNPs. The resulting AgNPs exhibited uniform spherical morphology, with average particle sizes of 5.30 nm (black tea), 8.74 nm (linden), 7.20 nm (cherry laurel), 6.32 nm (kale), and 9.44 nm (melocan). Antifungal assays against five *Phytophthora* species revealed that kale-derived AgNPs were most potent, with EC₅₀, MIC, and MFC values ranging from 9.28–30.84 µg mL⁻¹, 200–300 µg mL⁻¹, and 200–400 µg mL⁻¹, respectively. These results suggest that plant-extract-synthesized AgNPs offer a sustainable approach to managing *Phytophthora* diseases, warranting further research.

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Bitki Ekstraktı Bazlı AgNP Sentez Optimizasyonu için Plackett–Burman ve Box–Behnken Tasarımlarının Kullanımı: *Phytophthora* Türlerine Karşı Antifungal Potansiyelinin Ortaya Çıkarılması

ÖZET

Bu çalışma, siyah çay, ıhlamur, karayemiş, yaprak lahana ve melocan sulu ekstraktları kullanılarak gümüş nanoparçacıklar (AgNP'ler) için deneysel bir istatistiksel tasarım ile yeşil bir sentez yöntemini optimize etmiştir. Bu bitki ekstraktları biyo-indirgeyici ajanlar olarak işlev görmüştür. Ekstraktlardaki toplam ve bireysel fenolik bileşikler ultraviyole-görünür (UV–Vis) spektroskopi ve ultra yüksek performanslı sıvı kromatografisi (UHPLC) kullanılarak ölçülmüştür. AgNP verimleri, Plackett–Burman ve Box–Behnken tasarımlarının bir kombinasyonu ile maksimize edilmiştir. Sentezlenen AgNP'ler UV–Vis spektroskopisi, Fourier dönüşümlü kızılötesi (FT–IR) spektroskopisi, taramalı elektron mikroskobu (SEM)-enerji dağılımlı X-ışını spektroskopisi (EDS) ve geçirimli elektron mikroskopisi (TEM) ile karakterize edilmiştir. Optimum AgNP üretimi aşağıdaki koşullar altında elde edilmiştir (yanıt yüzey metodolojisi, RSM ile belirlenmiştir): 9.6 g bitki materyali, 80°C'de 20 dakika ekstraksiyon ısıtma, 10 mM AgNO₃, 2.5 mL ekstrakt, 800 W mikrodalga irritasyonu

Fitopatoloji

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Anahtar Kelimeler

Yeşil sentez
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Zehirlilik

ve 90 saniyelik reaksiyon süresi. FT-IR analizi, fenolik bileşiklerin AgNP indirgenmesi ve stabilize edilmesindeki rolünü göstermiştir. Elde edilen AgNP'ler 5.30 nm (siyah çay), 8.74 nm (ıhlamur), 7.20 nm (karayemiş), 6.32 nm (yaprak lahana) ve 9.44 nm (melocan) ortalama partikül boyutları ile tek tip küresel morfoloji sergilemiştir. Beş *Phytophthora* türüne karşı yapılan antifungal deneyler, yaprak lahana türü AgNP'lerin sırasıyla 9.28-30.84 µg mL⁻¹, 200-300 µg mL⁻¹ ve 200-400 µg mL⁻¹ arasında değişen EC₅₀, MIC ve MFC değerleri ile en güçlü olduğunu ortaya koymuştur. Bu sonuçlar, bitki ekstraktı ile sentezlenen AgNP'lerin *Phytophthora* hastalıklarının yönetiminde sürdürülebilir bir yaklaşım sunduğunu ve daha fazla araştırılması gerektiğini göstermektedir.

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INTRODUCTION

Türkiye's Black Sea Region, with its diverse geographical and ecological conditions, harbors a rich and unique flora (Davis et al., 1988). East of the Melet River (Altınordu, Ordu), the “Colchic flora” thrives at 700–800 meters altitude along the coast—a distinct forest ecosystem dominated by moisture-loving tree and shrub species. The region's flora includes abundant forest plants, notably lime trees (*Tilia dasystyla* subsp. *caucasica* (V. Engl.) Pigott). A dense understory of small trees and shrubs further characterizes these forests (Günel, 2013). Among these, cherry laurel (*Prunus laurocerasus* L.) is valued for its fruits, ornamental qualities, and protective uses (İslam & Deligöz, 2012). Kale (*Brassica oleracea* var. *acephala* (DC.) Schltdl.), a winter dietary staple, is widely consumed in the Western and Eastern Black Sea regions (Encü, 2010), while melocan (*Smilax excelsa* L.) is integrated into spring/summer diets and folk medicine (Baytop, 1999). Tea (*Camellia sinensis* (L.) Kuntze), a culturally significant beverage, is extensively cultivated from Fatsa (Ordu) to the Türkiye-Georgia border.

These plants are rich in bioactive compounds, underpinning their pharmacological properties. Linden (*T. dasystyla* subsp. *caucasica*) contains phenols, flavonoids, aldehydes, and terpenoids (Akyüz et al., 2014; Öz, 2022). Cherry laurel (*P. laurocerasus*) is a source of protein, sugars, ascorbic acid, minerals, antioxidants, phenols, and flavonoids (Kolaylı et al., 2003; Sahan et al., 2011; Karabegović et al., 2014). Melocan (*S. excelsa*) contains phenolics, flavonoids, and anthocyanins (Özsoy et al., 2008). Kale (*B. oleracea* var. *acephala*) provides glucosinolates, polyphenols, carotenoids, minerals, vitamins, and fatty acids (Šamec et al., 2019). Black tea (*C. sinensis*) is rich in flavonoids, amino acids, vitamins, phenolic acids, lipids, proteins, volatile compounds, carbohydrates, β-carotene, and fluoride (Naveed et al., 2018). These compounds contribute to immunomodulatory, antibacterial, antifungal, antiviral, antioxidant, and hepatoprotective effects (Yesilada et al., 1999; Kolaylı et al., 2003; Tuttu et al., 2017; Šamec et al., 2019; Naveed et al., 2018).

Growing interest in biological systems (e.g., plants, bacteria, fungi) for nanoparticle (NP) synthesis has spurred the development of green methods as sustainable alternatives to chemical approaches (Siddiqi et al., 2018). Plants, as renewable resources, offer a simple, eco-friendly route for synthesizing metallic nanoparticles (Shah et al., 2015; Siddiqi et al., 2018; Yılmaz et al., 2021). Phytochemicals in plant extracts—terpenoids, flavones, aldehydes, ketones, carboxylic acids, ascorbic acid, amides, and phenols—act as both reducing and stabilizing agents during NP synthesis (Ovais et al., 2018; Othman et al., 2019). Diverse plant parts (roots, seeds, bulbs, stems, bark, peels, petals, flowers) have been used for silver nanoparticle (AgNP) synthesis (Siddiqi et al., 2018). For example, fruit extracts of *Cnidium monnieri* (Ye et al., 2023), leaf extracts of *Phyllanthus urinaria*, *Pouzolzia zeylanica*, and *Scoparia dulcis* (Nguyen et al., 2020), and *Camellia sinensis* (white tea) (Karakaş et al., 2024), and peel extracts of *Citrus sinensis* (Miccky et al., 2023) and *C. limetta* (Trivedi et al., 2014) have demonstrated efficacy in AgNP synthesis. AgNP size and morphology can be controlled by tuning parameters like metal salt concentration, reaction time, pH, temperature, and biomaterial quantity (Sharma et al., 2022).

While traditional “one-variable-at-a-time” optimization is time-consuming, the Plackett–Burman design (PBD) offers a faster, more efficient alternative. For example, El-Sawaf et al. (2024) used PBD to identify critical factors (precursor concentrations, ratio, shaking speed, temperature, pH, incubation time) in the green synthesis of CuO/Ag/ZnO nanocomposites using *Ziziphus spina-christi* extract. Similarly, Fazil et al. (2024) optimized banana peel-AgNP synthesis via PBD, finding AgNO₃ concentration and incubation time as key factors. Siddiqi et al.

(2024) reported that only AgNO_3 concentration and incubation time significantly influenced *Salsola imbricata*-mediated AgNP synthesis. Statistical methods like PBD and response surface methodology (RSM) streamline optimization, enhancing AgNP synthesis efficiency (Halima et al., 2021; Laime-Oviedo et al., 2022). PBD identifies critical parameters, while RSM (often paired with Box–Behnken design, BBD) models optimal conditions for desired AgNP properties. This approach holds promise for agricultural and medical applications. Although PBD and RSM have been used to optimize AgNP synthesis from various plant materials, including ethanolic fractions (Laime-Oviedo et al., 2022), *Piper betle* and *Jatropha curcas* leaf extracts (Halima et al., 2021), and *Polygonum cognatum* (madimak) extracts (Türkkan & Gürel, 2024), their application to black tea, linden, cherry laurel, kale, and melocan remains unexplored, a gap addressed by this study.

The present study optimized AgNP synthesis from five plant extracts using PBD and BBD. The AgNPs were characterized via UV–Vis spectroscopy, FT–IR, SEM–EDS, and TEM to assess physicochemical properties. Their antifungal potential was evaluated against multiple *Phytophthora* species.

MATERIAL and METHOD

Plant Collection and Preparation

Plant specimens were collected from their natural habitats during the growing season. Specifically, kale (*Brassica oleracea* var. *acephala* – leaves), linden (*Tilia dasystyla* subsp. *caucasica* – flowers and leaves), and cherry laurel (*Prunus laurocerasus* – leaves) were obtained from Gülyalı, Ordu; black tea (*Camellia sinensis* – leaves) from Fındıklı, Rize; and melocan (*Smilax excelsa* – shoots and leaves) from Çarşamba, Samsun.

Chemicals and Reagents

Silver nitrate (AgNO_3), sodium hydroxide (NaOH), gallic acid (GA), Folin-Ciocalteu reagent, sodium bicarbonate, and all other chemicals were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steinheim, Germany).

Phytophthora Isolates

The *Phytophthora* isolates used in this study were obtained from the Batı Akdeniz Agricultural Research Institute (BATEM) and maintained in the mycology culture collection of the Department of Plant Protection, Faculty of Agriculture at Ordu University.

Preparation of Aqueous Extracts from Plants

Freshly collected plant specimens were washed with tap water to remove initial impurities and then rinsed three times with distilled water. The cleaned plants were cut into smaller pieces and dried at 60°C for 3–4 days to facilitate efficient extraction of bioactive compounds. To optimize the extraction process for silver nanoparticle (AgNP) synthesis, we investigated the influence of plant material amount (5, 7.5, or 10 g), extraction heating time (20 or 30 minutes), and extraction heating temperature (60, 70, or 80°C). For extraction, weighed portions of chopped plant material were added to individual 250 mL beakers containing 100 mL of distilled water and heated at the predetermined temperature for the indicated time. After cooling to room temperature, the extracts were filtered through Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 10 minutes to remove any remaining plant material. The resulting extracts were stored at 4°C until used as reducing and stabilizing agents in AgNP synthesis.

Phenolic Profile of Plant Extracts: Total Content and Individual Compounds

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu assay (Singleton & Rossi, 1965). Briefly, 600 μL of extract was diluted with 4 mL distilled water and mixed with 600 μL of 10% Folin-Ciocalteu reagent. After a 5-minute dark incubation, 300 μL of 2% sodium bicarbonate was added, and the mixture was allowed to stand for 2 hours. Absorbance was then measured at 760 nm using a UV–Vis spectrophotometer (Lambda 35, Perkin Elmer Inc., Hopkinton, MA, USA). Gallic acid (5–100 $\mu\text{g mL}^{-1}$) served as the standard for quantification, with results expressed as gallic acid equivalents (GAE) g kg^{-1} . Individual phenolic compounds were identified and quantified using ultra-high-performance liquid chromatography (UHPLC, Thermo Fisher Scientific Inc., Ultimate-3000, Waltham, MA, USA) coupled with a diode array detector (DAD 3000, Thermo Fisher Scientific Inc.), following a modified protocol from Ozturk et al. (2015). Dried plant material was suspended in methanol and centrifuged to remove debris. The supernatant was filtered and injected into the UHPLC system. Separation was achieved on a Hypersil GD column (Thermo Fisher Scientific Inc., USA) using a gradient of aqueous formic acid and methanol. Detection was set at 274 nm, with a 60-minute run time, 20 μL injection volume, and a 1.0 mL min^{-1}

flow rate. Concentrations were determined from peak areas compared to a standard curve of known phenolic standards and expressed as mg kg⁻¹ of dry plant material.

Synthesis of Silver Nanoparticles (AgNPs)

Plant extract (2.5–7.5 mL) was added dropwise to 25 mL of silver nitrate (AgNO₃) solution (1–10 mM). The mixture's pH was adjusted to 10 using 0.1 M sodium hydroxide (NaOH). Microwave irradiation (600–800 W, 30–90 seconds) reduced the silver ions (Ag⁺) to metallic silver nanoparticles (AgNPs), indicated by a color change from light yellow to brown or dark brown. AgNP formation was confirmed by UV–Vis spectroscopy (200–700 nm). AgNP yield, corresponding to 5–50 nm sized particles (Noroozi et al., 2012; Chowdhury et al., 2016), was estimated from the area under the spectral curve (350–420 nm) using the following equation (Eq. 1):

$$Y = \sum (a_i + a_{i+1}) / 2 \times (w_{i+1} - w_i)$$

where Y is the response (area), a is the absorbance, and w is the wavelength.

Optimization of Synthesized AgNP

To maximize AgNP yield and optimize the synthesis process, Plackett–Burman design (PBD) and the Box–Behnken design (BBD) were employed.

Plackett–Burman Design (PBD): Unveiling Key Factors

A Plackett–Burman design (PBD) was used to screen the most influential factors affecting plant-mediated AgNP synthesis. Seven variables were investigated: plant material quantity (g), extraction heating temperature (°C), extraction heating time (min), AgNO₃ concentration (mM), extract volume (mL), microwave power (W), and reaction time (sec). Each factor (A–G) was tested at two levels (+1 and –1), with upper and lower limits selected based on preliminary experiments. A 12-run PBD matrix was generated in Microsoft Excel, encompassing all high/low combinations (Table 1). AgNP yield, indirectly measured by UV–Vis spectroscopy (spectral area 350–420 nm), served as the response variable. The spectral area data were then analyzed using Minitab software (version 19.2; Minitab, Inc., USA).

PBD is based on the first-order linear model equation (Eq. 2) (Plackett & Burman, 1946):

$$Y = \beta_0 + \sum \beta_i x_i$$

where Y is the response (area), β_0 is the model intercept, x_i is the level of each independent variable, and β_i is the linear coefficient.

Table 1. Experimental factors and responses in Plackett–Burman design for AgNP synthesis

Çizelge 1. AgNP sentezi için Plackett–Burman tasarımındaki deneysel faktörler ve yanıtlar

Run	Independent variables							Response: Area (350–420 nm)				
	A ^a	B ^b	C ^c	D ^d	E ^e	F ^f	G ^g	Black tea	Linden	Cherry laurel	Kale	Melocan
1	10	60	20	1	7.5	400	30	-1.42	1.05	5.18	1.44	10.45
2	5	80	30	1	2.5	800	30	-1.95	2.36	3.88	-0.64	-2.29
3	5	60	30	10	2.5	400	90	28.50	17.79	26.49	13.92	27.71
4	10	60	20	1	7.5	400	30	1.12	1.54	5.05	2.06	10.34
5	5	80	20	10	2.5	800	30	24.87	16.55	27.17	11.19	21.80
6	5	60	30	1	2.5	400	90	-0.15	2.47	2.95	-1.38	0.69
7	10	60	30	10	2.5	400	30	22.21	15.53	23.55	16.71	22.53
8	5	80	20	10	7.5	400	30	18.83	18.38	22.74	13.10	16.86
9	10	60	30	1	7.5	800	90	4.97	4.49	15.18	10.10	26.99
10	10	80	20	10	2.5	800	90	59.07	52.95	56.07	43.04	55.16
11	5	80	20	1	7.5	800	90	0.62	3.76	7.74	5.83	7.77
12	10	80	30	10	7.5	800	90	50.36	45.88	43.71	32.38	35.98

^aAmount of plant material (g) (A), ^bExtraction heating temperature (°C) (B), ^cExtraction heating time (min.) (C),

^dConcentration of AgNO₃ (mM) (D), ^eExtract volume (mL) (E), ^fPower of microwave (W) (F), ^gReaction time (s) (G)

Regression analysis and ANOVA (analysis of variance) were employed to evaluate the significance of each factor's effect on the response variable. Statistical analysis revealed factors significantly affecting the yield of synthesized AgNPs.

Box–Behnken Design (BBD): Optimizing Key Factors

Following the Plackett–Burman design (PBD) analysis, a Box–Behnken design (BBD) was implemented to optimize the most influential factors affecting AgNP yield. This involved a 15-run experimental design, where each factor was tested at three levels: low (−1), medium (0), and high (+1) (Table 2). This allowed for a comprehensive analysis of the relationships between factors and AgNP yield, quantified as the spectral area ($\lambda = 350\text{--}420\text{ nm}$) using UV–Vis spectroscopy.

Minitab software (version 19.2; Minitab, Inc., USA) was used for experimental design, regression analysis, and graphical data representation. The response variable was fitted to a second-order polynomial model (Eq. 3) (Box & Behnken, 1960):

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j$$

where Y is the response, X_i is the coded level of the independent variable, β_0 is the regression coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient.

Statistical analysis of the BBD data revealed interactions between factors and their combined effects on AgNP yield. The quadratic and interaction terms provided critical insights into these complex relationships.

Table 2. Experimental factors and responses in Box–Behnken design for AgNP synthesis

Çizelge 2. AgNP sentezi için Box–Behnken tasarımındaki deneysel faktörler ve yanıtlar

Std ^a	Run	Independent variables			Response: Area (350–420 nm)				
		A ^b	D ^c	G ^d	Black tea	Linden	Cherry laurel	Kale	Melocan
5	1	5	5.5	30	15.11	16.43	18.64	12.59	18.73
7	2	5	5.5	90	27.45	29.32	33.80	25.07	31.80
3	3	5	10	60	40.82	26.56	36.67	25.46	38.62
9	4	7.5	1	30	4.56	3.83	3.73	2.59	3.25
15	5	7.5	5.5	60	20.02	20.12	23.34	17.22	22.63
13	6	7.5	5.5	60	21.43	20.79	22.59	18.05	24.40
14	7	7.5	5.5	60	20.57	21.02	22.46	17.30	23.29
1	8	5	1	60	2.72	2.96	3.55	2.94	4.67
6	9	10	5.5	30	17.12	17.086	19.06	12.65	18.08
8	10	10	5.5	90	35.27	31.77	36.09	25.85	39.37
2	11	10	1	60	3.44	3.11	4.98	3.35	5.83
12	12	7.5	10	90	58.47	46.45	57.75	45.06	54.52
11	13	7.5	1	90	4.92	5.82	6.06	5.60	7.54
10	14	7.5	10	30	27.73	19.20	29.03	19.88	29.50
4	15	10	10	60	43.38	32.78	39.14	28.61	42.61

^aStd is the standard order, ^bAmount of plant material (g) (A), ^cConcentration of AgNO₃ (mM) (D), ^dReaction time (s) (G)

Characterization of Synthesized AgNPs

Synthesized AgNPs were characterized using several techniques. Ultraviolet–Vis (UV–Vis) spectroscopy was performed by diluting 20 μL of AgNP solution with 2 mL of distilled water and analyzing the sample at a 1 nm resolution between 200 and 700 nm using a quartz cuvette and a distilled water blank for baseline correction. For Fourier Transform Infrared (FT–IR) spectroscopy (Spectrum 65, Perkin Elmer Inc., Hopkinton, MA, USA), 1 mg of AgNPs was mixed with 200 mg of potassium bromide (KBr), pressed into a pellet, and scanned in transmittance mode from 4000 to 400 cm^{-1} . Elemental analysis was performed using scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDS) (SU-1510, Hitachi High-Tech., Tokyo, Japan). Particle size and structure were confirmed by transmission electron microscopy (TEM) (HT7700, Hitachi High-Tech., Tokyo, Japan). Particle size distribution, determined from TEM images using ImageJ (National Institutes of Health, USA), was fitted to a Gaussian function using OriginPro software (2019b version 9.6.5.169, OriginLab Corporation, USA).

Antifungal Efficacy of Synthesized AgNPs Against *Phytophthora* Species

The antifungal efficacy of synthesized AgNPs against *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae*, and *P. palmivora* was determined following the method described by Gevrek et al. (2023). AgNPs were added to sterilized V8 agar media at concentrations of 50, 100, 200, 300, and 400 $\mu\text{g mL}^{-1}$ and cooled to 50°C. The modified medium was dispensed into Petri dishes, with unmodified V8 agar serving as a control. Mycelial discs (5 mm diameter) from 7-day-old fungal cultures were inoculated at the center of each Petri dish. Plates were incubated in darkness at 25°C until control plates were fully colonized (4–7 days). Fungal colony diameters were measured

perpendicularly to assess growth inhibition. The percentage of mycelial growth inhibition (MGI) was calculated using the formula: $MGI (\%) = [(dc - dt) / dc] \times 100$, where dc represents the colony diameter on control plates and dt represents the colony diameter on treated plates (measured in two perpendicular directions).

The fungicidal efficacy of the synthesized AgNPs was evaluated using EC_{50} values (the concentration inhibiting 50% of fungal growth), determined by probit analysis in SPSS (v. 22, IBM, Chicago, USA). The minimum inhibitory concentration (MIC), defined as the lowest concentration completely preventing fungal growth, was also determined. To confirm fungicidal activity and establish the minimum fungicidal concentration (MFC), agar disks from treated plates exhibiting no visible growth were subcultured onto fresh V8 agar and incubated at 25°C for 9 days.

RESULTS and DISCUSSION

Exploring the Bioactive Profile of Plant Extracts and Their Role in AgNP Synthesis

This study employed a biocompatible approach to synthesize highly efficient, spherical AgNPs using aqueous extracts of black tea, linden, cherry laurel, kale, and melocan as reducing and stabilizing agents. Total phenolic content, measured via the Folin-Ciocalteu (F-C) method (Singleton et al., 1999), varied across the extracts: black tea (4.07 g kg⁻¹ GAE), linden (4.87 g kg⁻¹ GAE), cherry laurel (6.66 g kg⁻¹ GAE), melocan (1.81 g kg⁻¹ GAE), and kale (5.56 g kg⁻¹ GAE). UHPLC analysis revealed diverse phenolic profiles for each extract (Table 3). Identified phenolic compounds, including aminobenzoic acid, protocatechuic acid, hydroxybenzoic acid, caffeic acid, coumaric acid, ferulic acid, and rutin, likely played a crucial role in the bioreduction of silver ions (Ag⁺) to AgNPs. These findings align with previous studies highlighting plant-derived phenolics as key agents in AgNP synthesis. For example, Kumar et al. (2012) attributed Ag⁺ reduction in *Terminalia chebula* extract to its polyphenol content, suggesting these compounds act as both reducing and stabilizing agents. Similarly, Ahmed and Sharma (2012) reported that phenolics in pineapple (*Ananas comosus*) exhibit strong antioxidant activity due to their free radical scavenging capacity. Firoozi et al. (2016) further demonstrated that phenolics in *Satureja intermedia* extract (leaves, stems, and flowers) facilitate Ag⁺ reduction to nanoparticles.

During microwave-assisted AgNP synthesis, the addition of plant extracts to aqueous AgNO₃ solution resulted in a color change from light yellow to dark brown, indicating AgNP formation via surface plasmon resonance (SPR). As shown in Figure 1(a–b), the AgNPs exhibited a prominent surface plasmon resonance (SPR) peak between 402 and 409 nm, consistent with the observed dark brown coloration. The sharp SPR peak confirmed Ag⁺ reduction and suggested high monodispersity (Konwarh et al., 2011). While SPR peak characteristics are influenced by factors beyond size distribution—such as shape, morphology, composition, and the surrounding dielectric environment (Kelly et al., 2003; Stepanov, 2004)—the observed narrow peak width implies a relatively uniform AgNP size distribution.

Table 3. Phenolic acid content in plant extracts

Çizelge 3. Bitki ekstraktlarındaki fenolik asit içeriği

Phenolic compounds	Black tea	Linden	Cherry laurel (mg kg ⁻¹ fw)	Kale	Melocan
4-Aminobenzoic acid	292.68	46.38	0.94	2.25	0.64
Protocatechuic acid	0.24	77.90	0.54	9.41	0.45
4-Hydroxybenzoic acid	4.21	-	-	2215.3	0.37
Catechin	-	5.76	3.40	10.07	0.26
Chlorogenic acid	-	-	0.73	-	-
Caffeic acid	6.54	24.83	10.92	4.88	7.79
Epicatechin	7.07	7.27	1.62	0.83	-
<i>p</i> -Coumaric acid	30.78	10.26	0.78	5.03	3.55
Ferulic acid	3.82	52.80	5.18	7.20	-
Rutin	42.99	0.18	18.07	1.86	11.85

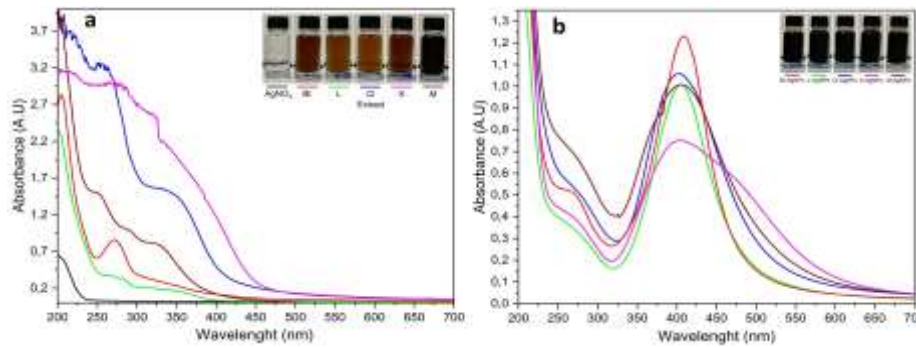


Figure 1. UV-Vis absorption spectra of (a) aqueous extracts of black tea (Bt), linden (L), cherry laurel (Cl), kale (K), and melocan (M), and (b) their corresponding synthesized AgNPs (Bt-AgNPs, L-AgNPs, Cl-AgNPs, K-AgNPs, M-AgNPs) under optimized conditions.

Şekil 1. Siyah çay (Bt), ıhlamur (L), karayemiş (Kl), yaprak lahanası (K) ve melocan (M) sulu ekstraktlarının (a) ve bunlara karşılık gelen sentezlenmiş AgNP'lerin (BT-, L-, KL-, K- ve M-AgNP'ler) optimize edilmiş koşullar altında (b) UV-Vis absorpsiyon spektrumları.

Statistical Optimization of The Synthesized AgNPs

Plackett-Burman design (PBD)

Table 1 presents the Plackett-Burman design (PBD) matrix and corresponding AgNP yields for each experimental run. The observed yields varied widely across plant species: black tea (−1.95 to 59.07), linden (1.05 to 52.95), cherry laurel (5.05 to 56.07), kale (−1.38 to 43.04), and melocan (−2.29 to 55.16), underscoring the need for optimization. Figure 2(a–e) shows the UV-Vis spectra from these runs.

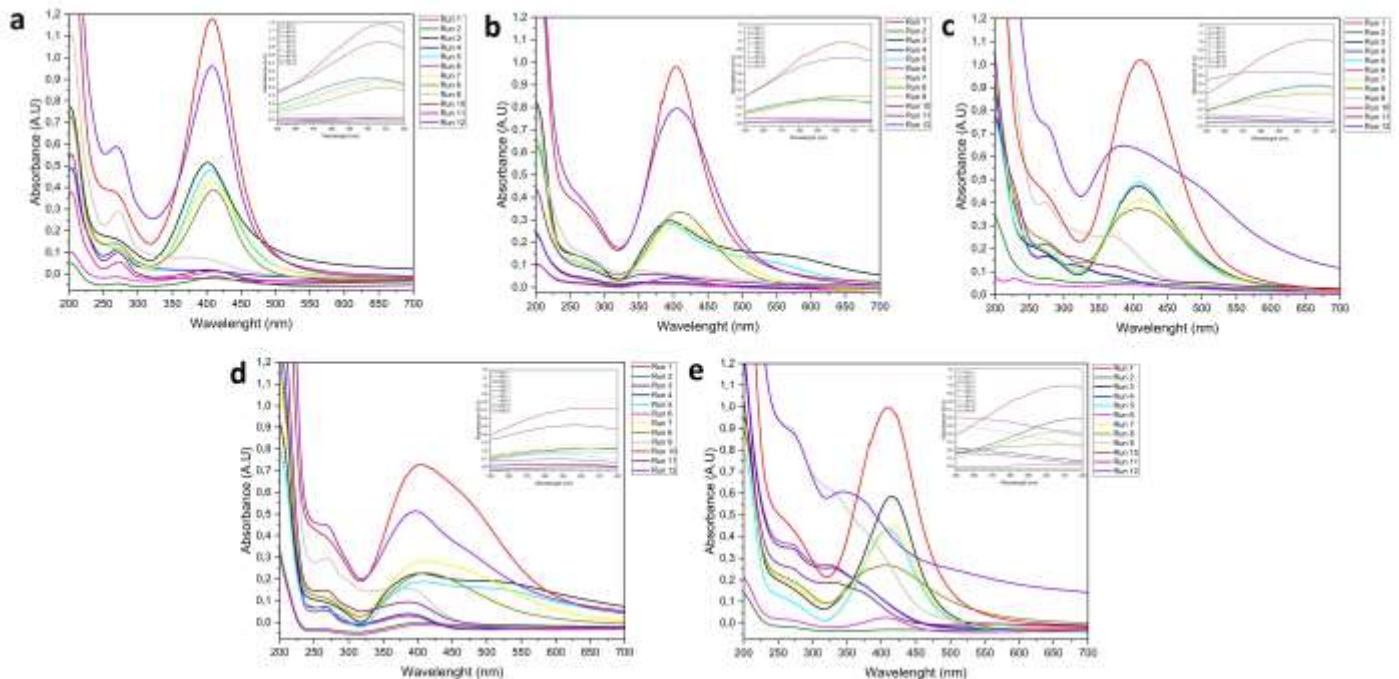


Figure 2. UV-Vis absorption spectra of silver nanoparticles synthesized using Plackett-Burman design parameters: Bt-AgNPs (a), L-AgNPs (b), Cl-AgNPs (c), K-AgNPs (d), and M-AgNPs (e).

Şekil 2. Plackett-Burman deney tasarımı parametreleri kullanılarak sentezlenmiş gümüş nanopartiküllerin UV-Vis absorpsiyon spektrumları: Bt-AgNP'ler (a), L-AgNP'ler (b), Cl-AgNP'ler (c), K-AgNP'ler (d) ve M-AgNP'ler (e).

Statistical analysis of the PBD data confirmed the model's significance (F and p -values), indicating a strong relationship between the investigated factors and AgNP yield (Table 4). Plant material quantity (A), AgNO_3 concentration (D), and reaction time (G) significantly and positively influenced AgNP yield ($p < 0.05$), with standardized effects ranging from 14.23–21.22, 12.72–25.05, and 11.78–15.70, respectively. Extraction heating temperature (B) also positively impacted yield, albeit with lower confidence (except for melocan, which exhibited

a negative effect). Conversely, extraction heating time (C), extract volume (E), and microwave power (F) adversely affected yield. These results highlight the critical role of plant species in determining key factors for AgNP biosynthesis. Previous studies report similar variations in influential parameters depending on plant material. For example, Trivedi et al. (2014) identified temperature, illumination, and pH as critical parameters for AgNP synthesis from citrus peel extract using PBD. Halima et al. (2021) found plant extract and AgNO₃ concentration to be most influential in PBD optimization of *Piper betle* and *Jatropha curcas* leaf extract-mediated AgNP synthesis. More recently, Fazil et al. (2024) utilized PBD to optimize banana peel-AgNP synthesis, determining that only AgNO₃ concentration and incubation time were statistically significant among the four factors investigated (AgNO₃ concentration, incubation temperature, incubation time, and plant/AgNO₃ ratio).

Table 4. Statistical analysis of Plackett–Burman design for AgNP yield optimization

Çizelge 4. AgNP verim optimizasyonu için Plackett–Burman tasarımının istatistiksel analizi

Variables	Effect	Coefficient	<i>t</i> -value	<i>F</i> -value	<i>p</i> -value	Confidense (%)
Black tea						
Model		17.253		24.464	0.0039	99.99
A:Amount of plant material (g)	19.32	9.659	4.488	20.141	0.011	98.91
B:Extraction heating temperature (°C)	16.59	8.294	2.348	5.513	0.079	92.13
C:Extraction heating time (min.)	-2.42	-1.210	-0.633	0.401	0.561	43.90
D:Concentration of AgNO ₃ (mM)	25.05	12.527	5.820	33.878	0.004	99.57
E:Extract volume (mL)	-8.57	-4.285	-2.154	4.642	0.097	90.25
F:Power of microwave (W)	-4.82	-2.409	-0.797	0.636	0.470	53.01
G:Reaction time (s)	15.70	7.849	4.290	18.402	0.013	98.73
Linden						
Model		15.230		23.650	0.0042	99.99
A:Amount of plant material (g)	21.22	10.609	5.786	33.483	0.004	99.56
B:Extraction heating temperature (°C)	25.69	12.843	4.268	18.219	0.013	98.70
C:Extraction heating time (min.)	-0.25	-0.124	-0.076	0.006	0.943	5.70
D:Concentration of AgNO ₃ (mM)	14.04	7.020	3.829	14.661	0.019	98.14
E:Extract volume (mL)	-7.90	-3.950	-2.331	5.435	0.080	91.99
F:Power of microwave (W)	-10.81	-5.406	-2.100	4.411	0.104	89.64
G:Reaction time (s)	15.68	7.839	5.029	25.294	0.007	99.27
Cherry laurel						
Model		19.977		31.805	0.0024	99.99
A:Amount of plant material (g)	14.78	7.388	4.805	23.093	0.009	99.14
B:Extraction heating temperature (°C)	9.57	4.784	1.896	3.595	0.131	86.92
C:Extraction heating time (min.)	-4.06	-2.031	-1.488	2.213	0.211	78.89
D:Concentration of AgNO ₃ (mM)	21.48	10.739	6.984	48.782	0.002	99.78
E:Extract volume (mL)	-5.87	-2.937	-2.067	4.273	0.108	89.24
F:Power of microwave (W)	0.99	0.496	0.230	0.053	0.830	17.04
G:Reaction time (s)	11.78	5.892	4.508	20.323	0.011	98.92
Kale						
Model		12.312		48.477	0.0010	99.99
A:Amount of plant material (g)	16.72	8.361	8.400	70.552	0.001	99.89
B:Extraction heating temperature (°C)	13.27	6.634	4.061	16.492	0.015	98.47
C:Extraction heating time (min.)	-2.14	-1.070	-1.211	1.466	0.293	70.73
D:Concentration of AgNO ₃ (mM)	12.72	6.361	6.390	40.829	0.003	99.69
E:Extract volume (mL)	-5.04	-2.518	-2.737	7.493	0.052	94.80
F:Power of microwave (W)	-3.46	-1.730	-1.238	1.532	0.284	71.65
G:Reaction time (s)	11.87	5.936	7.015	49.206	0.002	99.78
Melocan						
Model		19.500		11.730	0.0157	99.98
A:Amount of plant material (g)	14.23	7.116	3.012	9.075	0.039	96.05
B:Extraction heating temperature (°C)	-5.08	-2.542	-0.656	0.430	0.548	45.22
C:Extraction heating time (min.)	-8.75	-4.375	-2.086	4.351	0.105	89.47
D:Concentration of AgNO ₃ (mM)	21.60	10.799	4.572	20.899	0.010	98.98
E:Extract volume (mL)	-3.33	-1.665	-0.763	0.581	0.488	51.18
F:Power of microwave (W)	8.71	4.357	1.314	1.726	0.259	74.08
G:Reaction time (s)	12.45	6.223	3.099	9.605	0.036	96.38

Optimization of AgNP synthesis parameters using Box–Behnken design (BBD)

Based on the PBD results, a three-level Box–Behnken design (BBD) was used to optimize the three most significant factors: plant material quantity (A), AgNO₃ concentration (D), and reaction time (G). Fifteen experiments were conducted, and the resulting AgNP yields and UV–Vis spectra are presented in Table 2 and Figure 3(a–e). Response surface methodology (RSM) analysis showed a strong, highly significant model ($p < 0.0001$) for silver nanoparticle (AgNP) yield from black tea, linden, cherry laurel, kale, and melocan (Table 5). High F -values (314.43, 400.51, 148.42, 86.24, and 305.66, respectively) confirmed these strong effects. Plant material quantity (A), silver nitrate concentration (D), and reaction time (G) significantly influenced AgNP yield ($p < 0.05$, $p < 0.0001$, and $p < 0.0001$, respectively), except for plant material quantity in cherry laurel and kale. The interaction between silver nitrate concentration and reaction time (DG) was also significant ($p < 0.001$). While the quadratic term for plant quantity (A^2) was non-significant, the quadratic terms for silver nitrate concentration (D^2) and reaction time (G^2) were generally significant ($p < 0.05$). High R^2 values (0.99812, 0.9986, 0.9963, 0.9936, and 0.9982, respectively) demonstrated the model's excellent predictive power, approaching the ideal value of 1. Non-significant lack-of-fit F -values (3.72, 4.00, 17.99, 18.50, and 1.81, respectively) further confirmed the model's suitability. A second-order regression model was developed to predict AgNP yield based on these factors (Table 5).

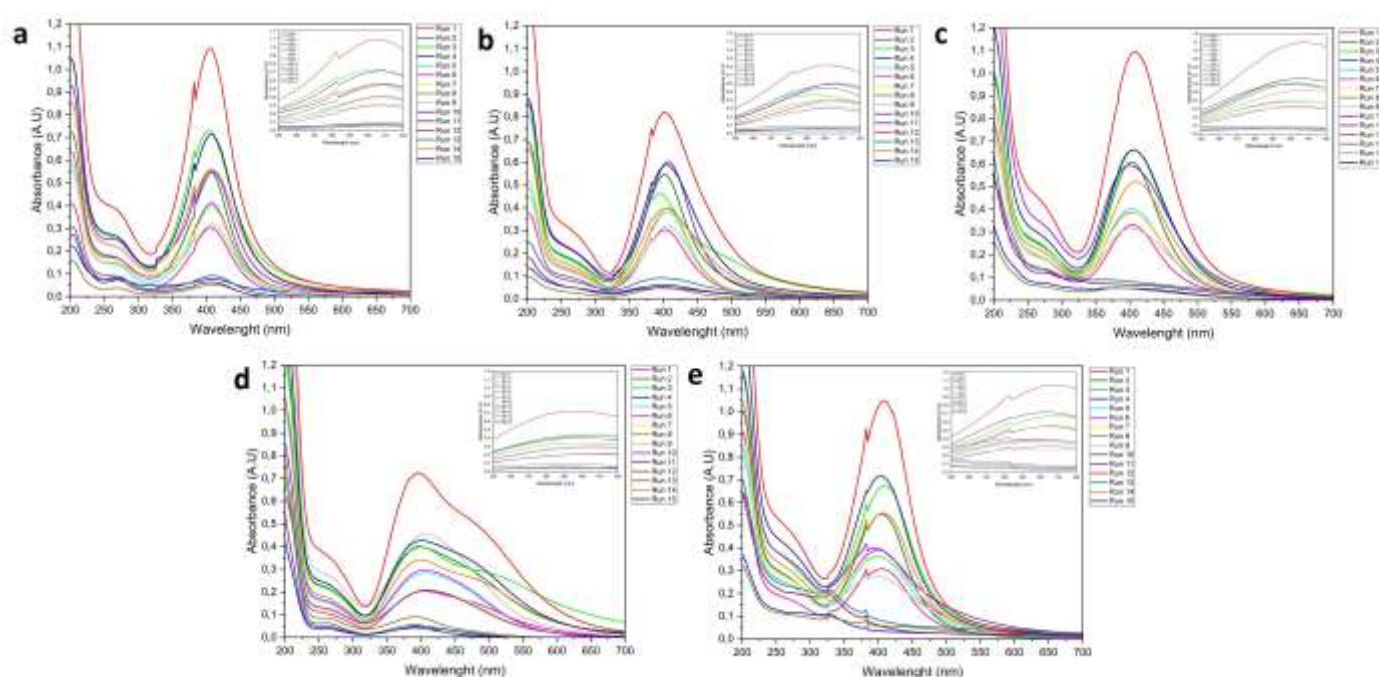


Figure 3. UV–Vis absorption spectra of silver nanoparticles synthesized using Box–Behnken design parameters: Bt-AgNPs (a), L-AgNPs (b), Cl-AgNPs (c), K-AgNPs (d), and M-AgNPs (e).

Şekil 3. Box–Behnken deney tasarımı parametreleri kullanılarak sentezlenmiş gümüş nanopartiküllerin UV–Vis absorpsiyon spektrumları: Bt-AgNP'ler (a), L-AgNP'ler (b), Cl-AgNP'ler (c), K-AgNP'ler (d) ve M-AgNP'ler (e).

The 3D and 2D response surface plots illustrate the key interactions influencing AgNP yield (Figure 4a–g). These visualizations depict the combined effects of plant material quantity, silver nitrate concentration, and reaction time. In general, maximizing silver nitrate concentration and reaction time, while maintaining plant material quantity at 7.5 g, resulted in higher AgNP yields (Figure 4a–c, 4e, and 4g). Further analysis suggests that plant material quantity had minimal impact on yield (Figure 4d and 4f). However, a synergistic interaction between silver nitrate concentration and reaction time was evident, with the highest yield achieved when both factors were at their maximum levels. These findings align with previous studies on AgNP synthesis using linden flower, hazelnut leaf, and madimak plant extracts (Gevrek et al., 2023; Yiğit & Türkkan, 2023; Türkkan & Gürel, 2024). Our results also reinforce the significance of optimizing "total energy" (power $\times$ time) for high-quality AgNP synthesis. This is consistent with the observations of Cai et al. (2017), who reported that insufficient total energy ($<700 \text{ W min } 100 \text{ mL}^{-1}$) limited complete silver ion reduction. Similarly, Nikaeen et al. (2020) observed a positive correlation between both silver nitrate concentration and reaction time with AgNP quality.

Table 5. Statistical analysis of Box–Behnken design for AgNP yield optimization

Çizelge 5. AgNP verim optimizasyonu için Box–Behnken tasarımının istatistiksel analizi

Source	Sum of squares	DF	Mean square	F-value	p-value	R ²	Adj. R ²	Pred R ²
Black tea								
Model	3751.36	9	416.82	314.43	0.000	0.9982	0.9951	0.9755
A:Amount of plant material (g)	21.53	1	21.53	16.24	0.01			
D:Concentration of AgNO ₃ (mM)	2993.65	1	2993.7	2258.32	< 0.0001			
G:Reaction time (s)	474.12	1	474.12	357.66	< 0.0001			
AD	0.8445	1	0.8445	0.637	0.461			
AG	8.44	1	8.44	6.37	0.053			
DG	230.59	1	230.59	173.95	< 0.0001			
A ²	2.77	1	2.77	2.09	0.2083			
D ²	4.07	1	4.07	3.07	0.1402			
G ²	17.8	1	17.8	13.42	0.0145			
Residual	6.63	5	1.33					
Lack of fit	5.62	3	1.87	3.72	0.219			
Pure error	1.01	2	0.50					
Corrected total	3757.99	14						
Y=20.67+1.64A+19.34D+7.70G+0.46AD+1.45AG+7.59DG+0.87A ² +1.05D ² +2.20G ²								
Linden								
Model	2189.60	9	243.29	400.51	0.000	0.9986	0.9961	0.9806
A:Amount of plant material (g)	11.2	1	11.2	18.44	0.0078			
D:Concentration of AgNO ₃ (mM)	1492.22	1	1492.2	2456.55	< 0.0001			
G:Reaction time (s)	403.75	1	403.75	664.66	< 0.0001			
AD	9.2	1	9.2	15.14	0.0115			
AG	0.8118	1	0.8118	1.34	0.2999			
DG	159.54	1	159.54	262.64	< 0.0001			
A ²	0.2619	1	0.2619	0.4312	0.5404			
D ²	76.77	1	76.77	126.39	< 0.0001			
G ²	27.75	1	27.75	45.68	0.0011			
Residual	3.04	5	0.61					
Lack of fit	2.60	3	0.87	4.00	0.206			
Pure error	0.43	2	0.22					
Corrected total	2192.63	14						
Y=20.64+1.18A+13.66D+7.10G+1.52AD+0.45AG+6.32DG+0.27A ² -4.56D ² +2.74G ²								
Cherry laurel								
Model	3353.69	9	372.63	148.42	0.000	0.9963	0.9896	0.9422
A:Amount of plant material (g)	5.47	1	5.47	2.18	0.1998			
D:Concentration of AgNO ₃ (mM)	2601.68	1	2601.7	1036.29	< 0.0001			
G:Reaction time (s)	500.08	1	500.08	199.19	< 0.0001			
AD	0.2732	1	0.2732	0.1088	0.7548			
AG	0.871	1	0.871	0.347	0.5814			
DG	174.05	1	174.05	69.33	0.0004			
A ²	1	1	1	0.4003	0.5548			
D ²	18.42	1	18.42	7.34	0.0423			
G ²	47.22	1	47.22	18.81	0.0075			
Residual	12.55	5	2.51					
Lack of fit	12.10	3	4.03	17.99	0.053			
Pure error	0.45	2	0.22					
Corrected total	3366.25	14						
Y=22.80+0.83A+18.03D+7.91G+0.26AD+0.47AG+6.60DG+0.52A ² -2.23D ² +3.58G ²								
Kale								
Model	1891.39	9	210.15	86.24	0.000	0.9936	0.9821	0.9007
A:Amount of plant material (g)	2.41	1	2.41	0.9901	0.3654			
D:Concentration of AgNO ₃ (mM)	1366.09	1	1366.1	560.62	< 0.0001			
G:Reaction time (s)	362.75	1	362.75	148.86	< 0.0001			
AD	1.88	1	1.88	0.7723	0.4197			
AG	0.132	1	0.132	0.0541	0.8252			
DG	122.97	1	122.97	50.46	0.0009			
A ²	2.6	1	2.6	1.07	0.3489			
D ²	9.38	1	9.38	3.85	0.1071			
G ²	20.48	1	20.48	8.4	0.0338			

Residual	12.18	5	2.44		
Lack of fit	11.76	3	3.92	18.50	0.052
Pure error	0.42	2	0.21		
Corrected total	1903.57	14			
$Y=17.52+0.55A+13.07D+6.73G+0.69AD+0.18AG+5.54DG-0.84A^2-1.59D^2+2.35G^2$					
Melocan					
Model	3282.11	9	364.68	305.66	0.000
A:Amount of plant material (g)	18.2	1	18.2	15.26	0.0113
D:Concentration of AgNO ₃ (mM)	2590.59	1	2590.6	2171.35	< 0.0001
G:Reaction time (s)	506.74	1	506.74	424.73	< 0.0001
AD	2	1	2	1.68	0.2516
AG	16.89	1	16.89	14.16	0.0131
DG	107.54	1	107.54	90.14	0.0002
A ²	7.17	1	7.17	6.01	0.0578
D ²	13.3	1	13.3	11.14	0.0206
G ²	17.26	1	17.26	14.47	0.0126
Residual	5.97	5	1.19		
Lack of fit	4.36	3	1.45	1.81	0.375
Pure error	1.61	2	0.80		
Corrected total	3288.08	14			
$Y=23.44+1.51A+18.00D+7.96G+0.71AD+2.05AG+5.19DG+1.39A^2-1.90D^2+2.16G^2$					

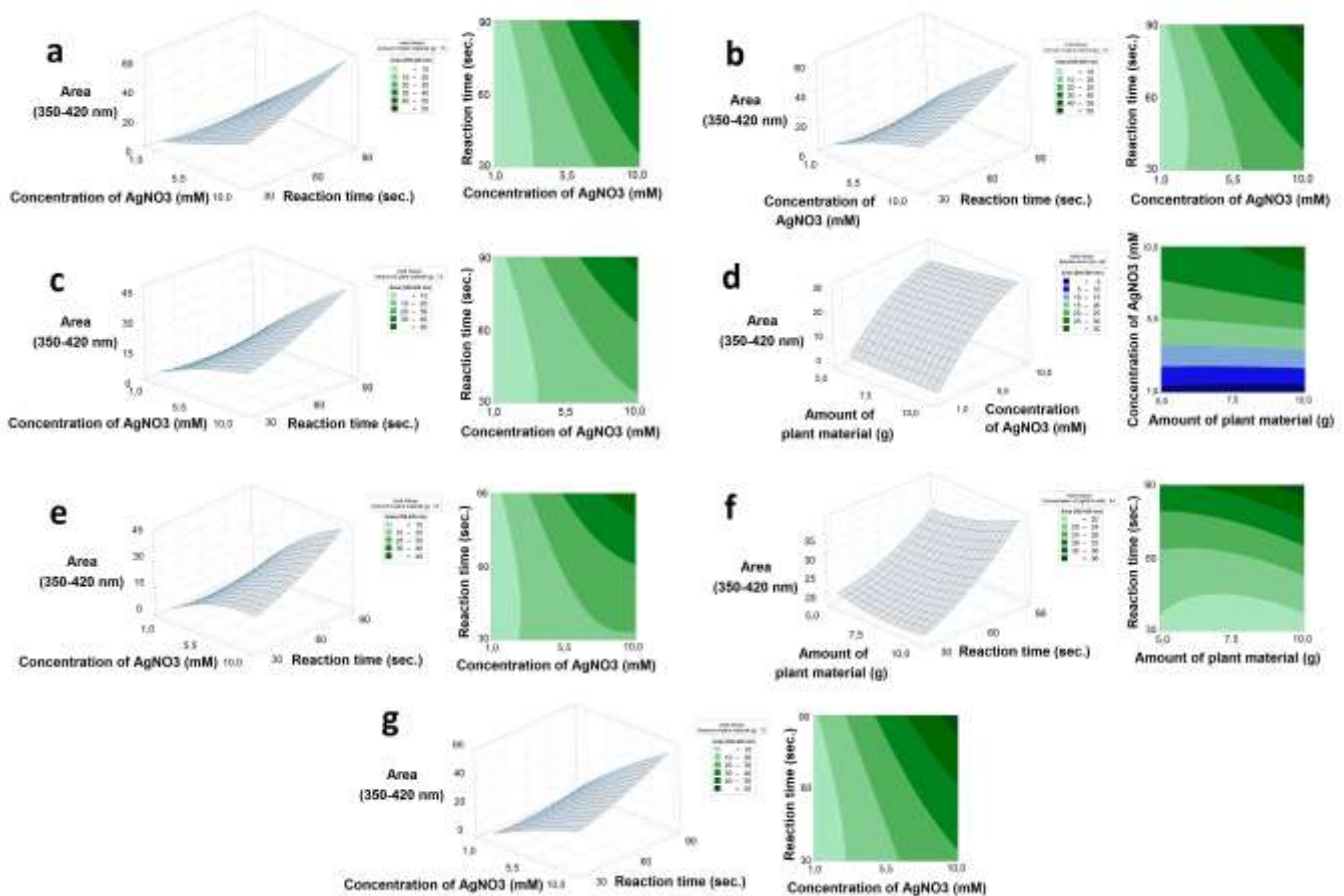


Figure 4. Influence of process parameters on AgNP yield (as determined by UV–Vis spectral area [350–420 nm]): (a–c, e, g) AgNO₃ concentration (mM) versus reaction time (s); (d, f) plant material quantity (g) versus AgNO₃ concentration (mM). Depicts three-dimensional (3D) response surface plots and their corresponding two-dimensional (2D) contour plots.

Şekil 4. İşlem parametrelerinin AgNP verimi üzerindeki etkisi (UV–Vis spektral alanı [350–420 nm] ile belirlenmiştir): AgNO₃ konsantrasyonuna (mM) karşı reaksiyon süresi (sn) (a–c, e, g); bitki materyali miktarına karşı AgNO₃ konsantrasyonu (mM) (d, f). Üç boyutlu (3D) yanıt yüzeyi grafiklerini ve bunlara karşılık gelen iki boyutlu (2D) kontür grafiklerini göstermektedir.

Validating the Model's Predictive Power

Model validation compared predicted AgNP yields (62.14 for black tea, 48.73 for linden, 58.35 for cherry laurel, 44.23 for kale, and 59.40 for melocan) with experimental yields obtained under optimal condition (9.6 g plant material, 80°C extraction heating temperature, 20 min extraction heating time, 10 mM AgNO₃ concentration, 2.5 mL extract volume, 800 W microwave power, and 90 s reaction time). Experimental yields of 63.35, 53.78, 61.95, 45.80, and 61.98, respectively, showed strong agreement with predictions, corresponding to 90.6% to 98.10% validation accuracy. This confirms the model's efficacy in predicting optimal AgNP synthesis conditions and highlights its robustness and potential for practical application in optimizing AgNP synthesis from various plant extracts.

Synthesized-AgNP Characterization

FT-IR spectroscopy

FT-IR analysis of the plant extracts revealed functional groups critical to AgNP synthesis (Figure 5). A broad peak between 3865.30 and 3217.3 cm⁻¹ corresponded to O-H stretching vibrations in hydroxyl groups, characteristic of phenolic compounds and aromatic structures (Nikaeen et al., 2020; Jyoti et al., 2016; Türkkan & Gürel, 2024). The sharp peak at 3618.46 cm⁻¹ was attributed to C-H stretching in alkanes (not alkynes, which typically appear near 3300 cm⁻¹). Peaks at 2931.8 cm⁻¹ (C-H stretching in aliphatic hydrocarbons) and the band spanning 2360.8–1743.7 cm⁻¹ (C≡N stretching in nitriles, C≡C in alkynes, and C=O stretching in carbonyl groups) further indicated diverse functional groups. The region 1589.3–1049.3 cm⁻¹ encompassed vibrations from C=C aromatic rings, C=O esters, C-O-C ethers, and C-N amines (Shameli et al., 2012; Adnan et al., 2020). Peaks below 887–663.5 cm⁻¹ confirmed aromatic C-H bending and phenyl group vibrations. For synthesized AgNPs, FT-IR spectra exhibited a new peak at 2090.8 cm⁻¹ (Figure 5), within the range reported for C≡C or metal-ligand interactions (Laime-Oviedo et al., 2023), suggesting involvement in Ag⁺ reduction and nanoparticle stabilization. Notably, this peak was absent in black tea-derived AgNPs, likely due to differences in extract composition. Overall, hydroxyl (–OH), amine (–NH₂), and carbonyl (–C=O) groups in the plant extracts were pivotal for AgNP formation, acting as both reducing and stabilizing agents.

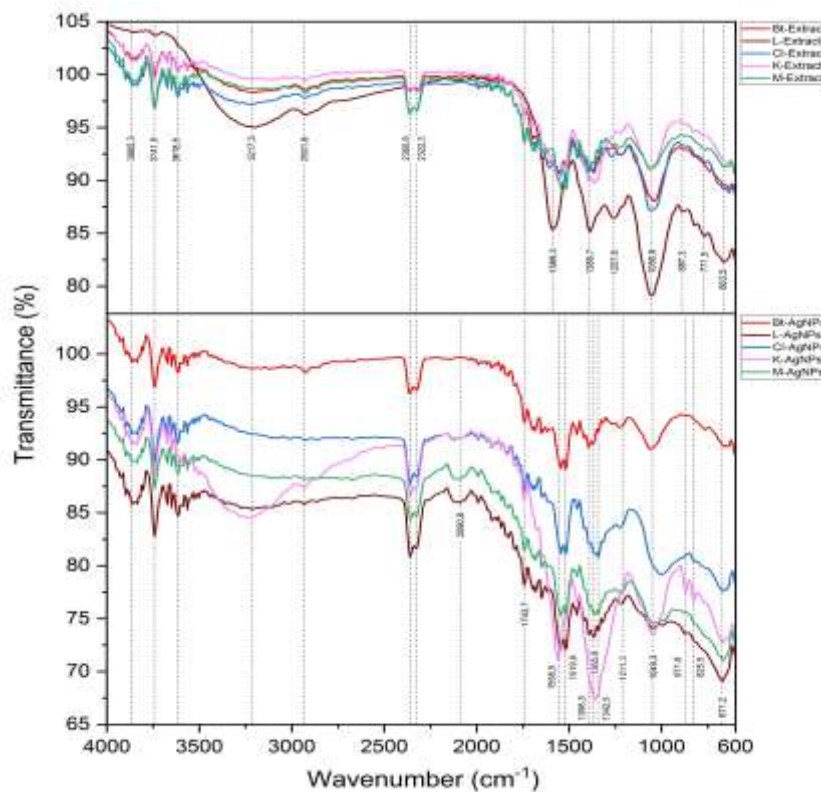


Figure 5. FT-IR spectra of plant extracts (black tea [Bt], linden [L], cherry laurel [Cl], kale [K], and melocan [M]) and their corresponding synthesized silver nanoparticles (Bt-, L-, Cl-, K-, and M-AgNPs).

Şekil 5. Bitki ekstraktlarının (siyah çay [Bt], ıhlamur [L], karayemiş [Cl], yaprak lahanası [K] ve melocan [M]) ve bunlara karşılık gelen sentezlenmiş AgNP'lerin (Bt-, L-, Cl-, K- ve M-AgNP'ler) FT-IR spektrumları.

Microscopic and elemental analysis

SEM (Figure 6a–e) and TEM (Figure 6f–j) analyses revealed spherical AgNPs with good dispersion and minimal aggregation. Particle size analysis showed sizes ranging from 3.00 to 25.00 nm, with average sizes of 5.30 ± 0.15 nm for black tea, 8.74 ± 0.42 nm for linden, 7.20 ± 0.17 nm for cherry laurel, 6.32 ± 0.15 nm for kale, and 9.44 ± 0.14 nm for melocan (Figure 6k–o). EDS analysis confirmed the presence of metallic silver (Ag) through a distinct peak at 3 keV (Figure 7a–e), further supported by the observed surface plasmon resonance (SPR) phenomenon, a characteristic signature of metallic AgNPs (Magudapathy et al., 2001; Vijayaraghavan et al., 2012; Karakaş et al., 2024; Türkkan & Gürel, 2024).

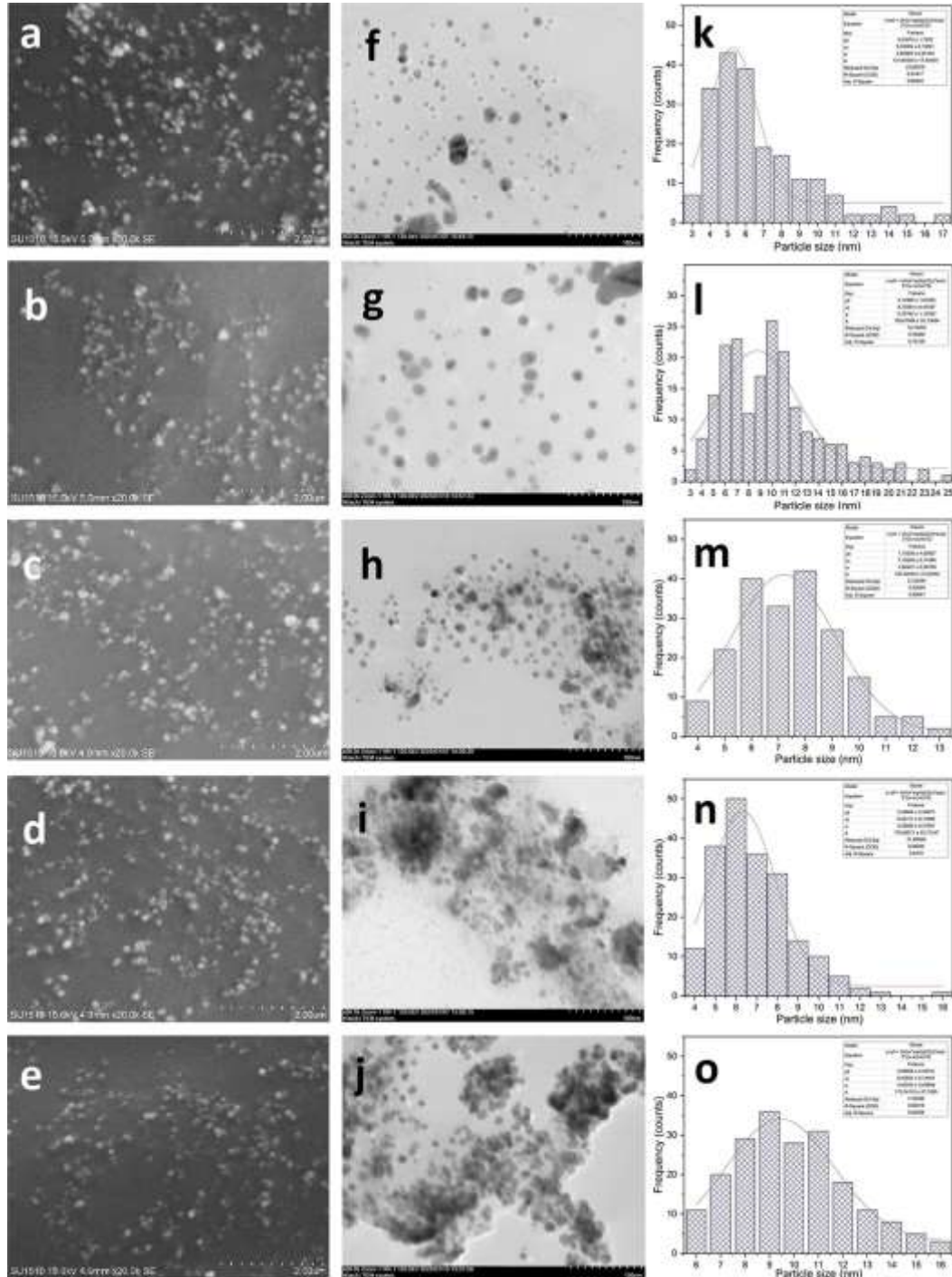


Figure 6. Characterization of Bt-, L-, Cl-, K-, and M-AgNPs. (a–e) SEM images, (f–j) TEM images, and (k–o) size distributions.

Şekil 6. Bt-, L-, Cl-, K- ve M-AgNP'lerin karakterizasyonu. (a–e) SEM görüntüleri, (f–j) TEM görüntüleri, ve (k–o) boyut dağılımları

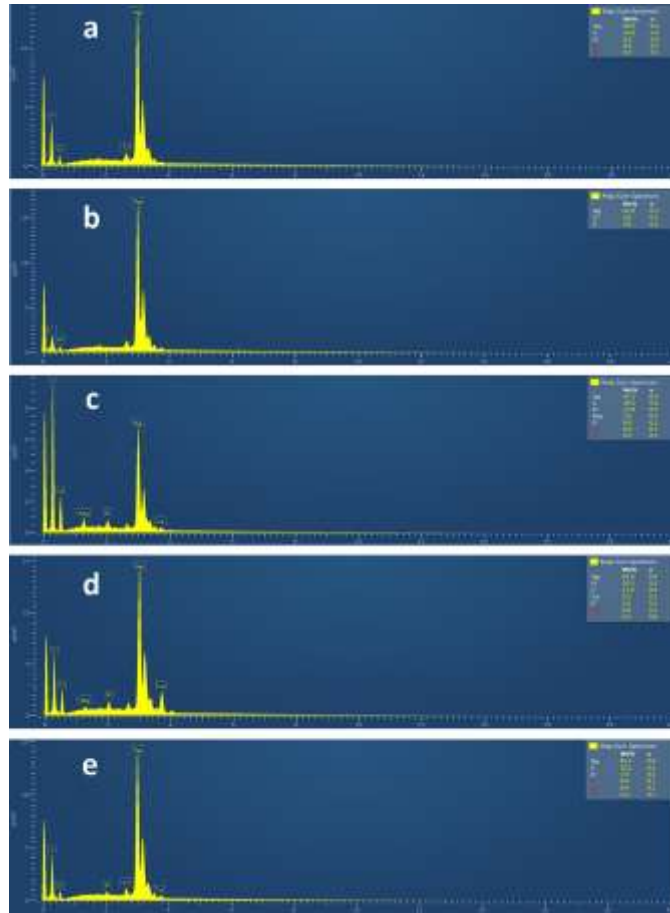


Figure 7. EDS spectra of (a) Bt-, (b) L-, (c) Cl-, (d) K-, and (e) M-AgNPs.

Şekil 7. Bt- (a), L- (b), Cl- (c), K- (d) ve M- (e) AgNP'lerin EDS spektrumları

Antifungal Activity of the Synthesized AgNPs

The antifungal efficacy of AgNPs synthesized from black tea, linden, cherry laurel, kale, and melocan was evaluated against five *Phytophthora* isolates (Table 6). AgNPs derived from different plant sources exhibited variable antifungal effects. Notably, linden- and kale-derived AgNPs showed superior activity, with *P. palmivora* being the most susceptible species. EC₅₀ values, reflecting antifungal potency, ranged from 0.76 to 1058.27 µg mL⁻¹ across isolates. Linden- and melocan- derived AgNPs did not completely inhibit any isolate at the highest tested concentration (400 µg mL⁻¹), whereas cherry laurel-derived AgNPs achieved complete inhibition of *P. cinnamomi* (Figure 8). Black tea-derived AgNPs at 300 µg mL⁻¹ fully inhibited three other *Phytophthora* isolates but failed to suppress *P. cinnamomi* and *P. citrophthora* even at 400 µg mL⁻¹. In contrast, kale-derived AgNPs completely inhibited all five *Phytophthora* isolates at 200 or 300 µg mL⁻¹. Regarding fungicidal activity, cherry laurel-derived AgNPs exhibited fungicidal effects against *P. cinnamomi* at 400 µg mL⁻¹. Black tea-derived AgNPs were fungicidal for *P. capsici* and *P. nicotianae* at 300 µg mL⁻¹ and for *P. palmivora* at 400 µg mL⁻¹. Kale-derived AgNPs showed fungicidal activity against *P. capsici* at 200 µg mL⁻¹ and against the remaining four *Phytophthora* isolates at 400 µg mL⁻¹. These findings align with prior reports on AgNP minimum fungicidal concentrations (MFCs). For instance, Yiğit et al. (2023) documented cherry laurel-AgNP MFCs exceeding 150 µg mL⁻¹ against oomycete and fungal pathogens, while Yiğit & Türkkan (2023) observed linden-AgNP MFCs ranging from 225 to 900 µg mL⁻¹ for *Phytophthora* species. Türkkan & Gürel (2024) reported Madimak-AgNP MFCs of 400–800 µg mL⁻¹ against related species (*P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae*, *P. megaspermae*/*P. palmivora*). In contrast, wormwood-derived AgNPs demonstrated stronger antifungal activity against *Phytophthora* species, with MICs well below 100 µg mL⁻¹ (Ali et al., 2007), emphasizing the critical role of plant source in shaping AgNP efficacy. The observed variability in antifungal performance underscores the intricate relationship between AgNP properties (e.g., size, shape, surface chemistry) and their biological targets. Further research is required to unravel how plant-specific phytochemicals modulate AgNP characteristics and interactions with pathogens. These variations highlight the importance of nanoparticle design in optimizing antimicrobial applications. Upon entering microbial cells, AgNPs disrupt essential cellular functions, ultimately leading to cell death (Buzea, 2007).

Table 6. *In vitro* antifungal activity of synthesized AgNPs against *Phytophthora* species

Çizelge 6. Sentezlenmiş AgNP'lerin *Phytophthora* türlerine karşı *in vitro* antifungal aktivitesi

Plant-AgNPs	<i>Phytophthora</i> spp.	Mycelial growth (cm) (400 µg mL ⁻¹)			EC ₅₀ ^b (µg mL ⁻¹)	MIC ^c (µg mL ⁻¹)	MFC ^d (µg mL ⁻¹)
Black tea-AgNPs	<i>P. capsici</i>	0.00	±	0.00 ^a	40.88	300	300
	<i>P. cinnomami</i>	1.61	±	0.12	36.67	>400	>400
	<i>P. citrophthora</i>	4.08	±	0.17	168.66	>400	>400
	<i>P. nicotianae</i>	0.00	±	0.00	45.73	300	300
	<i>P. palmivora</i>	0.00	±	0.00	48.53	300	400
Linden-AgNPs	<i>P. capsici</i>	0.95	±	0.12	3.69	>400	>400
	<i>P. cinnomami</i>	1.68	±	0.08	0.80	>400	>400
	<i>P. citrophthora</i>	1.72	±	0.02	9.60	>400	>400
	<i>P. nicotianae</i>	1.93	±	0.04	4.45	>400	>400
	<i>P. palmivora</i>	1.16	±	0.04	0.76	>400	>400
Chery laurel-AgNPs	<i>P. capsici</i>	1.20	±	0.05	58.52	>400	>400
	<i>P. cinnomami</i>	0.00	±	0.00	57.36	400	400
	<i>P. citrophthora</i>	4.77	±	0.08	225.37	>400	>400
	<i>P. nicotianae</i>	2.32	±	0.07	62.84	>400	>400
	<i>P. palmivora</i>	2.80	±	0.26	99.48	>400	>400
Kale-AgNPs	<i>P. capsici</i>	0.00	±	0.00	21.10	200	200
	<i>P. cinnomami</i>	0.00	±	0.00	21.62	300	400
	<i>P. citrophthora</i>	0.00	±	0.00	18.13	300	400
	<i>P. nicotianae</i>	0.00	±	0.00	30.84	300	400
	<i>P. palmivora</i>	0.00	±	0.00	9.28	300	400
Melocan-AgNPs	<i>P. capsici</i>	6.00	±	0.35	284.48	>400	>400
	<i>P. cinnomami</i>	7.88	±	0.20	199.14	>400	>400
	<i>P. citrophthora</i>	17.10	±	0.28	>400	>400	>400
	<i>P. nicotianae</i>	7.25	±	0.18	210.91	>400	>400
	<i>P. palmivora</i>	5.79	±	0.33	201.67	>400	>400

^aData are presented as means ± standard errors. ^bThe concentration that caused 50% reduction, ^cMinimum inhibitory concentration, ^dMinimum fungicidal concentration.

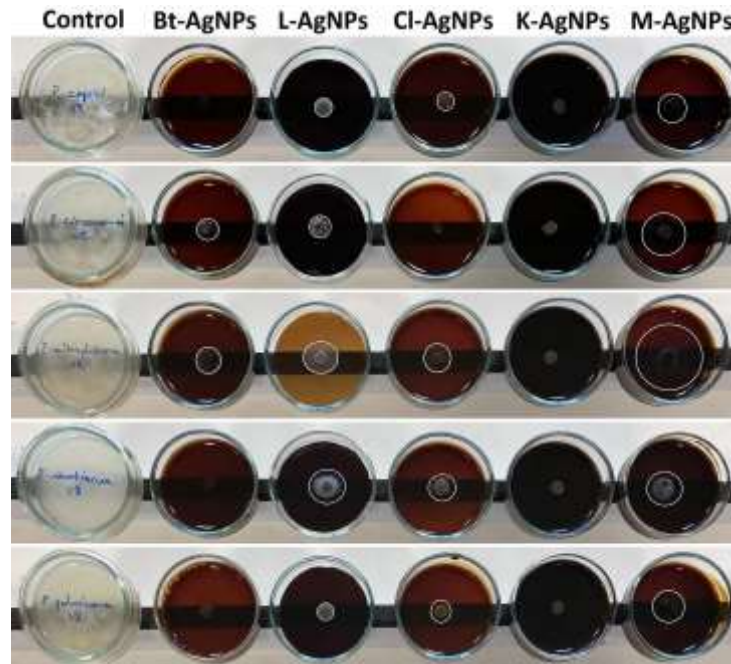


Figure 8. Inhibitory effects of AgNPs synthesized from black tea (Bt), linden (L), cherry laurel (Cl), kale (K), and melocan (M) on *Phytophthora* species at a concentration of 400 µg mL⁻¹.

Şekil 8. Siyah çay (Bt), ıhlamur (L), karayemiş (Cl), yaprak lahanası (K) ve melocan (M)'dan sentezlenmiş AgNP'lerin 400 µg mL⁻¹ konsantrasyonda *Phytophthora* türleri üzerindeki engelleyici etkisi.

CONCLUSION

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of black tea, linden, cherry laurel, kale, and melocan. UHPLC and UV–Vis analyses identified a diverse array of bioactive compounds, including phenolic acids (e.g., gallic acid) and putative flavonoids, which likely facilitated the reduction of Ag⁺ ions and stabilization of the nanoparticles. Through statistical optimization via Plackett–Burman and Box–Behnken designs, the following optimal synthesis conditions were established: 9.6 g of plant material, extraction heating at 80°C for 20 minutes, 10 mM AgNO₃, 2.5 mL extract volume, 800 W microwave power, and a 90-second reaction time. Characterization revealed uniform, spherical AgNPs with sizes ranging from 3.00 to 25.00 nm (determined by TEM), with plant-specific averages of 5.30 nm (black tea), 8.74 nm (linden), 7.20 nm (cherry laurel), 6.32 nm (kale), and 9.44 nm (melocan). The synthesized AgNPs exhibited significant antifungal activity against key *Phytophthora* pathogens (*P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae*, and *P. palmivora*), with kale-derived AgNPs showing exceptional dual fungistatic and fungicidal effects. These findings highlight the potential of plant-mediated AgNPs as a sustainable alternative for managing fungal diseases in agriculture. Future research should focus on field trials to assess practical efficacy, mechanistic studies to elucidate nanoparticle–pathogen interactions, and scalability assessments for large-scale agricultural applications.

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Contribution of the Authors as Summary

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

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

- Adnan, M., Azad, M. O. K., Madhusudhan, A., Saravanakumar, K., Hu, X., Wang, M. H. & Ha, C. D. (2020). Simple and cleaner system of silver nanoparticle synthesis using kenaf seed and revealing its anticancer and antimicrobial potential. *Nanotechnology*, 31(26), 265101. <https://doi.org/10.1088/1361-6528/ab7d72>
- Ahmad, N. & Sharma, S. (2012). Green Synthesis of silver nanoparticles using extracts of *Ananas comosus*. *Green and Sustainable Chemistry*, 2(4), 1-7. <https://doi.org/10.4236/gsc.2012.24020>
- Akyuz, E., Şahin, H., Islamoglu, F., Kolayli, S. & Sandra, P. (2014). Evaluation of phenolic compounds in *Tilia rubra* subsp. *caucasica* by HPLC–UV and HPLC–UV–MS/MS. *International Journal of Food Properties*, 17, 331-343. <https://doi.org/10.1080/10942912.2011.631252>
- Ali, M., Kim, B., Belfield, K. D., Norman, D., Brennan, M. & Ali, G. S. (2015). Inhibition of *Phytophthora parasitica* and *P. capsici* by silver nanoparticles synthesized using aqueous extract of *Artemisia absinthium*. *Phytopathology*, 105(9), 1183-1190. <https://doi.org/10.1094/PHYTO-01-15-0006-R>
- Baytop, T. (1999). Therapy with medicinal plants in Turkey (past and present). Publication of the Istanbul University, 312.
- Box, G. E. P. & Behnken, D. W. (1960). Simplex-sum designs: a class of second order rotatable designs derivable from those of first order. *The Annals of Mathematical Statistics*, 31(4), 838-864. <https://doi.org/10.1214/aoms/1177705661>
- Buzea, C., Pacheco, I. I. & Robbie, K. (2007). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2(4), MR17-MR71. <https://doi.org/10.1116/1.2815690>
- Cai, Y., Piao, X., Gao, W., Zhang, Z., Nie, E. & Sun, Z. (2017). Large-scale and facile synthesis of silver nanoparticles via a microwave method for a conductive pen. *RSC Advances*, 7(54), 34041-34048. <https://doi.org/10.1039/C7RA05125E>
- Chowdhury, S., Yusof, F., Faruck, M. O. & Sulaiman, N. (2016). Process optimization of silver nanoparticle synthesis using response surface methodology. *Procedia Engineering*, 148, 992-999. <https://doi.org/10.1016/j.proeng.2016.06.552>
- Davis, P., Tan, K. & Mill, R.R. (1988). Flora of Turkey and the East Aegean Islands and Supplement I. Edinburgh University Press, Edinburgh.
- El-Sawaf, A. K., El-Moslamy, S. H., Kamoun, E. A., & Hossain, K. (2024). Green synthesis of trimetallic CuO/Ag/ZnO nanocomposite using *Ziziphus spina-christi* plant extract: characterization, statistically

- experimental designs, and antimicrobial assessment. *Scientific Reports*, 14(1), 19718. <https://doi.org/10.1038/s41598-024-67579-5>
- Encu, S. (2010). *Lahana çeşitlerinin antioksidan kapasiteleri ve bileşenleri açısından değerlendirilmesi (Tez no 282642)*. [Yüksek Lisans Tezi, İstanbul Üniversitesi Fen Bilimleri Enstitüsü Kimya Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Günel, N. (2013). Türkiye’de iklimin doğal bitki örtüsü üzerindeki etkileri. *Acta Turcica Çevrimiçi Tematik Türkoloji Dergisi*, V(1), 1-22.
- Fazıl, M. M., Gul, A. & Jawed, H. (2024). Optimization of silver nanoparticles synthesis via Plackett–Burman experimental design: *in vitro* assessment of their efficacy against oxidative stress-induced disorders. *RSC Advances*, 14(29), 20809-20823. <https://doi.org/10.1039/d4ra02774d>
- Firoozi, S., Jamzad, M. & Yari, M. (2016). Biologically synthesized silver nanoparticles by aqueous extract of *Satureja intermedia* C.A. Mey and the evaluation of total phenolic and flavonoid contents and antioxidant activity. *Journal of Nanostructure in Chemistry*, 6, 357-364. <https://doi.org/10.1007/s40097-016-0207-0>
- Gevrek, C., Yiğit, U. & Türkkan, M. (2023). Optimization and antifungal activity of silver nanoparticles synthesized using the leaf extract of *Corylus colurna* L. (Turkish hazelnut). *Akademik Ziraat Dergisi*, 12(Özel Sayı), 159-172. <https://doi.org/10.29278/azd.1335259>
- Halima, R., Narula, A. & Sravanthi, V. (2021). Optimization of process parameters for the green synthesis of silver nanoparticles using Plackett–Burman and 3-level Box–Behnken Design. *Journal of Huazhong University of Science and Technology*, 50(3), 1-17.
- İslam, A. & Deligöz, H. (2012). Ordu ilinde karayemiş (*Laurocerasus officinalis* L.) seleksiyonu. *Akademik Ziraat Dergisi*, 1(1), 37-44.
- Jyoti, K., Baunthiyal, M. & Singh, A. (2016). Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. *Journal of Radiation Research and Applied Sciences*, 9(3), 217-227. <https://doi.org/10.1016/j.jrras.2015.10.002>
- Kale, R., Barwar, S., Kane, P. & More, S. (2018). Green synthesis of silver nanoparticles using papaya seed and its characterization. *International Journal for Research in Applied Science & Engineering Technology*, 6, 168-174. <https://doi.org/10.22214/ijraset.2018.2026>
- Karabegović, I.T., Stojicević, S.S., Velickovic, D.T., Zoran B. Todorovic, Z.B., Nada C. Nikolic, N.C., Miodrag L. & Lazic, M.L. (2014). The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (*Prunus laurocerasus*) leaf and fruit extracts. *Industrial Crops and Products*, 54, 142-148. <https://doi.org/10.1016/j.indcrop.2013.12.047>
- Karakaş, İ., Hacıoğlu, N. & Özdemir, B. E. (2024). Green synthesis and antibiofilm activity of silver nanoparticles by *Camellia sinensis* L. (White tea leaf). *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 27(2), 285-292. <https://doi.org/10.18016/ksutarimdoga.1297130>
- Kelly, K. L., Coronado, E., Zhao, L. L. & Schatz, G. C. (2003). The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *The Journal of Physical Chemistry B*, 107(3), 668-677. <https://doi.org/10.1021/jp026731y>
- Kolaylı, S., Kucuk, M., Duran, C., Candan, F. & Dinçer, B. (2003). Chemical and antioxidant properties of *Laurocerasus officinalis* Roem. (Cherry laurel) fruit grown in the Black Sea Region. *Journal of Agricultural and Food Chemistry*, 51, 7489-94. <https://doi.org/10.1021/jf0344486>
- Konwarh, R., Karak, N., Sawian, C. E., Baruah, S. & Mandal, M. (2011). Effect of sonication and aging on the templating attribute of starch for “green” silver nanoparticles and their interactions at biointerface. *Carbohydrate Polymers*, 83(3), 1245-1252. <https://doi.org/10.1016/j.carbpol.2010.09.031>
- Kumar, K. M., Sinha, M., Mandal, B. K., Ghosh, A. R., Kumar, K. S. & Reddy, P. S. (2012). Green synthesis of silver nanoparticles using *Terminalia chebula* extract at room temperature and their antimicrobial studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 91, 228-233. <https://doi.org/10.1016/j.saa.2012.02.001>
- Laime-Oviedo, L. A., Soncco-Cahui, A. A., Peralta-Alarcon, G., Arenas-Chávez, C. A., Pineda-Tapia, J. L., Díaz-Rosado, J. C. & Vera-Gonzales, C. (2022). Optimization of synthesis of silver nanoparticles conjugated with *Lepechinia meyenii* (Salvia) using Plackett–Burman Design and Response Surface Methodology—preliminary antibacterial activity. *Processes*, 10(9), 1727. <https://doi.org/10.3390/pr10091727>
- Laime-Oviedo, L. A., Arenas-Chávez, C. A., Yáñez, J. A. & Vera-González, C. A. (2023). Plackett–Burman design in the biosynthesis of silver nanoparticles with *Mutisia acuminata* (Chinchircoma) and preliminary evaluation of its antibacterial activity. *F1000Research*, 12. <https://doi.org/10.12688/f1000research.140883.1>
- Magudapathy, P., Gangopadhyay, P., Panigrahi, B. K., Nair, K. G. M. & Dhara, S. (2001). Electrical transport studies of Ag nanoclusters embedded in glass matrix. *Physica B: Condensed Matter*, 299(1-2), 142-146. [https://doi.org/10.1016/S0921-4526\(00\)00580-9](https://doi.org/10.1016/S0921-4526(00)00580-9)

- Naveed M., BiBi J., Kamboh A. A., Suheryani I., Kakar, I., Fazlani, S. A., FangFang, X., Kalhoro, S. A., Yunjuan L., Kakar, M. U, Abd El-Hack, M. E., Noreldin, A. E., Zhixiang, S., LiXia, C. & XiaoHui, Z. (2018). Pharmacological values and therapeutic properties of black tea (*Camellia sinensis*): A comprehensive overview. *Biomed Pharmacotherapy*, 100, 521-531. <https://doi.org/10.1016/j.biopha.2018.02.048>
- Nikaeen, G., Yousefinejad, S., Rahmdel, S., Samari, F. & Mahdavinia, S. (2020). Central composite design for optimizing the biosynthesis of silver nanoparticles using *Plantago major* extract and investigating antibacterial, antifungal and antioxidant activity. *Scientific Reports*, 10(1), 9642. <https://doi.org/10.1038/s41598-020-66357-3>
- Noroozi, M., Zakaria, A., Moksini, M. M., Wahab, Z. A. & Abedini, A. (2012). Green formation of spherical and dendritic silver nanostructures under microwave irradiation without reducing agent. *International Journal of Molecular Sciences*, 13(7), 8086-8096. <https://doi.org/10.3390/ijms13078086>
- Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in Middle eastern plants. *Frontiers in Microbiology*, 10, 911. <https://doi.org/10.3389/fmicb.2019.00911>.
- Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M. & Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied Microbiology and Biotechnology*, 102, 6799-6814. <https://doi.org/10.1007/s00253-018-9146-7>
- Öz, M. (2022). *Tilia rubra* DC. subsp. *caucasica* V.Engler (Kafkas İhlamuru) yaprak uçucu yağının kimyasal bileşimi. III. International Siirt Scientific Research Congress, Siirt, Türkiye, 18-19 November 2022, ss.14.
- Özsoy, N., Can, A., Yanardag, R. & Akev, N. (2008). Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chemistry*, 110(3), 571-583. <https://doi.org/10.1016/j.foodchem.2008.02.037>
- Öztürk, B., Yıldız, K. & Küçüker, E. (2015). Effect of pre-harvest methyl jasmonate treatments on ethylene production, water-soluble phenolic compounds and fruit quality of Japanese plums. *Journal of the Science of Food and Agriculture*, 95(3), 583-591. <https://doi.org/10.1002/jsfa.6787>
- Plackett, R. L. & Burman, J. P. (1946). The design of optimum multifactorial experiments. *Biometrika*, 33(4), 305-325. <https://doi.org/10.1093/biomet/33.4.305>
- Reddy, L. V. A., Wee, Y. J., Yun, J. S. & Ryu, H. W. (2008). Optimization of alkaline protease production by batch culture of *Bacillus* sp. RKY3 through Plackett–Burman and response surface methodological approaches. *Bioresource Technology*, 99(7), 2242-2249. <https://doi.org/10.1016/j.biortech.2007.05.006>
- Sahan, Y. (2011). Effect of *Prunus laurocerasus* L. (Cherry laurel) leaf extracts on growth of bread spoilage fungi. *Bulgarian Journal of Agricultural Science*, 17(1), 83-92.
- Šamec, D., Urlić, B. & Salopek-Sondi, B. (2018). Kale (*Brassica oleracea* var. *acephala*) as a superfood: Review of the scientific evidence behind the statement. *Critical Reviews in Food Science and Nutrition*, 59(15), 2411-2422. <https://doi.org/10.1080/10408398.2018.1454400>
- Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K. & Poinern, G. E. J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials*, 8(11), 7278-7308. <https://doi.org/10.3390/ma8115377>
- Shameli, K., Bin Ahmad, M., Jaffar Al-Mulla, E. A., Ibrahim, N. A., Shabanzadeh, P., Rustaiyan, A. & Zidan, M. (2012). Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. *Molecules*, 17(7), 8506-8517. <https://doi.org/10.3390/molecules17078506>
- Sharma, N. K., Vishwakarma, J., Rai, S., Alomar, T. S., AlMasoud, N. & Bhattarai, A. (2022). Green route synthesis and characterization techniques of silver nanoparticles and their biological adeptness. *ACS Omega*, 7(31), 27004-27020. <https://doi.org/10.1021/acsomega.2c01400>
- Siddiqi, K.S., Husen, A. & Rao, R.A.K. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology*, 16, 14. <https://doi.org/10.1186/s12951-018-0334-5>
- Siddiqui, A., Gul, A., Khan, H., Anjum, F. & Hussain, T. (2024). Bio-inspired synthesis of silver nanoparticles using *Salsola imbricata* and its application as antibacterial additive in glass ionomer cement. *Nanotechnology*, 35(35), 355101. <https://doi.org/10.1088/1361-6528/ad50e4>
- Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Singleton, V.L. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
- Stepanov, A. L. (2004). Optical properties of metal nanoparticles synthesized in a polymer by ion implantation: a review. *Technical Physics*, 49, 143-153. <https://doi.org/10.1134/1.1648948>
- Trivedi, P., Khandelwal, M. & Srivastava, P. (2014). Statistically optimized synthesis of silver nanocubes from peel extracts of *Citrus limetta* and potential application in waste water treatment. *Journal of Microbial & Biochemical Technology*, 4(004). <https://doi.org/10.4172/1948-5948.S4-004>
- Tuttu, G., Ursavaş, S. & Söyler, R. (2017). İhlamur çiçeğinin Türkiye'deki hasat miktarları ve etnobotanik kullanımı. *Anadolu Orman Araştırmaları Dergisi*, 3(1), 60-66

- Türkkan, M. & Gürel, Y. (2024). Plackett–Burman and Box–Behnken Designs for optimizing *Polygonum cognatum* Meissn-mediated AgNP synthesis: Antifungal activity against diverse *Phytophthora* spp. *Akademik Ziraat Dergisi*, 13(2), 272-286. <https://doi.org/10.29278/azd.1522321>
- Vijayaraghavan, K., Nalini, S. K., Prakash, N. U. & Madhankumar, D. J. M. L. (2012). Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. *Materials Letters*, 75, 33-35. <https://doi.org/10.1016/j.matlet.2012.01.083>
- Ye, M., Yang, W., Zhang, M., Huang, H., Huang, A. & Qiu, B. (2023). Biosynthesis, characterization, and antifungal activity of plant-mediated silver nanoparticles using *Cnidium monnieri* fruit extract. *Frontiers in Microbiology*, 14, 1291030. <https://doi.org/10.3389/fmicb.2023.1291030>
- Yeşilada, E., Sezik, E., Honda, G., Takashi, Y., Takeda, Y. & Tanaka, T. (1999). Traditional medicine in Turkey IX. Folk Medicine in North-West Anatolia. *Journal of Ethnopharmacology*, 64, 195- 210
- Yiğit, U. & Türkkan, M. (2023). Antifungal activity and optimization procedure of microwave-synthesized silver nanoparticles using linden (*Tilia rubra* subsp. *caucasica*) flower extract. *International Journal of Chemistry and Technology*, 7(1), 25-37. <https://doi.org/10.32571/ijct.1194356>
- Yiğit, U., Gürel, Y., İlhan, H. & Türkkan, M. (2023). Antifungal activity and optimization procedure of silver nanoparticles green synthesized with *Prunus laurocerasus* L. (Cherry laurel) leaf extract. *International Journal of Life Sciences and Biotechnology*, 6(1), 1–20. <https://doi.org/10.38001/ijlsb.1168628>
- Yilmaz, M., Yilmaz, A., Karaman, A., Aysin, F. & Aksakal, O. (2021). Monitoring chemically and green-synthesized silver nanoparticles in maize seedlings via surface-enhanced Raman spectroscopy (SERS) and their phytotoxicity evaluation. *Talanta*, 225, 121952. <https://doi.org/10.1016/j.talanta.2020.121952>

Tarımsal Atıklar Kullanılarak Tekstil Atık Sularındaki Boyar Maddelerin Adsorpsiyonla Arıtılması

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ÖZET

Tekstil endüstrisinin son yıllardaki hızlı gelişimi nedeniyle yüksek miktarda su tüketimi ve yüksek toksisiteli kimyasallar içeren atık suların çevreye salınımı, çevre kirliliği ve insan sağlığı için tehdit oluşturmaktadır. Özellikle, boyar maddeler gibi kimyasalların kanserojen, teratojen ve mutajen etkileri, su kirliliği sorununu daha da vahim hale getirmektedir. Bu çalışmada, tekstil atık sularında bulunan boyar maddelerin sulu çözeltilerden uzaklaştırılması için doğada atık olarak bulunan malzemelerin adsorban olarak kullanılabilirliği araştırılmıştır. Bu amaçla, asit kırmızısı 1 (AK1), asit mavisi 80 (AM80), parlak mavi G (PMG), safranin O (SO) ve Victoria saf mavisi BO (VSMBO) boyar maddelerinin adsorpsiyon potansiyeli incelenmiştir. Adsorban olarak yer fıstığı kabuğu, çam poleni, mandalina kabuğu, nar kabuğu, Antep fıstığı kabuğu, muz kabuğu ve bal kabağı kabuğu gibi doğada bol miktarda bulunan ve atık olarak değerlendirilebilecek malzemeler kullanılmıştır. Çalışma, iki farklı pH seviyesinde (orijinal pH ve pH=2) gerçekleştirilmiş, bu pH koşullarında adsorpsiyon verimliliği değerlendirilmiştir. Sonuç olarak pH=2'de en yüksek giderimin AK1 boyar maddesi için muz kabuğu (%86.07) ile, AM80 boyar maddesi için yer fıstığı kabuğu (%94.83) ve PMG boyar maddesi için ise nar kabuğu (%96.49) ile elde edilmiştir. Orijinal pH'da SO ve VSMBO boyar maddelerinin ise orijinal pH'da tüm adsorbanların etkili olduğu gözlemlenmiştir. Bu çalışmada, farklı pH seviyelerinin (orijinal pH ve pH=2) bu boyar maddelerin adsorpsiyon kapasiteleri üzerindeki etkisini, tek yönlü ANOVA analizi ile değerlendirilmiştir. AK1, AM80, SO ve VSM80 için anlamlı farklılıklar gözlemlenirken, PMG için anlamlı bir fark bulunmamıştır. Bu bulgular, tekstil atık sularının arıtımında çevre dostu ve düşük maliyetli çözümler sunmaktadır.

Tarımsal Biyoteknoloji

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Adsorption Treatment of Dyestuffs in Textile Wastewater Using Agricultural Wastes

ABSTRACT

Recent textile sector growth has resulted in excessive water consumption and wastewater releases into the environment that contain highly harmful compounds, endangering human health and the environment. Notably, the water pollution problem is exacerbated by the mutagenic, carcinogenic, and teratogenic properties of chemicals like dyes. This study aimed to determine whether waste materials from nature might be used as adsorbents to remove colors from aqueous solutions in textile effluent. Acid red 1 (AK1), acid blue 80 (AM80), bright blue G (PMG), safranin O (SO), and Victoria pure blue BO (VSMBO) dyes were examined for their adsorption capacity to achieve this goal. Materials that are abundant in nature and can be considered trash, such as peanut shells, pine pollen, tangerine peels, pomegranate peels, pistachio shells, banana peels, and pumpkin peels, were employed as adsorbents. The original pH and pH=2 were the two pH levels at which the study was conducted, and it was at these pH levels that the adsorption effectiveness was assessed. Consequently,

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the most excellent clearance at pH=2 was achieved with pomegranate peel (96.49%), peanut shell (94.83%), and banana peel (86.07%) for AK1 dye, AM80 dye, and PMG dye. For SO and VSMBO dyes, it was found that all adsorbents worked well at the initial pH. In this study, the effect of different pH levels (original pH and pH=2) on the adsorption capacities of these dyes was evaluated by one-way ANOVA analysis. While significant differences were observed for AK1, AM80, SO, and VSM80, no significant difference was found for PMG. These findings offer affordable, eco-friendly methods for treating wastewater from textile production. The results suggest that optimizing pH levels can enhance the effectiveness of certain adsorbents in removing dyes from wastewater. Further research may explore the long-term stability and regeneration potential of these materials to ensure sustainable applications in industrial settings. This tool offers a variety of features designed to enhance writing, including grammar checking, paraphrasing, and summarization. By utilizing advanced algorithms, it aims to assist users in producing clear and effective written communication.

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GİRİŞ

Hızlı sanayileşme ve kentleşme ile beraber çevreye salınan tehlikeli kimyasallar içeren atık sular arıtma işleminden sonra deniz, nehir gibi yakındaki sulara boşaltılarak ekosistemin ve insan sağlığının tahribatına neden olmaktadır (Cora ve ark., 2024; Thasneema ve ark., 2021). Bu yüzden su kirliliği endişe verici bir çevre sorunu haline gelmiştir. Özellikle tekstil fabrikaları tüm sanayi sektörleri arasında en fazla su kirliliğine neden olan sektör olarak kabul edilmektedir (Methneni ve ark., 2021). Tekstil endüstrilerinin boyama işlemlerinde büyük miktarda su tüketimi gerçekleşir (Rigueto ve ark., 2020). Bunun sonucunda, arıtılmadan veya yetersiz arıtma ile yüksek hacimde atık su çevreye salınır (Methneni ve ark., 2021). Üretilen renkli atık sular yüksek toksisiteleri nedeniyle kanserojen, teratojen ve hatta mutajen oldukları için sağlık açısından ciddi tehlike unsurlarıdır (Tzvetkov ve ark., 2020). Bu yüzden tekstil atık suları birçok ülkede günden güne artan endişe kaynağıdır (Thasneema ve ark., 2021).

Bu kimyasalların, insanlara ve suda yaşayan organizmalara yönelik oluşturduğu ciddi risk nedeniyle, atık sudan bu kimyasalları uzaklaştırmak için etkili bir yöntem geliştirmek zorunludur. Sentetik boyaları atık sulardan uzaklaştırmak için membran filtrasyonu, mikrobiyolojik veya enzimatik, adsorpsiyon, fotokatalizör gibi çeşitli yöntemler önerilmiştir (Liu ve ark., 2020; Kuo & Chen, 2012; Mi ve ark., 2019; Ajaz ve ark., 2019). Bu yöntemlerin arasında adsorpsiyon, boya giderimi için etkili bir tekniktir. Adsorpsiyon, yüksek verimliliği, düşük işlem maliyeti, tasarımı basitliği ve kullanım kolaylığı nedeniyle avantajlı bir teknolojidir (Tzvetkov ve ark., 2020). Sulu çözeltilerden boyaların uzaklaştırılması için düşük maliyetli, tekrar kullanılabilirliği yüksek ve proses gerektirmeyen adsorbanlar son zamanlarda dikkat çekmektedir. Tarımsal atık malzemelerden hazırlanan adsorbanlara, bol miktarda bulunmaları, yüksek sabit karbon içeriği ve gözenekli yapıları nedeniyle sıklıkla literatürde rastlanmaktadır (Bhatnagar & Minocha, 2010). Tarımsal atıklar, tarımsal üretimde yan ürün olarak oluşur ve doğru şekilde yönetilmediğinde çevresel sorunlara neden olabilir. Bu yüzden, bu atıkların etkili bir şekilde kullanılması önemlidir (Yıldız, 2024).

Kırmızı 2G olarak da bilinen asit kırmızı 1 (AK1), mürekkepleri, kağıtları, krep kağıtlarını ve hassas dokuları renklendirmek için kullanılan sentetik bir anyonik mono-azo boyadır (Alkoshab ve ark. 2024). İki sülfonat grubu içeren anyonik antrakinon boya olan asit mavisi 80 (AM80), tekstil endüstrisinin yanı sıra kağıt kimyasalları ve boyaları, mürekkep ve tonerler, deri işleme ürünleri, polimerler, kozmetik ve kişisel bakım ürünleri, yıkama ve temizlik ürünleri, biyositler vb. alanlarda kullanılır (Blanco ve ark., 2023). Parlak mavi G (BMG) Afrika Kıtası'nda, özellikle tekstil endüstrisinde yaygın olarak kullanılan, Coomassie Blue olarak da adlandırılan anyonik bir boyadır (Boukerche ve ark. 2023). Safranin O (SO) bilinen en eski suda çözünür sentetik ve katyonik bir boyadır. Genellikle pamuk, elyaf, deri, kağıt, ipek, tanen ve yünü boyamak için kullanılır. Ayrıca gıda ve tekstil endüstrilerinde renklendirici olarak yaygın olarak kullanılır (Çetinkaya ve ark., 2023). Victoria saf mavisi BO (VPBO), Basic blue 7 olarak da bilinir, 2010 yılında Vietnam'dan ithal edilen kirli panga balığında (pangasius bocourti) bulunan bir boyadır (Dubreil ve ark. 2021).

Daha önceki yapılan çalışmalarda, yer fıstığı kabuklarının kadmiyum ve nikelin arıtılması için adsorban olarak kullanılması (Pessôa ve ark., 2024), yer fıstığı kabuğu ve portakal kabuğundan elde edilen adsorbanlar ile fosfatın uzaklaştırılması (Zhao ve ark., 2023), muz kabuğundan hazırlanan biyokömürün bakır (Cu^+) ve kurşun (Pb^{2+}) adsorpsiyonu (Amin ve ark., 2018), mandalina kabuğunun sulu çözeltiden sekiz ağır metal iyonunun (Cd , Co , Cr , Cu , Mn , Ni , Pb ve Zn) uzaklaştırılması (Abdić ve ark., 2018) gibi çalışmalara rastlanmıştır. Boyaların tarımsal atıklar ile adsorpsiyonuna dair yapılan çalışmalar incelendiğinde işlenmemiş zeytin posası ile (Akar ve ark., 2009), fındık kabuğu-odun talaşı karışımı (Ferrero, 2007), yumurta kabuğu (Mobarak ve ark., 2023), ayçekirdeği kabuğu (Thinakaran ve ark., 2008), muz, portakal ve nar kabukları karışımı (Tolkou ve ark. 2024) gibi adsorbanların kullanıldığı görülmüştür.

Çalışmanın amacı, doğada tarımsal atık olarak bulunan bazı adsorbanların tekstil endüstrisinde kullanılan boyarmaddelerin gideriminde kullanılabilirliğini araştırmaktır. Bu amaçla yer fıstığı kabuğu, çam poleni, mandalina kabuğu, nar kabuğu, Antep fıstığı kabuğu, muz kabuğu, bal kabağı kabuğu kullanılarak asit kırmızısı 1 (AK1), asit mavisi 80 (AM80), parlak mavi G (BMG), safranin O (SO) ve victoria saf mavisi BO (VSMBO) boyalarının sulu ortamdan adsorpsiyon ile uzaklaştırma kapasiteleri incelenmiştir. Bu amaçla farklı pH'larda boyaların adsorpsiyon verileri karşılaştırılmıştır.

MATERYAL ve METOD

Kullanılan Kimyasallar

AK1, AM80, BMG, SO ve VSM boyar maddeler Sigma–Aldrich'den satın alınmıştır. pH ayarı için kullanılan sodyum hidroksit ve hidroklorik asit (%37, $d=1,19 \text{ g mL}^{-1}$) Riedel de Haen'den temin edilmiştir. Kullanılan boyar maddelerin kimyasal yapıları Çizelge 1.'de verilmiştir.

Cihazlar

Boyar maddelerin kantitatif tayinleri Thermo Genesys 10S marka UV/görünür bölge spektrofotometresi ile yapılmıştır. Çalışmalarda kullanılan saf su için Human Corp. Zeneer Power I marka cihaz kullanılmıştır. Çözelti hazırlama ve deneysel çalışma basamaklarında WiseClean marka ultrasonikatör, Memmert UN110 model etüv, Retsch MM 400 model öğütücü ve Hettich Rotofix 32 A model santifürij kullanılmıştır.

Standart Çözeltilerin Hazırlanması

Çalışmalarda kullanılan AK1, AM80, BMG, SO ve VSMBO boyar maddelerinin 200 mg L^{-1} derişiminde stok çözeltileri saf suda çözülerek hazırlanmıştır. Adsorpsiyon çalışmalarında kullanılacak boya çözeltileri stok çözeltilerden seyreltilerek hazırlanmıştır. $0,1 \text{ M}$ ve 1 M 'lık derişimlerde HCl ve NaOH çözeltileri saf su ile çözülerek hazırlanmış ve pH ayarları bu çözeltilerle yapılmıştır. Çalışmalar boyunca kullanılan tüm çözeltiler günlük olarak hazırlanmış ve $+4 \text{ }^\circ\text{C}$ 'de buzdolabında saklanmıştır.

Adsorbanların Hazırlanması

Çalışmada adsorban olarak kullanılan yer fıstığı kabuğu, mandalina kabuğu, nar kabuğu, antep fıstığı kabuğu, muz kabuğu ve bal kabağı kabuğu yerel bir marketten satın alınmış ve çalışmaya başlamadan önce saf su ile birkaç kez yıkama yapıldıktan sonra $50 \text{ }^\circ\text{C}$ 'de etüvde kurutulmuştur. Kurutma işlemi sonrasında ise laboratuvar öğütücüsü ile öğütülmüş $150 \text{ }\mu\text{m}$ 'lık elekten elenmiştir. Çalışmada kullanılan çam polenleri ise Kastamonu bölgesinden toplanmıştır. Çam polenlerine ise herhangi bir işlem yapılmamıştır.

Adsorpsiyon Çalışmaları

Adsorpsiyon çalışmaları tüm boyar maddeler için kesikli (batch) sistemde gerçekleştirilmiştir. Çözelti pH'ı adsorpsiyon çalışmalarında önemli parametrelerden biridir. Bu nedenle boyar maddelerin adsorpsiyon çalışmalarında asidik ve bazik olmak üzere 2 farklı pH'da çalışılmıştır. Boyar maddelerin pH'ları yaklaşık pH 5,5-7 aralığında olduğundan, ikinci pH olarak asidik bir pH ($\text{pH}=2$) seçilmiştir. 25 mg L^{-1} derişiminde hazırlanan boyar maddelerden 25 mL hacminde alınarak, $0,1 \text{ g}$ adsorban ile 1 saat boyunca 250 RPM 'de manyetik karıştırıcıda karıştırılmıştır. Daha sonra 3000 RPM hızda 3 dakika boyunca santrifüjlenmiştir. Elde edilen süpernetant ile boyar maddelerin kantitatif tayini, boya çözeltilerinin max. dalga boyunda UV-görünür bölge spektrofotometresi ile yapılmıştır.

Adsorpsiyon verimini belirlemek için adsorpsiyon kapasiteleri, q_d (mg g^{-1}) aşağıdaki eşitlikteki gibi hesaplanmıştır.

$$q_d = (C_0 - C) \frac{V}{m} \quad (1)$$

Eşitlikte,

q_d :Adsorpsiyon kapasitesi (mg g^{-1}),
 C_0 :Boyar maddenin başlangıç konsantrasyonu (mg L^{-1}),
 C_s :Boyanın denge zamanındaki konsantrasyonu (mg L^{-1}),
 V :Boyar madde çözeltisinin hacmi,
 M :Adsorban miktarını (g)

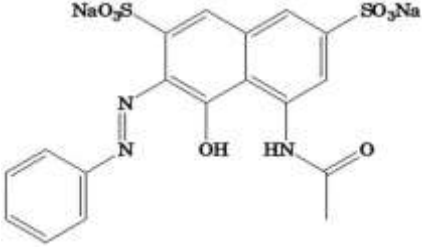
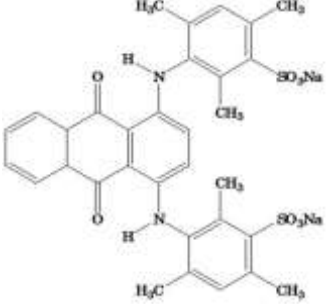
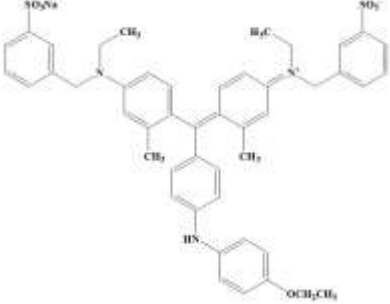
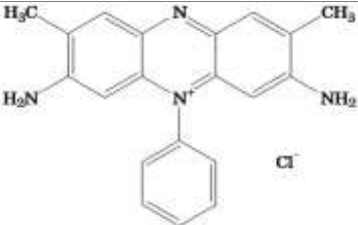
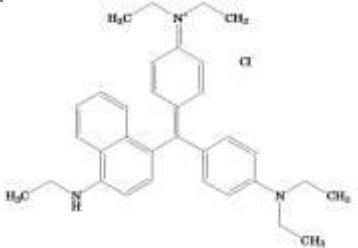
göstermektedir.

Boyar maddenin çözeltiden uzaklaştırılma yüzdesi aşağıdaki denklemle hesaplanmıştır:

$$\%Giderim = \frac{C_0 - C_s}{C_0} \quad (2)$$

Çizelge 1. Kullanılan boyar maddeler

Table 1. Dyes used

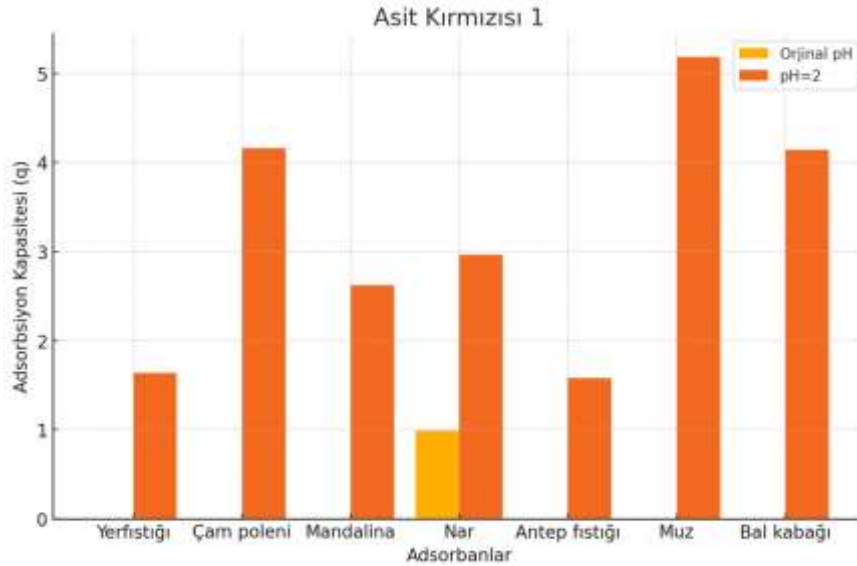
Boyar Maddeler	Kimyasal Yapıları	Molekül Ağırlığı (g mol^{-1})	Dalga Boyu (nm)
AK1		509.42	530
AM80		678.68	620
PMG		854.00	465
SO		350.85	520
VSMBO		514.14	600

Adsorpsiyon Verilerinin İstatistiksel Analizi

Bu çalışmada pH seviyelerinin adsorpsiyon kapasitesi üzerindeki etkisini test etmek için tek yönlü ANOVA analizi yapılmıştır. Analizlerde, pH bağımsız değişken (orijinal ve pH=2) olarak incelendi. AK1, AM80, PMG, SO ve VSMBO boyar maddelerinin farklı adsorpsiyon kapasitesinin pH değişimine bağlı olup olmadığı test edilmiştir. ANOVA testleri Python 3.10 ile yapılmıştır.

BULGULAR ve TARTIŞMA

Bu çalışmada, adsorpsiyon yöntemi ile AK1, AM80, PMG, SO ve VSMBO boyar maddelerinin sulu ortamdan uzaklaştırılması için yer fıstığı kabuğu, çam poleni, mandalina kabuğu, nar kabuğu, Antep fıstığı kabuğu, muz kabuğu, bal kabağı kabuğunun adsorban olarak kullanılabilirliği araştırılmıştır. Adsorpsiyon çalışmalarında çözelti pH'ı olarak, pH=2 ve boyar maddelerin orijinal pH'ları (AK1 pH=6.80; AM80 pH=5.80; PMG pH=5.91; SO pH=5.80; VSMBO pH=5.82) seçilmiştir. Şekil 1.'de AK1, Şekil 2.'de AM80, Şekil 3.'te PMG, Şekil 4.'de SO ve Şekil 5.'te VSMBO boyar maddelerinin hazırlanan adsorbanlar ile adsorpsiyon çalışmalarına ait adsorpsiyon kapasiteleri verileri grafik olarak verilmiştir. Çizelge 2.'de adsorpsiyon çalışmalarından elde edilen adsorpsiyon kapasitesi ve % giderim değerleri, Çizelge 3.'de AK1, AM80, PMG, SO ve VSMBO boyar maddeleri için orijinal pH ve pH=2'de adsorpsiyon kapasiteleri arasındaki farkların tek yönlü ANOVA analizi sonuçları verilmiştir.



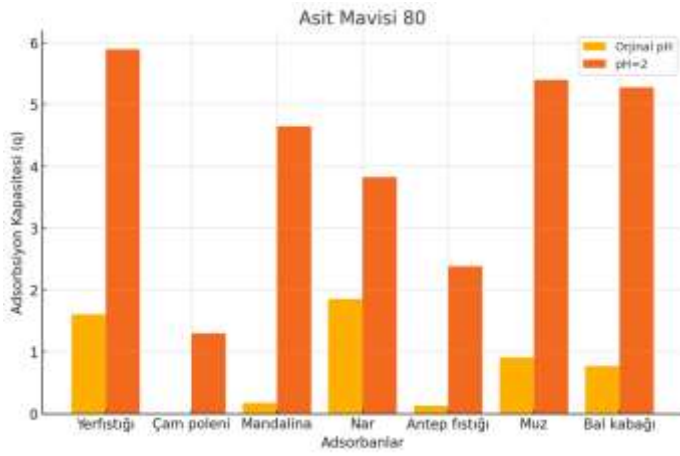
Şekil 1. AK1'in adsorpsiyonu için kapasite değerlerine ait grafik
Figure 1. Graph of capacity values for adsorption of AK1

Şekil 1. ve Çizelge 2'deki verilere bakıldığında, AK1 boyar maddesinin sulu ortamdan adsorpsiyon yöntemi ile uzaklaştırılması için yapılan deneylerde pH 2'de çam poleni ve bal kabağı kabuğundan % 68; muz kabuğunda ise %86 gibi yüksek bir verim elde edilmiştir. Yer fıstığı, Antep fıstığı, nar ve mandalina kabuklarında adsorpsiyon verimi düşüktür. Tüm adsorbanlar için ise orijinal pH'da AK1 boya çözeltisi ortamdan uzaklaştırılamamıştır. Çizelge 3'e bakıldığında AK1 boyar maddesinin ANOVA analiz sonucunda F-istatistiği 32.26 ve $p=0.00010$ olarak bulunmuştur. pH seviyelerinin AK1'in adsorpsiyon kapasitesi üzerinde anlamlı bir etkisi vardır.

AM80 boyar maddesinin adsorpsiyon çalışmasından elde edilen kapasite ve % giderim verilerine bakıldığında (Şekil 2 ve Çizelge 2); yer fıstığı, nar, muz ve bal kabağı kabukları ile boyanın orijinal pH'ında bir miktar adsorpsiyon gözlenmiştir. pH 2'de ise yer fıstığı kabuğunda %94 gibi çok yüksek giderim elde edilmiştir. Muz ve bal kabağı kabuğu ile yapılan çalışmada da %80'in üzerinde bir adsorpsiyon verimi elde edilmiştir. Çam poleni ve Antep fıstığı kabuğunda her iki pH'da da adsorpsiyon kapasitesi değerleri yüksek değildir. Çizelge 3'e bakıldığında AM80 boyar maddesinin ANOVA analiz sonucunda F-istatistiği 22.40 ve $p=0.00049$ olarak bulunmuştur. pH seviyelerinin AM80'nin adsorpsiyon kapasitesi üzerinde anlamlı bir etkisi vardır.

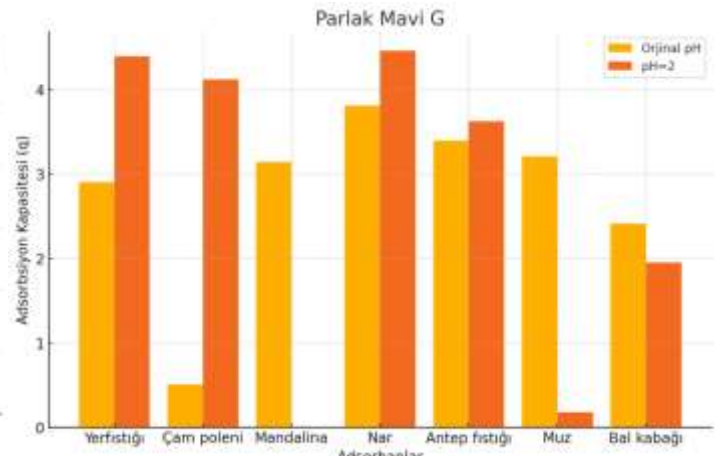
Şekil 3'te verilen PMG boyar maddesinin adsorpsiyon kapasitesi verileri incelendiğinde çam poleni ve muz kabuğu dışında çalışılan her iki pH'da da adsorpsiyonun gerçekleştiği söylenebilir. Sonuçlara genel olarak bakıldığında pH 2'deki adsorpsiyon kapasiteleri orijinal pH'lardaki kapasiteden daha yüksektir. PMG adsorpsiyonu için en yüksek uzaklaştırma verimi nar kabuğu (%96.49) olarak bulunmuştur. Yer fıstığı kabuğu, çam poleni ve Antep fıstığı kabuğunun adsorban olarak kullanıldığı çalışmalarda da oldukça yüksek verim elde edilmiştir. pH 2'de

mandalina ve muz kabuğunda ise kayda değer bir adsorpsiyon gözlemlenmemiştir. Çizelge 3'e bakıldığında PMG boyar maddesinin ANOVA analiz sonucunda F-istatistiği 0.01237 ve $p=0.9133$ olarak bulunmuştur. pH seviyelerinin PMG'nin adsorpsiyon kapasitesi üzerinde anlamlı bir etkisi yoktur. Bu durum, orijinal pH ve pH=2 seviyelerinde adsorpsiyon kapasitelerinin birbirine oldukça yakın olduğunu göstermektedir.



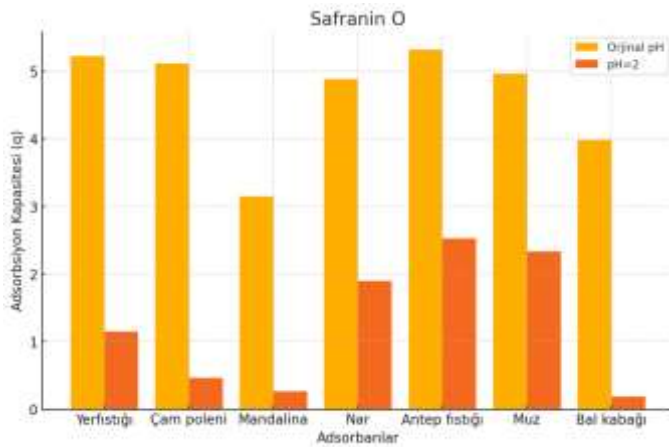
Şekil 2. AM80'in adsorpsiyonu için kapasite değerlerine ait grafik

Figure 2. Graph of capacity values for adsorption of AM80



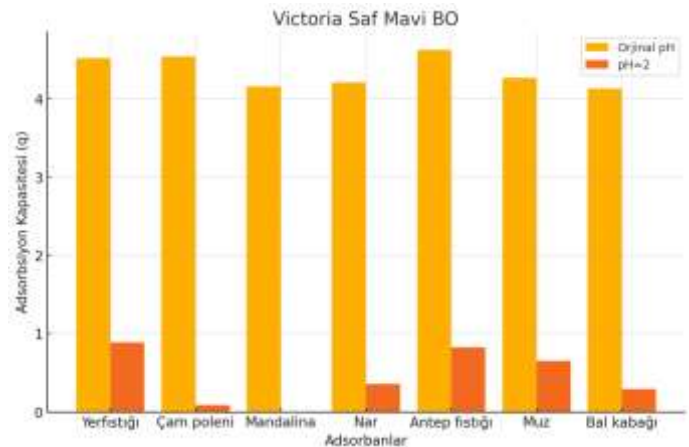
Şekil 3. PMG'nin adsorpsiyonu için kapasite değerlerine ait grafik

Figure 3. Graph of capacity values for adsorption of PMG



Şekil 4. SO'nun adsorpsiyonu için kapasite değerlerine ait grafik

Figure 4. Graph of capacity values for adsorption of SO



Şekil 5. VSMBO'nun adsorpsiyonu için kapasite değerlerine ait grafik

Figure 5. Graph of capacity values for adsorption of VSMBO

SO boyar maddesinin hazırlanan adsorbanlar ile yapılan adsorpsiyon çalışmasında, çözeltinin orijinal pH'ında adsorpsiyon kapasiteleri oldukça yüksektir. Yer fıstığı kabuğu, çam poleni ve Antep fıstığı kabuğunda %90'dan fazla boya giderimi gerçekleştiği görülmektedir (Çizelge 2). Nar ve muz kabuklarında ise yaklaşık %85'lik bir giderim gerçekleşmektedir. Şekil 4'teki verilere bakıldığında ise asidik pH'larda kapasite verileri oldukça düşüktür. Çizelge 3'e bakıldığında SO boyar maddesinin ANOVA analiz sonucunda F-istatistiği 49.00 ve $p=0.00058$ olarak bulunmuştur. pH seviyelerinin SO'nun adsorpsiyon kapasitesi üzerinde anlamlı bir etkisi vardır. Şekil 5'teki kapasite değerlerine bakıldığında pH 2'de çalışmada kullanılan tüm adsorbanlar için adsorpsiyonun gerçekleşmediği söylenebilir. VSMBO boyar maddesinin orijinal pH'ında ise tüm adsorbanlar için yüksek bir adsorpsiyon verimi elde edilmiştir. Yer fıstığı kabuğu, çam poleni ve Antep fıstığı kabuğunda %90'ın üzerinde giderim sağlanırken; mandalina, nar, muz ve bal kabağı kabuklarında %80'in üzerinde boya çözelti ortamından uzaklaştırılmıştır. Çizelge 3'e bakıldığında VSMBO boyar maddesinin ANOVA analiz sonucunda F-istatistiği 646.86 ve $p=8.299 \times 10^{-12}$ olarak bulunmuştur. pH seviyelerinin VSMBO'nun adsorpsiyon kapasitesi üzerinde anlamlı bir etkisi vardır.

SONUÇ ve ÖNERİLER

Tekstil endüstrisinde kullanılan boyar maddelerden AK1, AM80, PMG, SO ve VSMBO'nun atık sulardan adsorpsiyon yöntemi ile uzaklaştırılması için yer fıstığı kabuğu, çam poleni, mandalina kabuğu, nar kabuğu, Antep fıstığı kabuğu, muz kabuğu ve bal kabağı kabuğunun adsorban olarak uygunluğunun araştırılmıştır. Adsorpsiyon çalışmaları için 2 farklı pH'da çalışılmıştır. Boya çözeltilerinin orijinal pH'ları ve pH=2 ile çalışma pH'ı olarak seçilmiş, diğer adsorpsiyon parametreleri sabit tutularak çalışılmıştır.

Çizelge 2. Adsorpsiyon çalışmalarından elde edilen adsorpsiyon kapasitesi ve % giderim değerleri

Table 2. Adsorption capacity and % removal values obtained from adsorption studies

		Original pH		pH=2	
		q (mg g ⁻¹)	% Giderim	q (mg g ⁻¹)	% Giderim
AK1	Yerfıstığı	0	0	1.64	27.20
	Çam poleni	0	0	4.16	68.99
	Mandalina	0	0	2.63	43.54
	Nar	0.99	16.34	2.96	49.14
	Antep fıstığı	0	0	1.58	26.14
	Muz	0	0	5.19	86.07
	Bal kabağı	0	0	4.15	68.77
AM80	Yerfıstığı	1.61	25.72	5.90	94.83
	Çam poleni	0	0	1.30	20.87
	Mandalina	0.17	2.72	4.65	74.75
	Nar	1.86	29.70	3.82	61.48
	Antep fıstığı	0.13	2.02	2.38	38.30
	Muz	0.91	14.50	5.40	86.87
	Bal kabağı	0.77	12.24	5.28	84.86
PMG	Yerfıstığı	2.91	48.82	4.40	95.06
	Çam poleni	0.51	8.52	4.12	89.09
	Mandalina	3.14	52.79	0	0
	Nar	3.81	64.02	4.47	96.49
	Antep fıstığı	3.40	57.09	3.63	78.34
	Muz	3.21	53.93	0.18	3.78
	Bal kabağı	2.41	40.53	1.95	42.14
SO	Yerfıstığı	5.22	92.90	1.15	20.76
	Çam poleni	5.11	90.96	0.46	8.39
	Mandalina	3.15	56.02	0.26	4.72
	Nar	4.88	86.82	1.90	34.27
	Antep fıstığı	5.32	94.65	2.53	45.61
	Muz	4.96	88.30	2.33	42.05
	Bal kabağı	3.98	70.81	0.18	3.33
VSMBO	Yerfıstığı	4.52	90.94	0.89	58.19
	Çam poleni	4.54	91.31	0.09	5.58
	Mandalina	4.16	83.64	0	0
	Nar	4.21	84.65	0.35	23.27
	Antep fıstığı	4.62	93.08	0.82	54.07
	Muz	4.27	85.86	0.65	42.68
	Bal kabağı	4.13	83.12	0.29	19.08

Çizelge 3. AK1, AM80, PMG, SO ve VSMBO boyar maddeleri için orijinal pH ve pH=2'de adsorpsiyon kapasiteleri arasındaki farkların tek yönlü ANOVA analizi sonuçları

Table 3. Results of one-way ANOVA analysis of the differences between adsorption capacities at original pH and pH=2 for AK1, AM80, PMG, SO and VSMBO dyes

Boyars Madde	F-İstatistiği	p	Anlamlılık
AK1	32.26	0.00010	Anlamlı
AM80	22.40	0.00049	Anlamlı
PMG	0.01237	0.9133	Anlamlı değil
SO	49.00	0.00058	Anlamlı
VSMBO	646.86	8.299 x 10 ⁻¹²	Anlamlı

AK1 boyarmaddesinin en yüksek giderimi (%86.07) pH=2'de muz kabuğu ile gerçekleşmiştir. AM80 boyarmaddesinin adsorpsiyon çalışmalarında ise yine pH=2'de en yüksek giderim yer fıstığı (%94.83) daha sonra muz kabuğu (%86.87) ve bal kabağı (%84.86) ile olmuştur. PMG boyarmaddesinde ise pH=2'de en yüksek giderim nar kabuğu (%96.49) daha sonra yer fıstığı (%95.06) ve çam poleni (%89.09) ile olmuştur. AK1, AM80 ve PMG boyarmaddesinin asidik pH'ında adsorbanın yüzeyi pozitif yüklenir. Pozitif iyonlarla çevrili adsorban yüzeyi ile negatif iyonlarla yüklü boyarmadde arasında elektrostatik etkileşim meydana gelir. pH arttıkça adsorban çevresinde OH⁻ iyonlarının artması sebebiyle adsorpsiyon kapasitesi azalmaktadır. SO boyarmaddesinde en iyi adsorpsiyon orijinal pH'da sırasıyla Antep fıstığı kabuğu (%94.65), yer fıstığı kabuğu (%92.90), çam poleni (%90.96), muz kabuğu (88.30) ve nar kabuğu (86.82) ile gerçekleşmiştir. VSMBO boyarmaddesinin adsorpsiyon çalışmalarına bakıldığında orijinal pH'da sırasıyla Antep fıstığı kabuğu (%93.08), çam poleni (%91.31), yer fıstığı kabuğu (%90.94), muz kabuğu (%85.86), nar kabuğu (%84.65), mandalina kabuğu (%83.64), bal kabağı (%83.12) ile adsorpsiyonu gerçekleşmiştir. Asidik pH değerlerinde, SO ve VSMBO boyarmaddelerinin iyonları ile H₃O⁺ iyonları arasındaki rekabet nedeniyle adsorpsiyon kapasitesinin düşük olduğu bulunmuştur. Bazik pH'da ise adsorbanın yüzeyi negatif yüklerle çevrildiğinden dolayı boya çözeltisi ile elektrostatik çekim kuvveti artar ve dolayısıyla kapasitenin arttığı görülmüştür. AK1, AM80, PMG, SO ve VSMBO'nun adsorpsiyonuna pH'ın etkisi incelemek için yapılan tek yönlü ANOVA analiz sonuçlarına göre AK1, AM80, SO ve VSMBO için pH değişiminin adsorpsiyon kapasitesine etkisi oldukça önemlidir. PMG için pH değişiminin adsorpsiyon üzerinde önemli bir etkisi gözlenmemiştir.

Bu çalışmalar sonucunda pH=2'de AK1 boyarmaddesinin adsorpsiyonunda muz kabuğunun, AM80 boyarmaddesinin adsorpsiyonunda yer fıstığı, muz kabuğu ve bal kabağının, PMG boyarmaddesinin adsorpsiyonunda nar kabuğu, yer fıstığı ve çam poleninin kullanılabilirliği bulunmuştur. Orijinal pH'da SO ve VSMBO boyarmaddesinin uzaklaştırılmasında tüm adsorbanların kullanılabileceği gözlemlenmiştir. Sonuç olarak, bu boyaların adsorpsiyonu için çevrede atık olarak bulunan malzemelerin adsorban olarak kullanımı, kirlilik kontrolü ve çevre koruma açısından büyük önem taşımaktadır.

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

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KAYNAKLAR

- Abdić, Š., Memić, M., Šabanović, E., Sulejmanović, J., & Begić, S. (2018). Adsorptive Removal of Eight Heavy Metals from Aqueous Solution by Unmodified and Modified Agricultural Waste: Tangerine Peel. *International Journal of Environmental Science and Technology*, 15, 2511–2518. <https://doi.org/10.1007/s13762-018-1645-7>.
- Ajaz, M., Rehman, A., Khan, Z., Nisar, M.A., & Hussain, S. (2019). Degradation of Azo Dyes by *Alcaligenes Aquatilis* 3c and Its Potential Use in the Wastewater Treatment. *AMB Express*, 9, 64. <https://doi.org/10.1186/s13568-019-0788-3>.
- Akar, T., Tosun, I., Kaynak, Z., Ozkara, E., Yeni, O., Sahin, E.N., & Tunali Akar, S. (2009). An Attractive Agro-Industrial by-Product in Environmental Cleanup: Dye Biosorption Potential of Untreated Olive Pomace. *Journal of Hazardous Materials*, 166 (2-3), 1217-1225. <https://doi.org/10.1016/j.jhazmat.2008.12.029>.
- Alkoshab, M.Q., Al-Amrani, W.A., Drmoseh, Q.A., & Onaizi, S.A. (2024). Zeolitic Imidazolate Framework-8/Layered Triple Hydr(Oxide) Composite for Boosting the Adsorptive Removal of Acid Red 1 Dye from Wastewater. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 699, 134637. <https://doi.org/10.1016/j.colsurfa.2024.134637>.
- Amin, M.T., Alazba, A.A., & Shafiq, M. (2018). Removal of Copper and Lead Using Banana Biochar in Batch Adsorption Systems: Isotherms and Kinetic Studies. *Chemical Engineering*, 43, 5711–5722. <https://doi.org/10.1007/s13369-017-2934-z>.
- Bhatnagar, A., & Minochaa, A.K. (2010). Biosorption Optimization of Nickel Removal from Water using *Punica Granatum* Peel Waste. *Colloids and Surfaces B: Biointerfaces*, 76, 544–548. <https://doi.org/10.1016/j.colsurfb.2009.12.016>.
- Blanco, L., Martínez-Rico, O., Domínguez, Á., & González, B. (2023). Removal of Acid Blue 80 from Aqueous Solutions Using Chitosan-Based Beads Modified with Choline Chloride:Urea Deep Eutectic Solvent and FeO. *Water Resources and Industry*, 29, 100195. <https://doi.org/10.1016/j.wri.2022.100195>.

- Boukerche, I., Boutamine, Z., & Bekrou, S. (2023). Investigation of Photocatalytic Degradation of an Anionic Dye Brilliant Blue G 250 by Nanocatalyst of ZnO-EG Prepared Under Ultrasonic Waves. *Desalination and Water Treatment*, 284, 251–267. <https://doi.org/10.5004/dwt.2023.29236>.
- Cora, M., Durukan, İ., Uluçam Atay, G., & Kılıç, A.O. (2024). Atık Sulardan İzole Edilen Gram Negatif Bakterilerin Antibiyotik Direnç Profillerinin Belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 27(3), 520-527. <https://doi.org/10.18016/ksutarimdog.vi.1248203>.
- Çetinkaya, S., Kaya, S., Aksu, A., Çetintaş, H.İ., Jalbani, N.S., Erkan, S., & Marzouki, R. (2023). Equilibrium And DFT Modeling Studies for the Biosorption of Safranin O Dye from Water Samples Using Bacillus Subtilis Biosorbent. *Journal of Molecular Structure*, 1276, 134761. <https://doi.org/10.1016/j.molstruc.2022.134761>.
- Dubreil, E., Laurentie, M., Delmas, J.M., Danion, M., Morin, T., Hurtaud-Pessel, D., Viel, A., Sanders, P., & Verdon, E. (2021). Tissue Distribution, Metabolism, and Elimination of Victoria Pure Blue Bo in Rainbow Trout: Main Metabolite as an Appropriate Residue Marker. *Chemosphere*, 262, 127636. <https://doi.org/10.1016/j.chemosphere.2020.127636>.
- Ferrero, F. (2007). Dye removal by low cost adsorbents: Hazelnut shells in comparison with wood sawdust. *Journal of Hazardous Materials*, 142 (1-1), 144-152. <https://doi.org/10.1016/j.jhazmat.2006.07.072>.
- Kuo, W.S., & Chen, W.Y. (2012). Solar Photocatalytic Degradation of Azo Dye in Aqueous TiO₂ Suspension Assisted by Fresnel Lens. *International Journal of Photoenergy*, 1, 1-7. <https://doi.org/10.1155/2012/303586>.
- Liu, S., Xu, X., Kang, Y., Xiao, Y., & Liu, H. (2020). Degradation and Detoxification of Azo Dyes with Recombinant Ligninolytic Enzymes from Aspergillus Sp. with Secretory Overexpression in Pichia Pastoris. *Royal Society Open Science*, 7, 200688. <https://doi.org/10.1098/rsos.200688>.
- Methneni, N., Morales-González, J.A., Jaziri, A., Mansour, H.B., & Fernandez-Serrano, M. (2021). Persistent Organic and Inorganic Pollutants in the Effluents from the Textile Dyeing Industries: Ecotoxicology Appraisal Via a Battery of Biotests. *Environmental Research*, 196, 110956. <https://doi.org/10.1016/j.envres.2021.110956>.
- Mi, X., Shang, Z., Du, C., Li, G., Su, T., Chang, X., Li, R., Zheng, Z., & Tie, J. (2019). Adsorption of an Anionic Azo Dye Using Moringa Oleifera Seed Protein-Montmorillonite Composite. *Journal of Chemistry*, 1, 1-8. <https://doi.org/10.1155/2019/8464815>.
- Mobarak, M.B., Pinky, N.S., Chowdhury, F., Hossain, M.S., Mahmud, M., Quddus, M.S., Jahan, S.A., & Ahmed, S. (2023). Environmental Remediation by Hydroxyapatite: Solid State Synthesis Utilizing Waste Chicken Eggshell and Adsorption Experiment with Congo Red Dye. *Journal of Saudi Chemical Society*, 27 (5), 101690. <https://doi.org/10.1016/j.jscs.2023.101690>.
- Pessôa, N.T., Sales, D.C.S., Nascimento, G.E.D., Santos, J.H.L., Silva, M.N.S., Napoleão, D.C., Rodríguez-Díaz, J.M., & Duarte, M.M.M.B. (2024). Effective Adsorption of Cadmium and Nickel Ions in Mono and Bicomponent Systems Using Eco-Friendly Adsorbents Prepared from Peanut Shells. *Environmental Research*, 247, 118220. <https://doi.org/10.1016/j.envres.2024.118220>.
- Rigueto, C.V.T., Piccin, J.S., Dettmer, A., Rosseto, M., Dotto, G.L., Schmitz, A.P.O., Perondi, D., Freitas, T.S.M., Loss, R., & Geraldi, C.A.Q. (2020). Water Hyacinth (Eichhornia Crassipes) Roots, an Amazon Natural Waste, as an Alternative Biosorbent to Uptake a Reactive Textile Dye From Aqueous Solutions. *Ecological Engineering*, 150, 105817. <https://doi.org/10.1016/j.ecoleng.2020.105817>.
- Thasneema, K.K., Dipin, T., Thayyil, S.M., Sahu, P.K., Messali, M., Rosalin, T., Elyas, K.K., Saharuba, P.M., Anjitha, T., & Hadda, T.B. (2021). Removal of Toxic Heavy Metals, Phenolic Compounds and Textile Dyes From Industrial Waste Water Using Phosphonium Based Ionic Liquids. *Journal of Molecular Liquids*, 323, 114645. <https://doi.org/10.1016/j.molliq.2020.114645>.
- Thinakaran, N., Baskaralingam, P., Pulikesi, M., Panneerselvam, P., & Sivanesan, S. (2008). Removal of Acid Violet 17 from Aqueous Solutions by Adsorption Onto Activated Carbon Prepared from Sunflower Seed Hull. *Journal of Hazardous Materials*, 151 (2-3), 316-322. <https://doi.org/10.1016/j.jhazmat.2007.05.076>.
- Tolkou, A.K., Tsoutsas, E.K., Katsoyiannis, I.A., & Kyzas, G.Z. (2024). Simultaneous Removal of Anionic and Cationic Dyes on Quaternary Mixtures by Adsorption Onto Banana, Orange and Pomegranate Peels. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 685, 133176. <https://doi.org/10.1016/j.colsurfa.2024.133176>.
- Tzvetkov, G., Kaneva, N., & Spassov, T. (2017). Room-temperature Fabrication of Core-Shell Nano-Zn/Pollen Grain Biocomposite for Adsorptive Removal of Organic Dye from Water. *Applied Surface Science*, 400, 481-491. <https://doi.org/10.1016/j.apsusc.2016.12.225>.
- Yıldız, H. (2024). Tarım Atıklarından Aktif Karbon Üretimi ve Atıksudan Boya Giderimi: Karakterizasyon, Kinetik ve Denge Çalışmaları. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi* 27 (6), 1269-1281. <https://doi.org/10.18016/ksutarimdog.vi.1477888>.
- Zhao, Y., He, X., Qi, K., Zada, A., & Pan, J. (2023). Study on the Adsorption of Phosphate Over Biochar-Based Adsorbents from Peanut Shell and Orange Peel in Water. *Desalination and Water Treatment*, 308, 102-109. <https://doi.org/10.5004/dwt.2023.29810>.



The Effect of Different Stabilisers on the Quality Properties of Tahini Milk

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ABSTRACT

This study aimed to explore the chemical, physical, rheological, and sensory attributes of tahini milk produced with varying ratios of stabilizers, ranging from 0% (without a stabilizer) to other ratios including 0.2%, 0.08%, 0.06%, 0.04%, and 0.02% of κ -carrageenan; 0.2% of xanthan gum; 0.2% of carboxymethyl cellulose; 0.2% of salep; 0.2% of guar gum; and 0.2% of carob gum. Tahini milk is produced by incorporating tahini, sugar, and various stabilizers into semi-skimmed milk. Although the addition of different stabilizers did not affect its pH or acidity values, there were significant differences in the emulsion stability values, with the tahini milk exhibiting non-Newtonian flow behaviour. The addition of stabilisers was observed to have a significant effect on the colour and appearance, consistency, mouthfeel, and general acceptability scores of the tahini milk samples. Yet it did not affect the taste and odour or creaminess scores. The sample with 0.04% κ -carrageenan presented the highest scores in sensory evaluation. Based on the given results, tahini milk with the addition of 0.04% κ -carrageenan is recommended for production and consumption.

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ÖZET

Bu çalışmanın amacı, %0 (stabilizatörsüz) ile %0,2, %0,08, %0,06, %0,04 ve %0,02 κ -karragenan, %0,2 ksantan gam, %0,2 karboksimetil selüloz, %0,2 salep, %0,2 guar gam ve %0,2 keçiboynuzu gam gibi farklı oranlarda stabilizatör kullanılarak üretilen tahinli sütün kimyasal, fiziksel, reolojik ve duyuşal özelliklerini incelemektir. Tahinli süt, yarım yağlı süte tahin, şeker ve çeşitli stabilizatörlerin eklenmesiyle üretilmiştir. Farklı stabilizatörlerin eklenmesi pH veya asitlik değerlerini etkilemese de emülsiyon stabilite değerlerinde önemli farklılıklar olmuştur ve tahinli süt Newtonyen olmayan akış davranışı göstermiştir. Stabilizatör ilavesinin tahinli süt örneklerinin renk ve görünüm, kıvam, ağız hissi ve genel kabul edilebilirlik puanları üzerinde önemli bir etkiye sahip olduğu, ancak tat ve koku ve yağlılık puanlarını etkilemediği görülmüştür. 0.04 κ -karragenan içeren numune duyuşal değerlendirmede en yüksek puanları almıştır. Elde edilen sonuçlara göre, %0,04 κ -karragenan ilaveli tahinli süt, üretim ve tüketim için tavsiye edilmektedir.

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INTRODUCTION

Stabilizers increase the viscosity and water absorption capacity of foods. They also improve texture and sensory properties (Akin et al., 2019). Derived from the cell wall of red seaweed (*Rhodophyceae*), carrageenan is a polysaccharide characterized by hydrophilic linear sulphated galactans (Sedayu et al., 2019). It is divided into three primary categories based on repeating disaccharide units into three primary divisions: lambda (λ), kappa (κ), and iota (ι). Their interactions with various food components confer different properties such as water

retention, thickening, gelling, and stabilization in food products (Udo et al., 2023). Xanthan gum is a cream-colored, odorless, free-flowing powder. Its remarkable solubility in both cold and hot water leads to the development of viscosity, even at low concentrations of the substance (Gumus et al., 2010). Classified as a heteropolysaccharide, xanthan has a primary structure composed of repeating pentasaccharide units. The main chain is formed by β -D-glucose units linked at positions 1 and 4, mirroring the chemical structure of cellulose (Garcia-Ochoa et al., 2000). Because of its distinctive rheological properties, carboxymethyl cellulose (CMC) is widely used in various fields, including food formulations, product innovation and processing. This cellulose derivative is characterized by the addition of carboxymethyl groups to the hydroxyl groups of the glucose unit, which gives it a unique attribute (Mohod & Gogate, 2011).

Salep is a stabilizer obtained from the roots of the wild orchid (*Orchidaceae*). Salep drink is usually prepared by adding salep and sugar to milk in winter, and it is consumed as a hot drink (Bozdoğan & Yaşar, 2016). Salep is the main ingredient of Maraş ice cream. Its functional properties vary depending on the glucomannan content. As the glucomannan content increases, so does its stabilizing function. Glucomannan is also a water-soluble fibre that improves health (Yaşar & Bozdoğan, 2018). Locust bean gum, derived from *Ceratonia siliqua* L., an ancient Mediterranean plant, and known as carob gum, is now used in the production of many foods (Nasrallah et al., 2023). The locust bean contains galactose and mannose, which form galactomannan. It finds extensive industrial applications (Barak & Mudgil, 2014). Guar gum originates from the endosperm found within the seeds of *Cyamopsis tetragonolobus*, belonging to the legume family. When guar gum, a hydroxyl group-rich polymer, is added to water, it forms hydrogen bonds that impart significant viscosity and thickness to the solution (Thombare et al., 2016).

Sesame is a plant of the Pedaliaceae family. *Sesamum indicum* L. is cultivated to produce sesame seeds, which contain approximately 4.40% moisture, 21.00% protein, 54.26% fat, and 4.41% ash. They encompass a blend of unsaturated fatty acids, such as oleic and linoleic acids, alongside saturated fatty acids, including palmitic and stearic acids (Ünal & Yalçın, 2008). The numerous sesame varieties distributed worldwide have seed colors ranging from white to black (Kurt, 2018). Sesame is rich in fat, protein, minerals, vitamins, and dietary fiber. The sesame oil obtained through traditional extraction methods is particularly abundant in unsaturated fatty acids, fat-soluble vitamins, and amino acids. Sesame seeds contain approximately 21.9% protein and 61.7% fat, along with essential minerals such as iron (Fe) and calcium (Ca) (Wei et al., 2022). The Arabic term 'tahini', alternatively referred to as 'tahina' or 'tahana', is extensively consumed in nations like Syria, Lebanon, and Jordan, and its popularity has spread throughout the Middle East, Central Asia, and Africa. Its appeal has grown particularly in recent years, attributed to its role as a versatile condiment that enhances vegetarian culinary creations (Labban & Sumainah, 2021). The Turkish Food Codex describes it as follows: "Tahini: A product obtained by grinding sesame seeds (*Sesamum indicum* L.) in a mill after separating their shells, drying and oven-roasting according to the technique." It must contain at least 50% fat and 20% protein, at most 1.5% moisture, 3.2% ash and 2.4% acid (in oleic acid)" (TFK, 2015). Roasting enhances the flavor, color, and texture of sesame-based products, thereby improving their overall acceptability (Sert & Mercan, 2019). In Türkiye, tahini is mainly used to make halva. It is eaten with grape molasses for breakfast, especially in the winter months. It is also widely used in baking, as a topping for desserts like pumpkin pie, as a sauce, and in making hummus (Bayrakçı, 2018; Karakuş & Yaşar, 2025).

Oil-in-water (O/W) emulsions are prevalent in various food products, including ice cream, yogurt, and mayonnaise. The physical stability of these emulsions plays a crucial role in determining food quality and must be maintained for the desired shelf life. Several factors influence emulsion stability, including the properties of surface-active components, formulation variables, droplet size, and particle distribution (Kowalska & Żbikowska, 2016). Sesame tahini represents a colloidal suspension primarily consisting of hydrophilic solids dispersed in sesame oil. To utilize tahini effectively as an ingredient in various food products or to develop new creamy formulations, it is essential to enhance its colloidal stability. Understanding the rheological properties of tahini and their relationship with particle size is critical for optimizing its functional application (Çiftçi et al., 2008). One effective strategy to enhance emulsion stability is the incorporation of thickeners that increase viscosity. For instance, κ -carrageenan forms a robust network by interacting with casein micelles, thereby reducing the necessity for hydrophobic interactions and disulfide bonds among proteins. This interaction effectively inhibits droplet aggregation, thereby improving the stability of particulate emulsions (Xu et al., 2024).

Milk is recognized as one of the most versatile and valuable foods and is an essential part of the human diet. Approximately 80% of the world's population consumes dairy products, which provide essential macro- and micronutrients such as high-quality proteins, fats, calcium, potassium, phosphorus, vitamin D, riboflavin, and vitamin B12 (Linehan et al., 2024). This study aimed to develop a new functional food product by combining tahini and milk. Stabilizers such as κ -carrageenan, xanthan gum, carboxymethyl cellulose (CMC), carob gum, salep and guar gum were selected to increase the emulsion stability of the product. Although stabilizers are essential for product stability, there is no research on their use in the production of tahini milk. The aim of this study was to

investigate the effects of κ -carrageenan, xanthan gum, CMC, carob gum, salep, and guar gum on the physicochemical, rheological, and sensory properties of tahini milk.

MATERIAL and METHOD

Material

Tahini (Aktahin, Osmaniye, Türkiye), sugar (Torku, Konya, Türkiye), homogenized semi-skimmed UHT milk (Dost Milk, Istanbul, Türkiye) and stabilizers (κ -carrageenan, xanthan gum, carob gum, CMC, and guar gum) (Tate & Lyle, Türkiye) were purchased from the market, and salep was supplied from Kahramanmaraş, Türkiye.

Metod

Tahini Milk Production

Preliminary trials showed that the use of κ -carrageenan, salep, xanthan, carob gum, CMC, and guar gum at a concentration of 0.2% was effective in tahini milk. However, tahini milk produced with 0.2% κ -carrageenan showed a very firm and non-flowing structure. Based on the results of these trials, κ -carrageenan concentrations of 0.02%, 0.04%, 0.06%, and 0.08% were found to be more suitable, and production was carried out at these concentrations. The materials used in the production of tahini milk are presented in Table 1. A portion of 1000 g homogenized semi-skimmed UHT milk was used as the base into which 120 g of tahini and 120 g of sugar were added, each making up 9.68% of the mixture. Different stabilizers, labelled A to K, were added separately at different concentrations: A (0%, no stabilizer added), B (0.02% κ -carrageenan), C (0.04% κ -carrageenan), D (0.06% κ -carrageenan), E (0.08% κ -carrageenan), F (0.2% κ -carrageenan), G (0.2% xanthan gum), H (0.2% CMC), I (0.2% salep), J (0.2% locust bean gum), and K (0.2% guar gum). The homogenization process involved using an Ultra Turrax homogenizer (Janke & Kunkel KG, IKA, Werk, Germany) operating at 15000 rpm for 3 minutes. Subsequently, the homogenized tahini milk underwent exposure to a temperature of 90°C for 20 minutes through heat treatment, followed by a second round of homogenization at 15000 rpm for 3 minutes. The resultant product was gradually cooled to ambient temperature, then dispensed into 1-liter glass bottles, safely sealed, and preserved at 4°C until analysis.

Table 1. Materials used in tahini milk production

Çizelge 1. Tahinli süt üretiminde kullanılan malzemeler

Tahini Milks	Amount of Milk (%)	Amount of Sugar (%)	Amount of Tahini (%)	Amount of Stabilizer (%)
A	80.65%	9.68%	9.68%	0%
B	80.65%	9.68%	9.68%	0.02% κ -carrageenan
C	80.65%	9.68%	9.68%	0.04% κ -carrageenan
D	80.65%	9.68%	9.68%	0.06% κ -carrageenan
E	80.65%	9.68%	9.68%	0.08% κ -carrageenan
F	80.65%	9.68%	9.68%	0.2% κ -carrageenan
G	80.65%	9.68%	9.68%	0.2% xanthan gum
H	80.65%	9.68%	9.68%	0.2% CMC
I	80.65%	9.68%	9.68%	0.2% salep
J	80.65%	9.68%	9.68%	0.2% carob gum
K	80.65%	9.68%	9.68%	0.2% guar gum

Compositional analysis of tahini milk

The titratable acidity values of tahini milk were determined using the titrimetric method, as specified in AOAC (2005) guidelines, and the outcomes were represented as a proportion of lactic acid content. The pH values were measured using the Orion Star™ A 211 pH meter. Dry matter ratios were determined gravimetrically in accordance with AOAC (2005) standards, and the results were reported as a percentage of dry matter. The analysis of tahini fat content conformed to the Soxhlet method as outlined in the AOAC (2005) guidelines. Protein and nitrogen levels were assessed using the micro-Kjeldahl method in accordance with AOAC (2005) standards. Crude fiber content was calculated by following AOAC (2005) procedures, and ash content was determined using the wet combustion method specified in the AOAC (2005) guidelines. The results were calculated as a percentage.

Emulsion stability analysis of tahini milk

Forty grams of tahini milk were placed into 50 ml centrifuge tubes, and the samples were heat-processed in a water bath maintained at 80°C for 30 minutes. The centrifuge tubes containing tahini milk were then cooled under tap water for 15 minutes. The chilled tubes were placed into a centrifuge and underwent centrifugation at

4000 × g for a period of 10 minutes. The separated oil and water were taken with a Pasteur pipette and measured in mL. Emulsion stability (ES) was calculated with the following formula (Akbulut & Coklar, 2008).

$$1. ES (\%) = 100 - (AS + AY)$$

$$2. AS (\%) = \text{Amount of water separated (ml)} \times 2.5$$

$$3. AY (\%) = \text{Amount of oil separated (ml)} \times 2.5 \times d$$

$$4. d = \text{Specific gravity of oil}$$

Sensory analysis of tahini milk

The sensorial analysis was performed by 15 trained individuals (7 males, 8 females, who were aged 20-60 years). Samples were taken from the refrigerator, brought to room temperature, and presented to them. The panel members independently judged the sensory analysis using hedonic scoring (Meilgaard et al. 1999).

Rheological analysis of tahini milk

The rheological assessment of tahini milk samples involved using a Thermo Scientific Haake GmbH rheometer, operating under shear rates ranging from 0 to 100 1/s. This controlled stress rheometer was outfitted with a TCP/P Peltier temperature control unit, and rheological measurements were conducted employing a cone and plate sensor (diameter=3.5 cm, angle=2°), affixed to the rheometer. The experimental analyses were replicated in two sets, following the methodology outlined by Karaca et al. (2009).

Statistical analysis of tahini milk

The data from the tahini milk samples underwent analysis of variance, which was conducted using the SPSS 18.0 software package. Subsequently, a Duncan multiple comparison test was executed to identify significant group differences at confidence intervals of 95% and 99%.

RESULTS and DISCUSSION

Composition and emulsion stability of tahini milk

According to Table 2, the composition analysis of sample A showed that the dry matter, fat, protein, crude fibre, and ash values of tahini were determined as 24.66%, 6.58%, 5.65%, 0.38%, and 2.83%, respectively.

Table 2. Composition of tahini milk

Çizelge 2. Tahinli sütün bileşimi

Dry matter (%)	24.66±0.75
Fat (%)	6.58±0.45
Protein (%)	5.65±0.04
Crude fibre (%)	0.38±0.02
Ash (%)	2.83±0.04

The pH levels and titratable acidity of tahini milk beverages exhibited a narrow range, with values falling between 6.82 and 6.85 and between 0.11 and 0.13, respectively, in terms of lactic acid (Table 3). Notably, the incorporation of various stabilizers demonstrated no statistically significant impact on both pH and titratable acidity values in tahini milk, ($p > 0.0068$). Such a finding is consistent with the earlier investigation conducted by Yasar et al. (2009), who noted that the composition, titratable acidity, and pH of a drink containing salep, carob gum, guar gum, and their combinations remained unaffected. Similarly, Karaca & Guven (2016) reported that the utilization of diverse stabilizers, including salep, guar gum, CMC, alginate, and locust bean gum, exerted no discernible influence on the pH levels of ice cream.

The emulsion stability of tahini milk (A) without a stabilizer addition (0%) was 91.25%, while that of the other tahini milk was reported to be 100%. The addition of different stabilizers exerted a statistically significant impact on the emulsion stability of tahini milk ($p < 0.0082$). While fat separation occurred in sample A without any stabilizer addition, no fat separation occurred in the other samples (Table 3). Moreover, as given in Figure 1, the incorporation of κ-carrageenan prevented the formation of any sediment at the base of the tubes containing samples B, C, D, E, and F. Without a stabilizer, samples of xanthan gum, CMC, salep, and locust bean gum showed sediment accumulation at the bottom of the tubes. Hence, the tahini milk with the addition of κ-carrageenan is not expected to form sediment, while the other samples are expected to accumulate sediment during storage. In

conclusion, xanthan gum, CMC, salep, and locust bean gum are not appropriate for the manufacturing of tahini milk due to the likelihood of sediment formation.

Table 3. The pH, titratable acidity and emulsion stability of tahini milk

Çizelge 3. Tahinli sütün pH, titrasyon asitliği ve emülsiyon stabilitesi

Tahini Milk	pH	Titration Acidity (% lactic acid)	Emulsion Stability (%)
A	6.84±0.01	0.12±0.01	91.25±2.02 ^b
B	6.85±0.01	0.11±0.01	100±0.00 ^a
C	6.83±0.01	0.12±0.01	100±0.00 ^a
D	6.83±0.02	0.13±0.01	100±0.00 ^a
E	6.84±0.01	0.12±0.01	100±0.00 ^a
F	6.85±0.03	0.12±0.01	100±0.00 ^a
G	6.83±0.01	0.11±0.01	100±0.00 ^a
H	6.84±0.02	0.12±0.01	100±0.00 ^a
I	6.84±0.01	0.13±0.01	100±0.00 ^a
J	6.82±0.01	0.12±0.01	100±0.00 ^a
K	6.83±0.03	0.12±0.02	100±0.00 ^a

^{a,b}Statistically distinct letters within the same column indicate significant differences in means (p<0.05).

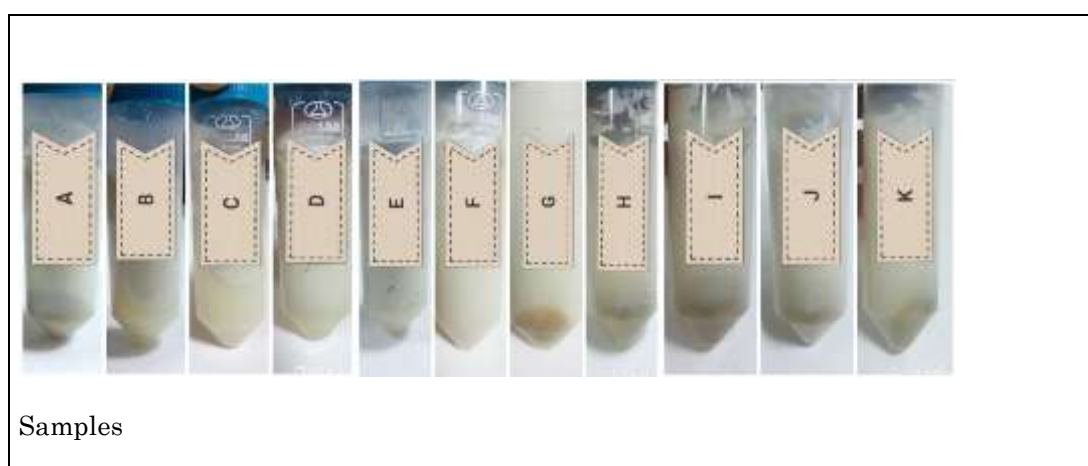


Figure 1. View of emulsion stability results

Şekil 1. Emülsiyon stabilite sonuçlarının görünümü

κ-Carrageenan interacts with casein micelles in milk to form a strong network, which reduces hydrophobic interactions and disulfide bond formation between proteins (Lu et al., 2025). This interaction enhances the stability of milk-tahini emulsions. In contrast, other stabilizers tested did not exhibit the same stabilizing effect.

The stability of the emulsion is primarily governed by the variety and amount of the stabilizer employed, along with the pH value of the medium (Shi et al., 2023). Stabilizers, comprising diverse polysaccharides, employ varying mechanisms to stabilize emulsions due to differences in their chemical composition. Certain polysaccharides possess amphiphilic properties, characterized by acetyl and methoxy groups in addition to hydrophilic hydroxyl groups. Through adsorption at the polysaccharide-oil-water interface, these compounds effectively reduce interfacial tension, resulting in the formation of stable droplets (Shao et al., 2020). Emulsions crafted with κ-carrageenan exhibited the swiftest digestion rate and the most extensive digestion extent among the formulations studied (Shi et al., 2023).

Alpaslan & Hayta (2002) investigated the mixtures of tahini molasses, adding tahini in concentrations of 0% to 6%. Results showed that the emulsion stability of the mixture ranged from 37.5% to 51.3%, with a clear trend of increased stability as a result of higher molasses additions. Similarly, in a study by Akbulut et al. (2012), tahini-honey mixtures were produced by incorporating different ratios of pine honey (3%, 6%, and 9%) into regular tahini and Bozkır tahini. The findings demonstrated a direct correlation between the emulsion stability of the tahini-honey mixture and the proportional increase in honey content.

Rheological properties of tahini milk

The rheological properties of tahini milk with and without a stabilizer (control) were investigated at shear rates ranging from 0-100 1/s. Figure 2 illustrates the shear stress values of the samples plotted against the shear rate and the resulting graphical representation.

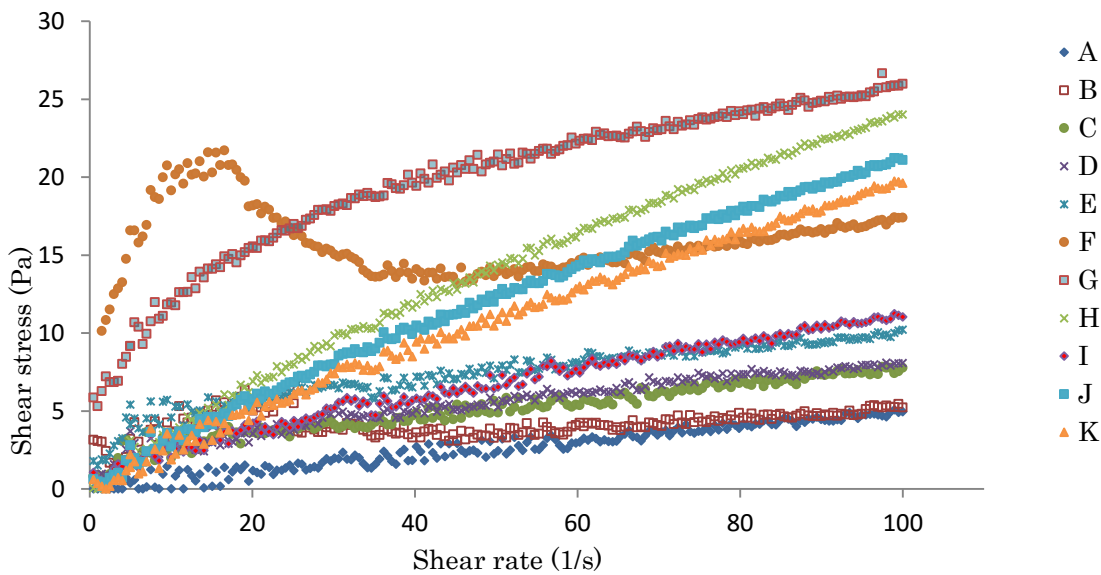


Figure 2. Variation of shear rate-shear stress values of tahini milk
Şekil 2. Tahinli sütün kayma hızı-kayma gerilimi değerlerinin değişimi

As depicted in the chart, the values of shear rate and shear stress for the tahini milk samples with stabilizer additives do not change linearly (Figure 2). In general, the tahini milk samples show non-Newtonian pseudoplastic flow behaviour; however, sample F demonstrates anomalous flow behaviour. Toker et al. (2013) and Şanlıdere Aloglu et al. (2018) investigated the rheological properties of ice cream mixtures. They revealed shear rate-shear stress values and determined that ice cream samples exhibited non-Newtonian pseudoplastic flow behaviour. Yasar et al. (2009) utilized salep, guar gum, and carob gum stabilizers in the production of the salep milk drink. Their study focused on examining the impact of these stabilizers on the rheological properties of salep. The findings revealed that the samples exhibited non-Newtonian flow behaviour, as the shear rate-shear stress values were not subject to a linear change.

The shear rate/viscosity values of tahini milk were plotted as shown in Figure 3. As illustrated in the provided graph, the viscosity values decrease as the shear rate increases, confirming that the tahini milk samples have non-Newtonian flow characteristics.

The rheological data of tahini milk samples were successfully described by the Ostwald-de.

5. Waele model ($\tau=k\dot{\gamma}^n$) except for the sample F with 0.2% κ -carrageenan added.

Shear stress (τ) is represented as Pa, Shear rate ($\dot{\gamma}$) as 1/s, and the coefficient of viscosity (k) as Pa sⁿ.

The Ostwald-de Waele model values of the tahini milks are presented in Table 4. The R² values of the Ostwald-de Waele model varied between 0.9763 and 0.9991, except for sample F with the addition of 0.2% κ -carrageenan. The sample F with 0.2% κ -carrageenan added to tahini milk did not fit the Ostwald-de Waele model, as given in Table 4. Hence, this sample was described by the polynomial equation given below.

$$6. Y = 8.341 + 1.218x + 0.1093x^2 - 0.01929x^3 + 0.001007x^4 - 2.704e-05x^5 + 4.2e-07x^6 - 3.813e-09x^7 + 1.882e-11x^8 - 3.907e-14x^9$$

$$R^2 = 0.9846, \quad x = \dot{\gamma} \text{ in (1/s)}, \quad y = \tau \text{ (Pa)}$$

As the concentrations of stabilizers added to the tahini milk samples increased, the coefficient of consistency (k) values increased, and the flow index (n) values in the sample (F) with 0.2% κ -carrageenan ranged from 0.33 to 0.90.

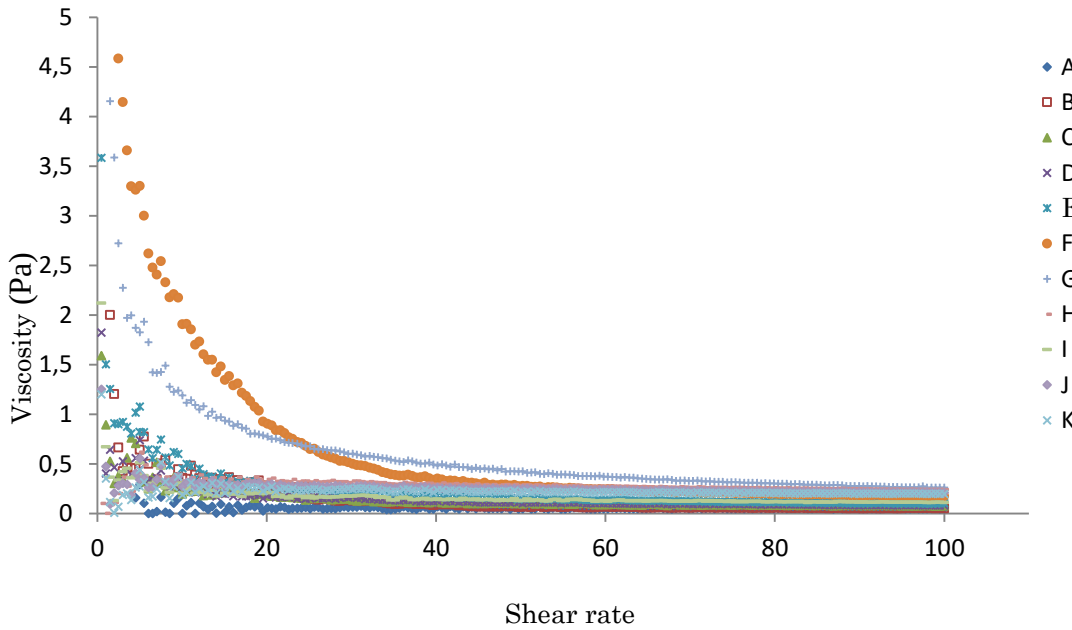


Figure 3. Variation in shear rate-viscosity values of tahini milk
Şekil 3. Tahinli sütün kayma hızı-viskozite değerlerindeki değişim

Hydrocolloids used in the categorization of ingredients in foods can be bifurcated into two groups: anionic (κ -carrageenan, CMC, pectin) and neutral (LBG, xanthan, and guar). Anionic hydrocolloids interact with positively charged groups on the surface of casein micelles, forming a stronger casein network and leading to earlier coagulation of caseins. In contrast, neutral hydrocolloids elevate the viscosity of the continuous phase. The inclusion of these substances enhances the thickness of food products (Yousefi & Jafari, 2019; Arab et al., 2023). Yaşar et al. (2009) investigated the rheological characteristics of salep milk beverages, revealing that the addition of stabilizers (such as corab gum, salep, and guar gum) increased the consistency coefficient. They also documented the flow behavior index ranging from 0.22 to 0.45 at 25 °C and from 0.29 to 0.43 at 50 °C. Moreover, Pon et al. (2015) produced ice cream by adding stevia at different ratios and investigated the effect of stevia on rheological properties. They observed that the consistency coefficient exhibited an ascending trend as the concentration of stevia sugar increased, spanning the range of 0.20 to 0.34. Additionally, the study revealed that the flow index varied between 0.42 and 0.58. Akbulut et al. (2012) initiated a study to examine the rheological attributes of tahini, which showed non-Newtonian pseudoplastic flow properties. The viscosity coefficients of the samples ranged from 9.34 to 37.53, while the flow indices ranged from 0.4226 to 0.7266.

Table 4. Ostwald-de Waele Model values for tahini milk
Çizelge 4. Tahinli süt için Ostwald-de Waele Modeli değerleri

Tahini Milk Samples	k	n	R ²
A	0.077	0.90	0.9763
B	0.092	0.80	0.9815
C	0.826	0.48	0.9769
D	0.835	0.49	0.9881
E	2.153	0.33	0.9796
F	15.94	-0.002	0.016
G	5.80	0.33	0.9963
H	0.635	0.79	0.9990
I	0.518	0.66	0.9933
J	0.519	0.81	0.9991
K	0.381	0.86	0.9982

Sensory properties of tahini milk

The sensory characteristics of dairy products have a significant impact on the consumer's food choices and acceptance attitudes (Ozdemir & Ozcan 2020). Statistical analysis revealed a significant impact of various

stabilizing agents on the appearance characteristics of tahini milk ($p<0.0064$). The panelists gave sample C the highest scores for its vibrant colour and appealing appearance, closely trailed by sample I (Table 5). Although the colour profile exhibited uniformity across all tahini milk variants, distinctive differences in appearance, notably in sample F, were observed. The inclusion of 0.2% κ -carrageenan resulted in a transformative effect, endowing the mixture with a solid yoghurt-like consistency, rendering it unsuitable for consumption in its liquid form. Altun (2024) documented that employing distinct stabilizing agents altered the color and appearance scores of ice cream.

Table 5. Sensory properties of tahini milk
Çizelge 5. Tahinli sütün duyuşsal özellikleri

Tahini Milk	Colour and Appearance (1=Worst 9= Best)	Consistency (1=Overflow 9= Excessive viscosity)	Taste and Odour (1= Worst 9= Best)	Creaminess (1= Leanest 9= Fattest)	Mouthfeel (1= Least mouth filling 9= Most mouth- filling)	General acceptability (1= Worst 9= Best)
A	6.15±1.42 ^{bc}	4.10±1.48 ^f	6.55±1.57	3.27±0.57	4.35±1.75 ^d	5.80±0.63 ^{bc}
B	6.46±1.37 ^{bc}	4.65±1.38 ^e	6.05±1.57	3.17±0.53	4.45±1.14 ^{cd}	5.89±0.76 ^{bc}
C	7.30±1.34 ^a	7.45±1.15 ^a	6.30±2.05	3.24±0.47	5.45±1.70 ^c	6.94±0.66 ^a
D	6.10±1.65 ^{bc}	5.05±1.70 ^d	6.15±1.38	3.18±0.87	5.55±1.35 ^{bc}	6.30±0.92 ^b
E	5.30±1.52 ^{cd}	4.10±0.78 ^f	6.30±1.75	3.14±0.79	6.20±1.76 ^{ab}	5.74±1.16 ^{bc}
F	4.75±1.74 ^d	3.10±1.48 ^g	6.75±1.51	3.13±0.64	6.65±1.72 ^a	4.59±1.03 ^d
G	5.15±1.92 ^{cd}	4.20±1.23 ^{ef}	6.25±1.68	3.10±0.89	6.30±1.78 ^{ab}	5.47±1.00 ^c
H	6.50±1.23 ^{bc}	6.90±1.07 ^{ab}	6.55±1.87	3.07±1.10	5.70±1.59 ^b	6.24±1.85 ^b
I	6.75±1.37 ^{ab}	6.10±1.71 ^c	7.15±1.03	3.00±0.74	5.40±1.16 ^{bc}	6.14±0.97 ^b
J	6.00±1.23 ^c	6.30±1.38 ^b	6.75±1.55	3.09±1.08	6.15±1.38 ^{ab}	6.18±0.99 ^b
K	6.45±1.57 ^{bc}	5.90±1.44 ^{cd}	6.55±1.76	3.49±1.01	5.65±1.69 ^{bc}	6.29±1.04 ^b

^{a-f} Statistically distinct letters within the same column indicate significant differences in means ($p<0.05$).

The consistency scores of the tahini milks were significantly affected by different stabilizers ($p<0.0058$), with the highest score attributed to sample C (Table 5). The panelists stated that tahini milk samples A and B were low in viscosity, while D was slightly too thick. Samples E and G were also described as very viscous, while sample F was too firm.

The taste and odour scores of tahini milks fell within the range from 6.05 to 7.15, and the application of various stabilizing agents exhibited no statistically significant impact on these attributes ($p>0.074$) (Table 5). The proximity of taste and odour in tahini milk is attributed to the robust aroma of tahini used in its production, coupled with the minimal stabilizer content. This aligns with findings by Azarikia & Abbasi (2010), who observed no significant statistical impact on the odour of doogh, an acidic milk drink, when different stabilizers were introduced. The levels of stabilizers used are limited in their effects on the taste and odour profile of camel milk yogurt. Even at low concentrations, the camel milk yogurt samples with added stabilizers retained the characteristic taste and aroma associated with camel milk (Ibrahim & Khalifa, 2015).

Creaminess is defined as the determinant effect of a thick, smooth, slippery, and soft liquid in the mouth, serving as an indicator of richness and high quality. The perception of creaminess is influenced by various structural factors, including rheology, oil droplet size, percentage of incorporated air, and the number and size of ice crystals (BahramParvar et al., 2013). The panellists rated the creaminess of tahini milk samples between 3.00 and 3.49 (Table 5). However, statistical analysis revealed that the effect of different stabilizers on the richness scores of the tahini milk samples was not significant ($p>0.062$). Consistent with the evaluations conducted by the panelists, the tahini milk samples did not convey an excessive sense of creaminess, and the samples were assessed as similar.

Sample F had the highest mouthfeel score with 6.65, followed by G with 6.30. The least favorable rating was assigned to sample A, which lacked the addition of any stabilizer (Table 5). The panelists found sample F to be firmer with a good mouthfeel. Furthermore, they pointed out that the sample with 0.2% xanthan gum had a good mouthfeel due to its higher consistency. They also noted that the sample with no added stabilizer (A) was viscous yet lacking mouthfeel.

The total acceptability score of tahini milk was highest in sample C, while sample F received the lowest score (Table 5). Statistical analysis indicated a significant impact of different stabilizers on the total acceptability score of tahini milk ($p<0.0078$). This aligns with findings from Koksoy & Kiliç (2004), who, in their analysis of ayran with various stabilizers, observed that different stabilizers significantly influenced its overall acceptability, emphasizing their key role in shaping the beverage's sensory perception. The sensory attributes of buffalo milk-based sandesh can be enhanced by the addition of stabilizing agents such as carrageenan, carboxymethyl cellulose (CMC), or sodium alginate prior to the manufacturing process. Of these stabilizers, carrageenan has been shown

to be the most suitable for optimizing the sensory quality of the final product (Sanyal et al., 2011). The values presented in Table 5 appear to resemble the findings of this study.

CONCLUSION

The introduction of various stabilizers exhibited a negligible impact on the pH levels and titratable acidity of tahini milk samples. However, the addition of stabilizers was found to prevent fat separation in the milk samples. κ -carrageenan did not cause sedimentation, whereas xanthan gum, CMC, salep, and locust bean gum did. The shear rate-shear stress values of the stabilized milk samples showed non-Newtonian flow properties, with the consistency coefficient increasing with higher stabilizer concentrations. The value of the flow index of tahini milk with added κ -carrageenan ranged from 0.33 to 0.90. In summary, incorporating xanthan gum, CMC, salep, and carob gum in tahini milk production is not advisable due to their propensity for sedimentation. The sensorial tests revealed that the addition of stabilizers affected the colour, appearance, consistency, mouthfeel, and overall approval of the tahini milks. Nevertheless, the scores for taste, odour, fat, and stickiness had no significant impact on the outcome. The sensory assessment outcomes reveal a preference for the tahini milk sample enriched with 0.04% κ -carrageenan, indicating its potential suitability for integration into manufacturing processes.

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

Statement of Conflict of Interest

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

REFERENCES

- Akbulut, M., & Coklar, H. (2008). Physicochemical and rheological properties of sesame pastes (Tahin) processed from hulled and unhulled roasted sesame seeds and their blends at various levels, *Journal of Food Process Engineering*, 31, 488–502. <https://doi.org/10.1111/j.1745-4530.2007.00162.x>
- Akbulut, M., Saricoban, C., & Ozcan, M. M. (2012). Determination of rheological behavior, emulsion stability, color, and sensory of sesame pastes (tahin) blended with pine honey. *Food and Bioprocess Technology*, 5, 1832–1839. DOI 10.1007/s11947-011-0668-6
- Alpaslan, M., & Hayta, M. (2002). Rheological and sensory properties of pekmez (grape molasses)/tahin (sesame paste) blends. *Journal of Food Engineering*, 54(1), 89–93. [https://doi.org/10.1016/S0260-8774\(01\)00197-2](https://doi.org/10.1016/S0260-8774(01)00197-2)
- Altun, İ. (2024). Peynir altı suyunda üretilen ekzopolisakkaritlerin stabilizatör olarak kullanımının dondurmanın bazı fiziksel, duyuşsal ve mikrobiyolojik özelliklerine etkileri. *Journal of the Institute of Science and Technology*, 14(1), 257–270. <https://doi.org/10.21597/jist.1347920>
- Akin, S.M., Goncu, B., & Akin, B. M. (2019). Designing an industrial protocol to develop a new fat-reduced ice cream formulation by replacing stabilizers with microbial transglutaminase enzyme. *Mljekarstvo* 69(3), 162–171. Doi: 10.15567/mljekarstvo.2019.0302
- AOAC. (2005). *Official Methods of the AOAC*. Official Methods of Analysis of AOAC International, 18th Ed., AOAC International, Gaithersburg, MD.
- Arab, M., Yousefi, M., Khanniri, E., Azari, M., Ghasemzadeh-Mohammadi, V., & Mollakhalili-Meybodi, N. (2023). A comprehensive review on yogurt syneresis: Effect of processing conditions and added additives. *Journal of Food Science and Technology*, 60(6), 1656–1665. <https://doi.org/10.1007/s13197-022-05403-6>
- Azarikia, F., & Abbasi, S. (2010). On the stabilization mechanism of Doogh (Iranian yoghurt drink) by gum tragacanth. *Food Hydrocolloids*, 24(4), 358–363 <https://doi.org/10.1016/j.foodhyd.2009.11.001>
- Barak, S., & Mudgil, D. (2014). Locust bean gum: Processing, properties and food applications—A review. *International Journal of Biological Macromolecules*, 66, 74–80. <https://doi.org/10.1016/j.ijbiomac.2014.02.017>
- BahramParvar, M., Tehrani, M. M., & Razavi, S. M. (2013). Effects of a novel stabilizer blend and presence of κ -carrageenan on some properties of vanilla ice cream during storage. *Food Bioscience*, 3, 10–18. <https://doi.org/10.1016/j.fbio.2013.05.001>
- Bayrakci, H. (2018). *Determination of the effect of tahini on the quality of ice cream*, Master's Thesis, Institute of Science and Technology, Osmaniye Korkut Ata University, Osmaniye, Türkiye.

- Bozdoğan, A., & Yaşar, K., (2016). Increasing the nutritional value of sahlep. *Journal of Food Physics*, 28, 33-39.
- Çiftçi, D., Kahyaoglu, T., Kapucu, S., & Kaya, S. (2008). Colloidal stability and rheological properties of sesame paste. *Journal of Food Engineering*, 87(3), 428-435. <https://doi.org/10.1016/j.jfoodeng.2007.12.026>
- Garia-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. *Biotechnology Advances*, 18(7), 549-579. [https://doi.org/10.1016/S0734-9750\(00\)00050-1](https://doi.org/10.1016/S0734-9750(00)00050-1)
- Gumus, T., Sukru, D.A., Mirik, M., Arici, M., & Aysan, Y. (2010) Xanthan gum production of *Xanthomonas* spp. isolated from different plants. *Food Science and Biotechnology*, 19(1), 201-206. <https://doi.org/10.1007/s10068-010-0027-9>
- Ibrahim, A. H., & Khalifa, S. A. (2015). The effects of various stabilizers on physiochemical properties of camel's milk yoghurt. *Journal of American Science*, 11(1), 15-24. (ISSN: 1545-1003). <http://www.jofamericanscience.org>.
- Karaca, O. B., & Guven, M. (2016). The effects of the combined use of salep and locust bean gum with some stabilizers on the properties of Kahramanmaraş type ice creams. *Journal of International Scientific Publications*, 4, 327-338. ISSN 1314-8591.
- Karaca, O. B., Güven, M., Yasar, K., Kaya, S., & Kahyaoglu, T. (2009). The functional, rheological and sensory characteristics of ice creams with various fat replacers. *International Journal of Dairy Technology*, 62(1), 93-99. <https://doi.org/10.1111/j.1471-0307.2008.00456.x>
- Karakuş, V., & Yaşar, K. (2025). The effect of peanut tahini utilization on the mineral content of halva. *Kahramanmaraş Sütçü İmam Üniversitesi Mühendislik Bilimleri Dergisi*, 28(1), 285-291. <https://doi.org/10.17780/ksujes.1559065>
- Koksoy, A., & Kilic, M. (2004). Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran. *Food Hydrocolloids*, 18(4), 593-600. <https://doi.org/10.1016/j.foodhyd.2003.10.002>
- Kowalska, M., & Żbikowska, A. (2016). Study of stability of sesame oil-in-water emulsions determined using an optical analyzer and measurement of particle size and distribution. *Journal of Dispersion Science and Technology*, 37(10), 1408-1414. <https://doi.org/10.1080/01932691.2015.1111143>
- Kurt, C. (2018). Tohum Kabağı Renginin Susam Tohumlarında Bulunan Demir İçeriği Düzeyine Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 21(5), 686-690. DOI:10.18016/ksudobil.403601
- Labban, L., & Sumainah, G. (2021). The Nutritive and Medicinal Properties of Tahini: A Review. *International Journal of Nutrition Sciences*, 6(4), 172-179. [10.30476/ijns.2021.90294.1123](https://doi.org/10.30476/ijns.2021.90294.1123)
- Linehan, K., & Patangia, D. V., Ross, R. P., & Stanton, C. (2024). Production, Composition and Nutritional Properties of Organic Milk: A Critical Review. *Foods*, 13(4), 550 <https://doi.org/10.3390/foods13040550>
- Lu, Z., Sheng, Z., Zhou, H., He, J., Zhang, X., Zhang, Y., & Luo, J. (2025). Improvement of casein/κ-carrageenan composite gel properties: Role of locust bean gum concentration. *Food Hydrocolloids*, 158, 110547. <https://doi.org/10.1016/j.foodhyd.2024.110547>
- Meilgaard, M. C., Civille, G. V., & Carr, B. T. (1999). *Sensory evaluation techniques* (3rd ed), Boca Raton, FL: CRC Press.
- Mohod, A. V., & Gogate, P. R. (2011). Ultrasonic degradation of polymers: Effect of operating parameters and intensification using additives for carboxymethyl cellulose (CMC) and polyvinyl alcohol (PVA). *Ultrasonics Sonochemistry*, 18(3), 727-734. <https://doi.org/10.1016/j.ultsonch.2010.11.002> [Get rights and content](#)
- Nasrallah, K., Khaled, S., El Khatib, S., & Krayem, M. (2023). Nutritional, biochemical and health properties of Locust beans and its applications in the food industry: a review. *Journal of Food Science and Technology*, 61(4), 621-630. <https://doi.org/10.1007/s13197-023-05765-5>
- Ozdemir, T., & Ozcan, T. (2020). Effect of steviol glycosides as sugar substitute on the probiotic fermentation in milk gels enriched with red beetroot (*Beta vulgaris* L.) bioactive compounds. *Lwt*, 134, 109851. <https://doi.org/10.1016/j.lwt.2020.109851>
- Pon, S. Y., Lee, W. J., & Chong, G. H. (2015). Textural and rheological properties of stevia ice cream. *International Food Research Journal*, 22(4):1544-1549.
- Sanyal, M. K., & Pal, S. C., Gangopadhyay, S. K., Dutta, S. K., Ganguli, D., Das, S., & Maiti, P. (2011). Influence of stabilizers on quality of sandesh from buffalo milk. *Journal of Food Science and Technology*, 48, 740-744. <https://doi.org/10.1007/s13197-010-0176-0>
- Sedayu, B. B., Cran, M. J., & Bigger, S. W. (2019). A review of property enhancement techniques for carrageenan-based films and coatings. *Carbohydrate Polymers*, 216, 287-302. <https://doi.org/10.1016/j.carbpol.2019.04.021>
- Sert, D., & Mercan, E. (2019). Hava Sirkülasyonlu Fırın, Mikrodalga ve Halojen Lamba ile Kavurmanın Susamda Salmonella İnaktivasyonu ve Bazı Fizikokimyasal Özellikleri Üzerine Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 22, 212-221. DOI: 10.18016/ksutarimdoga.vi.539670

- Shao, P., Feng, J., Sun, P., Xiang, N., Lu, B., & Qiu, D. (2020). Recent advances in improving stability of food emulsion by plant polysaccharides. *Food Research International*, 137, 109376. <https://doi.org/10.1016/j.foodres.2020.109376>
- Shi, F., Chang, Y., Shen, J., Chen, G., & Xue, C. (2023). A comparative investigation of anionic polysaccharides (sulfated fucan, ι-carrageenan, κ-carrageenan, and alginate) on the fabrication, stability, rheology, and digestion of multilayer emulsion. *Food Hydrocolloids*, 134, 108081. <https://doi.org/10.1016/j.foodhyd.2022.108081>
- Şanlıdere Aloglu, H., Özcan, Y., Karasu, S., Çetin, B., & Sağdıç, O. (2018). Influence of transglutaminase treatment on the physicochemical, rheological, and melting properties of ice cream prepared from goat milk. *Mljekarstvo*, 68 (2), 126-138. doi: 10.15567/mljekarstvo.2018.0206
- Thombare, N., Jha, U., Mishra, S., & Siddiqui, M. Z. (2016). Guar gum as a promising starting material for diverse applications: A review. *International Journal of Biological Macromolecules*, 88, 361-372. <https://doi.org/10.1016/j.ijbiomac.2016.04.001>
- TFK. (2015). *2015/27 Turkish Food Codex Communiqué on Tahini* (No: 29385).
- Toker, O. S., Dogan, M., Canyılmaz, E., Ersöz, N. B., & Kaya, Y. (2013). The effects of different gums and their interactions on the rheological properties of a dairy dessert: a mixture design approach. *Food and Bioprocess Technology*, 6, 896-908. <https://doi.org/10.1007/s11947-012-0787-8>
- Udo, T., Mummaleti, G., Mohan, A., Singh, R. K., & Kong, F. (2023). Current and emerging applications of carrageenan in the food industry. *Food Research International*, 113369. <https://doi.org/10.1016/j.foodres.2023.113369>
- Ünal, M. K., & Yalçın, H. (2008). Proximate composition of Turkish sesame seeds and characterization of their oils. *Grasas y Aceites* 59: 23-26. <https://doi.org/10.3989/gva.2008.v59.i1.485>
- Wei, P., Zhao, F., Wang, Z., Wang, Q., Chai, X., Hou, G., & Meng, Q. (2022). Sesame (*Sesamum indicum* L.): A comprehensive review of nutritional value, phytochemical composition, health benefits, development of food, and industrial applications. *Nutrients*, 14(19), 4079. <https://doi.org/10.3390/nu14194079>
- Xu, H., Gao, Z., Huang, M., Fan, Q., Cui, L., Xie, P., & Wang, X. (2024). Static stability of partially crystalline emulsions stabilized by milk proteins: Effects of κ-carrageenan, λ-carrageenan, ι-carrageenan, and their blends. *Food Hydrocolloids*, 147, 109387. <https://doi.org/10.1016/j.foodhyd.2023.109387>
- Yaşar, K., & Bozdoğan, A. (2018). Effect of the use of different whey proteins on some properties of sahlep beverage prepared from functional sahlep powder. *Turkish Journal of Agriculture-Food Science and Technology*, 6(5), 520-523. <https://doi.org/10.24925/turjaf.v6i5.520-523.1590>
- Yaşar, K., Kahyaoglu, T., & Şahan, N. (2009). Dynamic rheological characterization of salep glucomannan/galactomannan-based milk beverages. *Food Hydrocolloids*, 23(5), 1305-1311. <https://doi.org/10.1016/j.foodhyd.2008.11.005>
- Yousefi, M., & Jafari, S. M. (2019). Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties. *Trends in Food Science & Technology*, 88, 468-483. <https://doi.org/10.1016/j.tifs.2019.04.015>

Pamuk Tarımının Çevresel Etkilerinin Yeniden İncelenmesi

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ÖZET

Son yıllarda pamuk yetiştiriciliğinin çevreye verdiği zararlar üzerine pek çok çalışma yapılmıştır ancak pamuk tarımının olası olumlu etkileri açısından bazı boşluklar bulunmaktadır. Bu çalışmada, pamuk tarımının olası olumlu çevresel etkilerini araştırmak amacıyla, 2012-2022 yılları arasında Türkiye'de tarımsal yangınlar ve pamuk yetiştiriciliği arasındaki ilişki analiz edilmektedir. Çalışma kapsamında ulusal istatistikler, uzaktan algılama veri setleri ve sabit etkiler panel veri yöntemi kullanılmıştır. Türkiye'de pamuk tarımı yapılan 21 il için gerçekleştirilen analiz sonucunda, pamuk yetiştiriciliğinin yaygınlaşmasının anız yangınlarını azalttığı sonucuna varılmıştır. Bu sonuç, pamuk tarımının çevresel etkilerine ilişkin gelecekte yapılacak çalışmalarda bu olgunun dikkate alınması gerektiğini ortaya koymaktadır.

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Anahtar Kelimeler

Pamuk

Anız Yangınları

Panel Veri

Türkiye

Re-examining the Environmental Effects of Cotton Farming

ABSTRACT

In recent years, extensive research has focused on the environmental impacts of cotton cultivation, primarily emphasizing its negative effects. However, potential positive environmental contributions remain underexplored. This study investigates the relationship between agricultural fires and cotton cultivation in Turkey from 2012 to 2022, aiming to identify potential environmental benefits of cotton farming. Utilizing national statistics, remote sensing datasets, and a fixed-effects panel data approach, the analysis examines 21 provinces where cotton cultivation is prevalent. The findings reveal that the expansion of cotton cultivation is associated with a significant reduction in stubble burning. These results reveal that this phenomenon should be taken into consideration in future studies on the environmental impacts of cotton farming.

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GİRİŞ

21. yüzyıl bilimsel literatüründe çevre çalışmaları önemli bir yer kaplamaktadır. İnsan eliyle çevreye verilen zararlar ve telafi yolları bilim insanları tarafından gün geçtikçe daha fazla araştırma konusu olmaktadır. Bu bağlamda günümüz tarımsal üretim pratiklerinin çevreye etkilerine dair bilimsel çalışmaların sayısı gitgide artmaktadır (Arthur, 2022; Chataut et al., 2023; Cozim-Melges et al., 2024; Kayışoğlu & Türksoy, 2023; Sommer et al., 2023; Udvardi et al., 2015). Öyle ki Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) mevcut tarımsal üretim pratiklerinin çevreye maliyetinin yaklaşık 12,7 trilyon dolar olduğunu hesaplamıştır (FAO, 2023).

Güncel tarımsal üretim pratiklerinin çevreye zararları ürün bazında incelendiğinde bazı ürünler özellikle öne çıkmaktadır. Bu ürünlerin başında pamuk gelmektedir. Son yıllarda pamuk tarımının çevreye etkileri sıkça araştırılan bilimsel konulardan biri olmuştur (Huang et al., 2022; Mahdei et al., 2023; Network, 2018; Pranay Raja, 2022; Trent, 2020; Zinyemba et al., 2021). Birleşmiş Milletler Çevre Programı'na (UNEP) göre, pamuk ekimi dünyadaki ekilebilir arazinin %2,5'ini kaplamaktadır ancak yılda 200.000 ton pestisit ile Dünya genelinde yıllık toplam kullanımın %16'sını, 8 milyon ton gübre ile de dünya genelinde yıllık toplam kullanımın %4'ünü kapsamaktadır (Arthur, 2022). Ayrıca pamuk tarımının toprak ve su kirliliğine yol açtığına dair bilimsel

araştırmalarda yapılmıştır (Dai & Dong, 2014). Pamuk tarımının çevresel yükünü azaltma vaadiyle ortaya çıkan “Better Cotton” (İyi pamuk) gibi pratiklerin de özellikle pestisit kullanımında uzun vadede bir iyileşme yaratmadığına dair çalışmalar da mevcuttur (Kranthi & Stone, 2020).

İnsan uygarlığının gelişmesinde ve bugünkü dünyamızın oluşmasında önemli rollerden birine sahip olan bitki pamuktur (Beckert, 2015; Onat et al., 2017). İnsanlık için böylesine önemli ve vazgeçilmez olan bir bitkinin çevresel maliyetine dair yapılan bu hesaplamalar bazı olguları gözden kaçırmaktadır. Bu çalışmada pamuk tarımının çevreye etkilerine dair son dönemde yaygınlaşan araştırmaların gözden kaçırdığı “olumlu” bir etki incelenmeye çalışılmıştır.

Çin, Hindistan ve Türkiye gibi ülkelerin tarımsal pratiklerinde “anız yangınları” gibi çevreye zararlı bir olgunun önemli bir yer kapladığı bilinmektedir (Demirdogen, 2024). Türkiye’de bu olgu özellikle mısır üretim alanlarında gözlemlenmektedir (Öztornacı, 2024). Üreticilerin mısır hasadından sonra artakalan anızlarını yakmaları sonucunda çevreye ciddi zararlar verilmektedir. Türkiye’de üreticilerin anız yakmamaları için eğitimden, para cezalarına kadar pek çok yöntem uygulanmaktadır (Demirdogen et al., 2023). Ancak bu önemlerin yanısıra üreticilere mısır haricinde alternatif ürünler de sunulmalıdır. Mısır ile benzer iklim isteklerine sahip olan (Lv et al., 2023), benzer alanlarda yetiştirilen (Maitra et al., 2021) ve üretici nezdinde mısırın muadili olan (Demirdögen et al., 2016) bitki pamuktur. Mısır yetiştiriciliğinin anız yangınlarına neden olabildiğine dair literatürde pek çok çalışma olmasına karşın (Demirdogen, 2024; Demirdogen et al., 2023; Öztornacı, 2024), pamuk yetiştiriciliğinin böyle bir etkisi olduğuna dair bilimsel bir veri bildiğimiz kadarıyla bulunmamaktadır.

Araştırma kapsamında incelenilen dönemde (2012-2022), Türkiye’de 21 ilde düzenli pamuk ekimi yapılmaktadır. Bu illerin tamamında mısır üretimi de yapılmaktadır (TÜİK, 2024b). Bu iller aynı zamanda Türkiye’de tarımsal yangınların en çok gözlemlendiği illerdir (FIRMS, 2024). Bu bilgiler ışığında ortaya şöyle bir soru çıkmaktadır: mısır ekilen alanlarda pamuk tarımı yapıldığı takdirde anız yangınları azalır mı? Araştırmamızın temel sorusunu oluşturan bu problem, son yıllarda pamuk tarımının çevre verdiği zararlara dair yapılan çalışmalarda gözden kaçan, dikkate alınmayan bir olgudur.

Bu çalışmada pamuk tarımının anız yangınları üzerinde olumlu bir etkisi olup olmadığı araştırılmıştır. Böylece son yıllarda pamuk tarımının çevreye zararlı etkileri hususunda yapılan çalışmalarda, çevreye böyle bir “olumlu” etkisi olabileceği gerçeğinin gözden kaçırıldığı ortaya koyulmuştur.

MATERYAL ve METOD

Bu çalışmanın ana materyalini 2012-2022 yılları arasında, Türkiye’de pamuk yetiştiriciliği yapılan 21 şehre ait veriler oluşturmaktadır. Bu 21 ile dair, mısır ve pamuk hasadının yapıldığı Ağustos, Eylül ve Ekim aylarındaki yangın sayıları, sıcaklık değerleri, yağış miktarları, yıllık pamuk ve mısır ekim alanları ve nüfus sayılarına dair veriler derlenmiştir (DB, 2024; FIRMS, 2024; TÜİK, 2024a, 2024b). Yangın verileri NASA’nın gerçek zamanlı yangın noktası veri seti FIRMS’den (VIIRS-NPP 375m) alınmıştır. NASA bu veri setinde yangınları üç güven seviyesinde sınıflandırmaktadır: düşük, orta ve yüksek. Analizimiz, muhtemel güneş ışığı yansımalarını veya küçük sıcaklık anomalilerini hariç tutarak, yüksek ve orta güven seviyelerine sahip yangınlara odaklanmaktadır. Sıcaklık ve yağış verileri ise Dünya Bankası’nın İklim Değişikliği Bilgi Portalı’ndan derlenmiştir. Son olarak pamuk ve mısır ekim alanları ile nüfus verileri ise Türkiye İstatistik Kurumu’ndan elde edilmiştir. Bu değişkenlere dair veriler Çizelge 1’de sunulmuştur.

Çizelge 1. Tanımlayıcı İstatistikler Ağustos, Eylül ve Ekim Ayı (2012-2022)

Table 1. Descriptive Statistics August, September and October (2012-2022)

Değişkenler	Birimler	Ortalama	S.D.	N	Özellik
Yangın sayısı	Adet (3 ay)	610,15	772,26	231	Bağımlı
Yağış	cm (3 ay)	22,62	12,42	231	Bağımsız
Sıcaklık	°C (3 ay)	23,08	2,16	231	Bağımsız
Nüfus	Kişi	1.334.365,71	918.575,58	231	Bağımsız
Pamuk ekim alanı	da	221.498,20	443.825,57	231	Bağımsız
Mısır ekim alanı	da	199.173,07	254.270,05	231	Bağımsız

Kaynak: (DB, 2024; FIRMS, 2024; TÜİK, 2024a, 2024b)

Araştırma kapsamında il düzeyinde yıllık gözlemleri içeren bir panel veri seti kullanılmıştır. Bu sayede pamuk üretimi ile anız yangınları arasındaki karmaşık ilişki incelenmiştir. Bu bağlamda iki temel sabit etki türü varsayılmıştır: zaman sabit etkisi ve birim (il) sabit etkisi. Zamana bağlı sabit etkiler zaman içinde değişen ancak iller arasında sabit olan faktörleri hesaba katarken, il düzeyindeki sabit etkiler her bir ille özgü olan ancak zaman içinde sabit kalan faktörleri kontrol etmektedir. Bu sabit etkileri dâhil ederek, zamansal veya ille özgü etkilerden

kaynaklanan yanlılıkları azaltmak ve böylece analizimizin sağlamlığını arttırmak amaçlanmıştır. Modelin yapısal çerçevesi aşağıdaki gibi tanımlanmıştır:

$$y_{dt} = \delta_d + \gamma_t + \beta X_{dt} + \varepsilon_{dt}$$

Modelimizde, y_{dt} , t yılı boyunca d bölgesinde meydana gelen yangınların toplam sayısını ifade etmektedir. X_{dt} değişkeni, sıcaklık, yağış, insan nüfusu, pamuk ve mısır ekim alanları parametrelerini içermektedir. δ_d terimi, gizli değişkenlerin yangınlar üzerindeki etkisini azaltmada çok önemli rolü olan bölge sabit etkisini temsil etmektedir. Bu kontrol değişkeninin dâhil edilmesi, bağımsız değişkenlerin yangın olaylarına göre değerlendirilmesinde ortaya çıkan potansiyel önyargıların azaltılmasında etkili olmaktadır. γ_t , tüm bölgeleri yıllık olarak aynı şekilde etkileyen kapsayıcı eğilimleri ve zamansal olarak değişen şokları yakalayan zamana bağlı sabit etkiyi ifade etmektedir. Son olarak, ε_{dt} hata terimidir.

BULGULAR

Bu araştırma anız yangınları ile pamuk yetiştiriciliği arasındaki ilişkiyi incelemektedir. Türkiye’de her yıl yaklaşık 5 milyon dekar alanda, yaklaşık 2 milyon ton pamuk yetiştiriciliği yapılmaktadır. Tam olarak 2022 yılında 5.731.613 da alanda, 2.750.000 ton kütlü pamuk üretilmiştir (TÜİK, 2024b). Bu üretimin tamamına yakını (%99,9’u) Türkiye’de 21 şehirde gerçekleşmektedir. En çok ekim alanına ve üretim miktarına sahip iller başta Şanlıurfa olmak üzere sırasıyla Diyarbakır, Aydın, Hatay ve Adana’dır (Çizelge 2).

Çizelge 2. Türkiye’de Pamuk ve Mısır Yetiştiriciliği Yapılan Şehirler (2022)

Table 2. Cotton and Maize Growing Cities in Turkey (2022)

	Pamuk		Mısır	
	Ekilen Alan (da)	Üretim (ton)	Ekilen Alan (da)	Üretim (ton)
Türkiye	5.731.613	2.750.000	9.118.849	8.500.000
Adana	302.836	144.471	866.983	888.348
Adıyaman	81.888	38.264	28.682	23.553
Antalya	47.343	21.669	26.182	18.656
Aydın	574.129	283.643	44.397	39.414
Balıkesir	1.660	698	45.909	40.181
Batman	13.085	6.186	56.396	60.266
Denizli	123.595	61.932	182.576	169.130
Diyarbakır	829.151	408.389	179.431	185.139
Gaziantep	59.074	26.865	63.695	44.286
Hatay	459.510	239.036	172.338	172.245
Kahramanmaraş	70.858	34.985	279.609	252.376
Kilis	4.120	1.931	5.531	4.027
Manisa	174.175	91.933	186.207	191.089
Mardin	101.977	54.193	673.193	568.580
Mersin	44.694	18.083	102.976	100.622
Muğla	7.140	3.382	13.585	8.278
Osmaniye	4.600	2.295	393.164	404.440
Siirt	11.080	6.551	9.560	11.100
İzmir	332.647	172.839	111.997	96.007
Şanlıurfa	2.424.783	1.102.859	1.211.372	870.822
Şırnak	62.200	29.392	42.718	31.179
21 Şehrin payı	99,98	99,99	51,5	49,17

Kaynak: (TÜİK, 2024b)

Çizelge 2 incelendiğinde Türkiye’de en çok pamuk yetiştirilen 21 ilde aynı zamanda mısır yetiştiriciliği de yapıldığı görülmektedir. Bu 21 il Türkiye’deki mısır ekilen alanların %51’ine ve üretimin %49,17’sine sahiptir. Bu durum pamuk ve mısır bitkilerinin yetiştiriciliğinin benzer iklim özelliklerini gerektirmesi dolayısıyla üreticiler açısından bu ürünlerinin birbirinin alternatifi olmasından kaynaklanmaktadır.

Üreticileri nezdinde birbirine alternatif bu iki üründen biri olan mısır için, anız yangınlarının önemli kaynaklarından biri olduğuna dair bilimsel literatürde pek çok çalışma bulunmaktadır. Bu nedenle bu araştırmada Türkiye’de mısır ve pamuk hasadının yapıldığı Ağustos, Eylül ve Ekim aylarında, ele alınan dönem ve şehirlerdeki yangın sayıları incelenmiştir.

Çizelge 3. Türkiye’de Ağustos, Eylül ve Ekim Aylarında Çıkan Yangınlar (2012-2022)

Table 3. Fires in August, September and October in Turkey (2012-2022)

Yıllar	Türkiye Geneli	21 Şehrin Toplamı	21 Şehrin Oranı (%)
2012	16.968	10.605	62,50
2013	29.982	15.768	52,59
2014	15.727	10.904	69,33
2015	36.028	21.061	58,46
2016	31.763	18.410	57,96
2017	21.028	13.123	62,41
2018	18.586	9.946	53,51
2019	18.939	11.078	58,49
2020	22.233	13.826	62,19
2021	16.185	11.327	69,98
2022	8.855	4.896	55,29
Ortalama	21.481	12.813	59,64

Kaynak: (FIRMS, 2024)

Çizelge 3 incelendiğinde Türkiye’de, ele alınan yıllar içerisinde pamuk ve mısır hasadının yapıldığı aylar olan Ağustos, Eylül ve Ekim aylarında her yıl ortalama yaklaşık 21 bin yangının çıktığı anlaşılmaktadır. Bu aylarda çıkan yangınların ortalama yaklaşık 13 bini (%60), incelediğimiz 21 ilde gözlemlenmektedir.

Bu veriler ışığında araştırma kapsamında anız yangınları ile mısır ve pamuk yetiştiriciliği arasındaki ilişki incelenmiştir. Bu ilişkiyi ortaya koymak için kullanılan regresyon modelinin sonuçları Çizelge 4’te verilmiştir. Regresyon analizinde yıl kuklaları ile sabit etkiler modeli kullanılmıştır. Model kapsamında toplam 5 değişken dikkate alınmıştır.

Çizelge 4. Regresyon Analizi Sonuçları

Table 4. Regression Analysis Results

Değişkenler	Regresyon katsayıları	Std. Hata
Yağış (cm)	-5.0884*	(2.8152)
Sıcaklık (°C)	215.5513**	(96.2271)
Nüfus (kişi)	0.0004	(0.0006)
Pamuk (da)	-0.0014**	(0.0012)
Mısır (da)	0.0011**	(0.0005)
Sabit	-4519.90	(2501.05)
Gözlem Sayısı (N)	231	

Çizelge 4’te sonuçları verilen regresyon analizi yağış miktarı ile anız yangınları arasında anlamlı ve ters yönlü bir ilişki olduğunu ortaya koymuştur (-5,0884*). Bu durum, yağış miktarı arttıkça anız yangınlarının azalabileceğini göstermektedir. Sıcaklık ile anız yangınları arasında ise pozitif yönlü ve anlamlı bir ilişki vardır (215.5513**). Sıcaklık arttıkça yangın sayısı da artmaktadır. Analiz sonucunda Nüfus değişkeninin anız yangınları ile anlamlı bir ilişkisi olmadığı sonucuna varılmıştır (0.0004). Beklendiği gibi, bu analizde odaklandığımız ana değişken olan pamuk ekim alanları ile anız yangınları arasında negatif yönlü ve anlamlı bir ilişki hesaplanmıştır (-0.0014**). Bu durum üreticilerin mısır alternatif olarak pamuğa yöneldikleri takdirde anız yangınlarının azalabileceği ihtimalini doğurmaktadır. Ayrıca mısır ekim alanları ile anız yangınları arasında pozitif yönlü ve anlamlı bir ilişki gözlenmektedir (0.0011**). Bu durum literatürdeki pek çok diğer çalışma ile tutarlı, beklenen bir olgudur.

TARTIŞMA

Yapılan regresyon analizi sonucunda pamuk tarımının anız yangınlarının azalmasıyla ilişkili olduğu tespit edilmiştir. Pamuk yetiştiriciliğinin çiftçiler nezdinde mısır yetiştiriciliğine alternatif teşkil etmesi ve mısır yetiştiriciliğinin anız yangınlarını arttırmaya yönelik etkisinin bilinmesi, pamuk tarımının anız yangınlarını azaltabileceği sonucunu doğurmaktadır. Literatürde daha önce gerçekleştirilen çalışmalar mısır yetiştiriciliğinin anız yangınlarını arttırdığını ortaya koymuştur. Bu araştırma kapsamında gerçekleştirilen analiz sonucunda elde edilen bulgular bilimsel literatür ile uyumludur. Literatürde daha önce yapılan çalışmalarda mısır yetiştiriciliğinin anız yangınlarına etki katsayısı sırasıyla 34.369, 24.290 ve 67.691 olarak hesaplanmıştır (Demirdogen, 2024). Bir başka çalışmada ise buğday+mısır üretiminin anız yangınlarına etki katsayısı 6.200 ve pamuk üretiminin katsayısı ise -6.307 olduğu saptanmıştır (Demirdogen et al., 2023). Bu çalışmada hesaplanan katsayılar literatürdeki diğer çalışmalarda dikkate alındığında çalışmanın temel hipotezini destekler niteliktedir.

Türkiye’de çiftçilerin anız yakmasının temel nedeni ekonomiktir (Yakupoğlu et al., 2022). Üreticiler hasattan artakalanları yok etmek için masraf yapmak zorundadırlar. Bu durum üreticilerin maliyetlerini arttırıcı önemli bir kalemdir. Bu nedenle çiftçiler anızları yakmak sureti ile masraf kalemlerinden birini azaltmayı tercih etmektedirler. Ancak çiftçilere alternatif tarımsal ürünler sunulursa bu durumun önleneme ihtimali bulunmaktadır. Anız yangınlarını azaltmak için kullanılabilecek bir diğer yöntem olan “para cezaları” bu araştırmada dikkate alınmamıştır. Çünkü Türkiye’de anız yangınlarına yönelik cezaların hem miktar hem de sayı bakımından etkisine yönelik ciddi sorunlar bulunmaktadır.

Anız yangınlarının azaltılmasına yönelik diğer tarım politikası araçları (aynı ve nakdi desteklemeler, biyokütle santrallerinin yaygınlaştırılması vb.) bu araştırmada yapılan analiz kapsamında dikkate alınmamıştır. Bu tür politika araçları ve etkileri bu araştırmanın odak noktası değildir ve araştırmanın temel hipotezi ile doğrudan ilişkileri bulunmamaktadır. Bu çalışma, pamuk tarımının çevre üzerinde daha önce dikkate alınmamış bir olumlu etkiye sahip olabileme ihtimaline odaklanmıştır.

SONUÇ ve ÖNERİLER

Bu araştırmada 21. yüzyıl bilimsel literatüründe sayıları gitgide artan pamuk tarımının çevreye zararlarına dair yapılan çalışmaların gözden kaçırıldığı bir “olumlu” çevresel etki ihtimali tartışılmaktadır. Bu amaç doğrultusunda, Türkiye’de anız yangınları ile pamuk yetiştiriciliği ilişkisi analiz edilmiştir. Bu analiz 2012-2022 yılları arasında düzenli pamuk tarımı yapılan 21 ili kapsamaktadır. Analiz kapsamında anız yangınlarını etkilemesi muhtemel diğer değişkenler de dikkate alınmış ve sabit etkiler panel veri yöntemi kullanılmıştır. Yapılan hesaplamalar neticesinde Türkiye’de pamuk tarımının anız yangınlarını azaltıcı bir etkisi olabileceği sonucuna varılmıştır. Bu sonuçlar son yıllarda pamuk tarımının çevresel etkilerine dair yapılan çalışmalarda bu olası “pozitif” etkinin de dikkate alınması gerektiğini ortaya koymuştur.

Araştırma neticesinde elde edilen sonuçlar literatürdeki önceki çalışmalarla karşılaştırılmıştır. Araştırma sonuçları önceki çalışmaları destekler nitelikte olsa da katsayı değerleri diğer çalışmalara kıyasla küçük hesaplanmıştır. Bu farkın başlıca nedeni veri mevcudiyeti olabilir. Mevcut durumda anız yangınlarına ilişkin kapsamlı ve ayrıntılı bir veri seti bulunmamaktadır. Önümüzdeki yıllarda Türkiye için, ayrıntılı mikro düzey veri setlerinin geliştirilmesi ve yayınlanması, pamuk tarımının çevresel etkilerinin daha incelikli bir şekilde analiz edilmesini sağlayacaktır. Devlet kurumları, çevre örgütleri ve akademik kurumlarla işbirliği çabaları, bu tür veri setlerinin geliştirilmesini kolaylaştırabilir. Ayrıca, çevre bilimi, tarım ekonomisi ve coğrafi bilgi sistemleri vb. bilimsel alanları birleştiren disiplinler arası yaklaşımların benimsenmesi, pamuk tarımının çevresel etkileri üzerine gelecekte yapılacak çalışmaların derinliğini ve doğruluğunu artırabilir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

- Arthur, R. (2022). Sustainable Fashion: Communication Strategy 2021 - 2024. Retrieved from https://wedocs.unep.org/bitstream/handle/20.500.11822/41076/Sustainable_Fashion_Strategy.pdf?sequence=3&isAllowed=y
- Beckert, S. (2015). Empire of cotton: a global history: Penguin Books.
- Chataut, G., Bhatta, B., Joshi, D., Subedi, K., & Kafle, K. (2023). Greenhouse gases emission from agricultural soil: A review. *Journal of Agriculture and Food Research*, 11, 100533. doi:<https://doi.org/10.1016/j.jafr.2023.100533>
- Cozim-Melges, F., Ripoll-Bosch, R., Veen, G. F., Oggiano, P., Bianchi, F. J. J. A., van der Putten, W. H., & van Zanten, H. H. E. (2024). Farming practices to enhance biodiversity across biomes: a systematic review. *npj Biodiversity*, 3(1), 1. doi:10.1038/s44185-023-00034-2
- Dai, J., & Dong, H. (2014). Intensive cotton farming technologies in China: Achievements, challenges and countermeasures. *Field Crops Research*, 155, 99-110. <https://doi.org/https://doi.org/10.1016/j.fcr.2013.09.017>
- DB. (2024). Climate Change Knowledge Portal. Retrieved from <https://climateknowledgeportal.worldbank.org/>
- Demirdöğen, A. (2024). Stubble burning: What determines this fire? *Environmental Development*, 51, 101029. doi:<https://doi.org/10.1016/j.envdev.2024.101029>
- Demirdöğen, A., Guldal, H. T., & Sanli, H. (2023). Monoculture, crop rotation policy, and fire. *Ecological Economics*, 203, 107611. doi:10.1016/j.ecolecon.2022.107611

- Demirdöğen, A., Olhan, E., & Chavas, J.-P. (2016). Food vs. fiber: An analysis of agricultural support policy in Turkey. *Food Policy*, 61, 1-8. doi:http://dx.doi.org/10.1016/j.foodpol.2015.12.013
- FAO. (2023). The State of Food and Agriculture 2023. Rome, Italy: FAO.
- FIRMS. (2024). Fire information for resource management system (FIRMS). LANCE: NASA Near Real-Time Data and Imagery. Retrieved from <https://www.earthdata.nasa.gov/learn/find-data/near-real-time/firms>
- Huang, W. B., Wu, F. Q., Han, W. R., Li, Q. Q., Han, Y. C., Wang, G. P., . . . Wang, Z. B. (2022). Carbon footprint of cotton production in China: Composition, spatiotemporal changes and driving factors. *Science of the Total Environment*, 821. doi:10.1016/j.scitotenv.2022.153407
- Kayıoğlu, Ç., & Türksoy, S. (2023). Tarımda Sürdürülebilirlik ve gıda güvenliği. *Bursa Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, 37(1), 289-303.
- Kranthi, K. R., & Stone, G. D. (2020). Long-term impacts of Bt cotton in India. *Nature Plants*, 6(3), 188-196. doi:10.1038/s41477-020-0615-5
- Lv, Q., Chi, B., He, N., Zhang, D., Dai, J., Zhang, Y., & Dong, H. (2023). Cotton-Based Rotation, Intercropping, and Alternate Intercropping Increase Yields by Improving Root-Shoot Relations. *Agronomy*, 13(2), 413.
- Mahdei, K. N., Esfahani, S. M. J., Lebailly, P., Dogot, T., Van Passel, S., & Azadi, H. (2023). Environmental impact assessment and efficiency of cotton: the case of Northeast Iran. *Environment Development and Sustainability*, 25(9), 10301-10321. doi:10.1007/s10668-022-02490-5
- Maitra, S., Hossain, A., Brestic, M., Skalicky, M., Ondrisik, P., Gitari, H., . . . Sairam, M. (2021). Intercropping—A Low Input Agricultural Strategy for Food and Environmental Security. *Agronomy*, 11(2), 343.
- Network, P. A. (2018). Is cotton conquering its chemical addiction? Retrieved from https://issuu.com/pan-uk/docs/cottons_chemical_addiction_-_update?e=28041656/62705601
- Onat, B., Arioğlu, H., Güllüoğlu, L., Kurt, C., & Bakal, H. (2017). Dünya ve Türkiye’de Yağlı Tohum ve Ham Yağ Üretimine Bir Bakış. *Kahramanmaraş Sütçü İmam Üniversitesi Doğa Bilimleri Dergisi*, 20, 149-153. <https://doi.org/10.18016/ksudobil.349197>
- Öztornacı, B. (2024). The relationship between agricultural fires and livestock farming. *Heliyon*, 10(22), e40455. doi:<https://doi.org/10.1016/j.heliyon.2024.e40455>
- Pranay Raja, B. (2022). Biopesticides use on cotton and their harmful effects on human health & environment. *International Journal of Clinical Microbiology and Biochemical Technology*, 5(1), 005-008. doi:10.29328/journal.ijcmbt.1001025
- Sommer, J., Zhang, Y., & Shandra, J. (2023). Ecologically unequal exchange, repression, and forest loss: How China's demand for agricultural products impacts the natural environment. *Environmental Development*, 46, 100866. doi:<https://doi.org/10.1016/j.envdev.2023.100866>
- Trent, S. (2020). Clothes and climate: Is cotton best? Retrieved from <https://ejfoundation.org/news-media/clothes-and-climate-is-cotton-best>
- TÜİK. (2024a). Adrese Dayalı Nüfus Kayıt Sistemi. Retrieved from <https://data.tuik.gov.tr/Kategori/GetKategori?p=nufus-ve-demografi-109&dil=1>
- TÜİK. (2024b). Bitkisel Üretim İstatistikleri. Retrieved from <https://biruni.tuik.gov.tr/medas/?kn=92&locale=en>
- Udvardi, M., Brodie, E. L., Riley, W., Kaeppler, S., & Lynch, J. (2015). Impacts of Agricultural Nitrogen on the Environment and Strategies to Reduce these Impacts. *Procedia Environmental Sciences*, 29, 303. doi:<https://doi.org/10.1016/j.proenv.2015.07.275>
- Yakupoğlu, T., Dindaroğlu, T., Rodrigo-Comino, J., & Cerdà, A. (2022). Stubble burning and wildfires in Turkey considering the Sustainable Development Goals of the United Nations. *Eurasian Journal of Soil Science*, 11(1), 66-76. doi:10.18393/ejss.993611
- Zinyemba, C., Archer, E., & Rother, H.-A. (2021). Climate Change, Pesticides and Health: Considering the Risks and Opportunities of Adaptation for Zimbabwean Smallholder Cotton Growers. *International Journal of Environmental Research and Public Health*, 18(1), 121.

Hirfanlı Baraj Gölünde Gökkuşağı Alabalığı (*Oncorhynchus mykiss* Walbaum, 1792) Yetiştiriciliği Yapan İşletmelerin Bakteriyel Hastalıklarının Araştırılması

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ÖZET

Bu çalışmada Kırşehir İlindeki Hirfanlı Baraj Gölünde yetiştiriciliği yapılan Gökkuşağı alabalığında, sıklıkla rastlanan bakteriyel hastalıkların taranması amaçlanmıştır. Bu amaçla 2023 yılında enfeksiyona dayalı ölüm bildirisi ile 5 farklı işletmeye gidilmiş ve balıklar kafeslerde klinik (dış) muayeneden geçirilmiştir. Yavaş hareket, yeme tepkisizlik, koyu vücut rengi, ekzoftalmi ve vücut yüzeyinde görülen ülserler ile tespit edilmiştir. Hastalık semptomu gösteren toplam 70 adet Gökkuşağı alabalığından karaciğer, dalak ve böbreklerde histopatolojik ve mikrobiyolojik muayeneler yapılmış ve örnekler alınmıştır. Bu örneklerden elde edilen saf suşlara biyokimyasal tanımlama testler uygulanmıştır. Biyokimyasal testler ile identifiye edilen suşların sekans analizi yapılmış ve analiz sonucu NCBI'daki referans bakterilere göre yorumlanmıştır. Sonuç olarak; *Aeromonas sobria*, *Pseudomonas fluorescens* ve *Hafnia alvei* bakterileri genetik özelliklerine göre tanımlama yapılmıştır. Ayrıca elde edilen suşların Kirby Bauer disk difüzyon yöntemi ile antibiyotik duyarlılıkları belirlenmiştir.

Fisheries

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 29.11.2024

Kabul Tarihi : 21.02.2025

Anahtar Kelimeler

Akuakültür

Hirfanlı baraj gölü

Gökkuşağı alabalığı

Bakteriyel hastalık

16s rRNA gen dizilimi

Investigation of bacterial diseases in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) farms in Hirfanlı Dam Lake

ABSTRACT

The aim of this study was to screen rainbow trout cultured in Hirfanlı Dam Lake, Kırşehir Province, for common bacterial diseases. For this purpose, five different facilities were visited in 2023, following reports of fish mortality due to infection. Fish in the cages underwent clinical (external) examination. Observed symptoms included slow movement, unresponsiveness to feed, darkened body color, exophthalmos, and occasional ulcers on the body surface. Histopathological and microbiological samples were collected from the liver, spleen, and kidneys of a total of 70 rainbow trout exhibiting disease symptoms. Biochemical identification tests were performed on pure bacterial strains obtained from these samples. The strains identified through biochemical tests were further analyzed using sequence analysis, and the results were compared with reference bacteria in the NCBI database. As a result, *Aeromonas sobria*, *Pseudomonas fluorescens*, and *Hafnia alvei* were identified based on their genetic characteristics. Additionally, the antibiotic susceptibilities of the bacterial strains were determined using the Kirby-Bauer disk diffusion method.

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GİRİŞ

Son yirmi yılda, dünya çapında su ürünleri yetiştiriciliği üretimi önemli bir büyüme sağlamış ve 2000 yılında yaklaşık 40 milyon ton olan, çoğunlukla insan gıdası olarak kullanılan toplam 87,5 milyon ton üretime ulaşarak %120'lik kayda değer bir artış göstermiştir. Küresel olarak, su ürünleri yetiştiriciliği üretimi ağırlıklı olarak Asya ülkelerinde, özellikle de üretimin %88,7'sine katkıda bulunan Çin'de yoğunlaşmıştır. Çin'in ardından, Avrupa (%4,39), Amerika (%4,35), Afrika (%2,33) ve Okyanusya (%0,33) gelmektedir (Agriculture Organization of the United Nations. Fisheries Department, 2000; FAO, 2022). Bu üretim içerisinde Gökkuşluğu alabalığı *O. mykiss*, farklı koşullarda su ürünleri yetiştiriciliği için son derece uyarlanabilir ve uygun olmasını sağlayan birçok faktörün kombinasyonu sayesinde yetiştiricilik için avantajlı bir tür olarak yerini almıştır (Sarıeyyüpoğlu ve ark., 2017).

Norveç, Şili ve Türkiye, kültür balığı türlerinin çeşitliliği açısından ilk on ülke arasında yer almakta ve özellikle somon yetiştiriciliği yoluyla toplam küresel su ürünleri üretiminin yaklaşık %1 ila %2'sine katkıda bulunmaktadır (Medina ve ark., 2020; Naylor ve ark., 2021). Türkiye'de su ürünleri yetiştiriciliği yeraltı suları, doğal göller, nehirler ve baraj gölleri gibi iç su kaynaklarında ve denizlerde yapılmaktadır. Türkiye'de Gökkuşluğu alabalığı (*O. mykiss*) üretimi 1970'lerde başlamış ve şu anda yıllık 165.683 ton üretimle (134.174 ton iç sulardan ve 31.509 ton denizlerden), alabalık yetiştiriciliğinde Avrupa'nın önde gelen ülkelerinden biri haline gelmiştir (FEAP, 2022). Ancak üretimdeki bu hızlı artış özellikle iç sularda tam olarak tanımlanmamış çeşitli hastalıkları beraberinde getirmiştir. Su ürünleri yetiştiriciliğindeki bakteriyel hastalıkların birçok ülkede önemli ekonomik ve sosyal etkileri olduğu bilinmektedir (Defoirdt ve ark., 2011).

Son yıllarda Dünyada olduğu gibi Türkiye'de de hızla artan su ürünleri yetiştiriciliği yeni üretim sahalarının da açılmasını tetiklemiştir. Çalışmaya konu olan Hirfanlı Baraj Gölü su havzası bakımından Ankara, Aksaray ve Kırşehir İllerinin idari sınırlarında olup; su ürünleri yetiştiriciliği sadece Kırşehir İline bağlı olan 5. ve 6. bölgede gerçekleşmektedir. Bu bölgelerde ilgili bakanlık tarafından toplam 3.300 da'lık su alanı üretime açılmış ve ilk tesis 2017 yılında kurulmuştur (Anonim, 2024). Baraj gölünde 2024 yılı itibarıyla 17 işletme faaliyet göstermektedir. Baraj gölünde su ürünleri yetiştiriciliği yakın zamanda başladığı için bu havza ile ilgili su ürünleri yetiştiriciliği açısından çok fazla çalışma yapılmamış olup; balık hastalıkları konusunda tek çalışma 2021 yılında gölde yaşayan sazan balıklarının parazitolojik, bakteriyolojik ve virolojik yönden incelenmesi yönünde olmuştur ve çalışmada bakteriyolojik analiz Vitek-2 sistemi ile yapılmıştır (Çilli ve ark., 2023).

Tatlı suda kültürü yapılan balık türlerinin de *Flavobacterium*, *Aeromonas*, *Yersinia*, *Renibacterium*, *Mycobacterium* ile *Streptococcus* ve *Lactococcus* türleri tarafından tehdit edildiği bildirilmiştir (Pridgeon ve Klesius 2012; Sudheesh ve ark., 2012; Özcan ve Sarıeyyupoglu, 2014; Özcan ve Gök, 2019). Ülkemizde de kültürü yapılan gökkuşluğu alabalıklarında (*Oncorhynchus mykiss*) farklı coğrafik bölgelerde enfeksiyona neden olan bakteriyel patojenlerin izolasyonu ve teşhisi ile ilgili pek çok çalışma bulunmaktadır (Sarıeyyüpoğlu, 1984; Timur ve ark., 1996; Karataş Düğenci ve Candan, 1997; Kılıç ve ark., 2007; Diler ve ark., 2000a; Diler ve ark., 2000b; Aydın ve ark., 2001; Altun ve ark., 2010; Özcan ve Sarıeyyüpoğlu, 2013; Özcan ve ark., 2014; Özcan ve Barış 2019; Özcan, ve Kuzucu 2019; İspir ve ark., 2021; Özcan ve Donat 2022).

Bu çalışmanın amacı yakın zamanda su ürünleri yetiştiriciliğinin başladığı Hirfanlı Baraj Gölünde işletmelerden hastalık semptomlu balıkların örnekleme yapılarak, bakteriyel enfeksiyona neden olan patojenlerin ortaya çıkarılmasıdır. Bu amaçla işletmelerde hastalık semptomu gösteren örneklerin patolojik incelemesinin ardından bakteriyel izolasyonu ve bu bakterilerin 16s rRNA gen sekans analizi ile tanımlaması yapılmıştır. Ayrıca izole edilen bakteri türlerine karşı antibiogram test uygulanmıştır.

MATERYAL ve METOD

Hirfanlı Baraj Gölünde faaliyet gösteren alabalık yetiştiricilik tesislerinden 2023 yılında hastalık bildirimine müteakip örnekleme yapılmış olup; 17 işletmenin 5'inden örnekler tesadüfi olarak seçilmiştir. İstatistiksel açıdan toplam işletme sayısının %29'unu temsil etmektedir. Örnekler öncelikle yavru ve porsiyonluk olarak ayrılmış ve her iki grubun kendi arasında standart sapması hesaplanmıştır. Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Balık hastalıkları laboratuvarına teşhis için gönderilen balık örnekleri (50-250 gr) makroskobik olarak muayene edildikten sonra vücut yüzeyi, dış bakteri florasını dekontamine etmek için %70 etil alkol kullanılarak muamele edilmiştir. Örneklerin patolojik muayenesi için öncelikle; deri, karaciğer, kalp, böbrek ve bağırsakları kasete alınarak preparatları hazırlanmış ve mikroskop altında incelenmiştir. Bakteriyolojik inceleme için ise örneklerde nekropsinin ardından karaciğer, dalak ve böbreklerinden, Tryptic Soy Agar (TSA) ve Beyin Kalp İnfüzyon Agar (BHIA) üzerine ekim yapılmıştır. 24°C'de 24-72 saat inkübasyondan sonra, balıklardan izole edilen bakteriler, izolasyonun saflığını kontrol etmek amacıyla aynı koşullar altında alt kültüre tabi tutulmuştur. Saflaştırılan bakteri kolonileri fizyolojik açıdan incelenmiştir. Ardından elde edilen saf bakteri kültürleri; %20 gliserol ilaveli Tryptic Soy Broth (TSB) ile -22 °C'de stoklanmıştır. Bakteriyel identifikasyon için ise daha önce

stoklanan bakteri örnekleri uygun besiyerlerinde canlandırma işleminden sonra pasajlanarak saf kültür haline getirilmiştir. Bakteri örneklerinin moleküler yöntemlerle teşhisinde ticari DNA izolasyon kiti kullanılmıştır (Vivantis GF-BA-100). Kit protokolüne göre elde edilen DNA örneklerinin saflığının belirlenmesi için NanoDrop cihazında ölçümleri yapılarak, DNA örneklerinin 260/280 nm dalga boyundaki absorbands değerleri 1,8-2,0 aralığında ve konsantrasyonu 100 ng/μl en yakın olan örnekler çalışmaya dahil edilmiştir. İzole edilen DNA örneklerinin tanımlanması için evrensel bakteriyel primer olarak adlandırılan İnternal Transcribed Spacer (ITS) gen bölgelerinden

BAK2-F:5'AGTTTGATC(A/C)TGGCTCAG/3'

BAK11-F: 5'GGACTAC(C/T/A)AGGGTATCTAAT/3' primer çiftleri kullanılmıştır.

DNA örneklerinin uygun PCR koşullarında çoğaltılması ve DNA bantlarının DNA gen dizi analizi için çoğaltılmasında PCR karışım protokolü (2 μL 10x PCR Buffer, 0,4 μL MgCl₂, 0,4 μL Primer F, 0,4 Primer R, 1 μL DNA (Template), 0,4 μL dNTP, 0,3 μL Taq polimeraz, 15,1 μL dH₂O) uygulandı (Kuzucu, 2023). Karışım, protokolün hazırlanmasının ardından bakteri DNA'larının çoğaltılması için; 95°C 5 dakika, 35 tekrar ile [5°C 30 sn.- 48°C 45 sn. 72°C 1dk.], 72°C 10 dakika ve +4°C'de tamamlandı. Elde edilen DNA örneklerinin amplifikasyonunun kontrolü için PCR ürünleri agaroz jel elektroforezinde yürütüldü.

Çalışmada DNA gen dizi analizi için AB3130XL16 kapiller cihazı (Hitachi, Japonya) kullanıldı. Jel elektroforezinde uygun bant gösteren örnekler sekans işlemi için seçilerek Exso-SAP pürifikasyonu; 2,5 μl PCR ürünü, 1 μl Exosap ile karıştırılarak, 37°C 30 dakika, 80°C'de 15 dakika ve +4 °C'de sonlandırılacak şekilde reaksiyon karışımı ve PCR protokolü uygulandı. ExsoSAP pürifikasyonundan sonra Cycle Sequencing PCR reaksiyon karışımı; 3,5 μl PCR ürünü, 2 μl Big Dye, 2 μl Buffer (5x), 0,5 μl dH₂O ve 2 μl Primer (F ve R, 2 ng/ul) şeklinde hazırlandı ve PCR protokolü uygulandı. Sekans öncesi tüm örnekler Sephadex pürifikasyonu yapılarak AB3130xl Genetic Analyzer cihazına yüklendi. Sephadex pürifikasyonu için; 1 gr Sephadex 14 ml deiyonize ultra saf su içerisinde çözülerek 700 μl alıcı sütun içerisine aktarıldı. 4600 rpm 2.5 dk santrifüj edilerek sephadex karışımının sıvı kısmı uzaklaştırıldı. Elde edilen kolona Sekans PCR ürünleri eklenerek 4600 rpm 2.5 dk santrifüj edildi. Santrifüj sonrası altta kalan kısım sekans cihazına yüklendi.

Sekans datalarının değerlendirilmesi için ham veriler Bioedit 7.2.5 software ile işlenerek NCBI - Basic Local Alignment Search Tools (BLAST) programı kullanılarak referans veri tabanları ile karşılaştırıldı. Tüm örnekler çift yönlü çalışarak konsensüs oluşturulup blastlandı. İdentifikasyonu yapılarak stoklanan suşların antibiyotik duyarlılığının tespiti için Kirby Bauer disk difüzyon yöntemi BD ticari markalı antibiyotik diskleri ile Muller-Hilton agarda uygulandı (Bauer ve ark., 1966).

Örnekleme yapılan tesisin baraj gölünü temsili ve örnekleme sayısının anlamlı bir sonuç oluşturup oluşturmadığı ile ilgili verilerin istatistiksel değerlendirmeleri IBM SPSS Statistics v25.0 yazılımı ile gerçekleştirildi (Anonim, 2001).

BULGULAR ve TARTIŞMA

Klinik ve Histopatolojik Bulgular

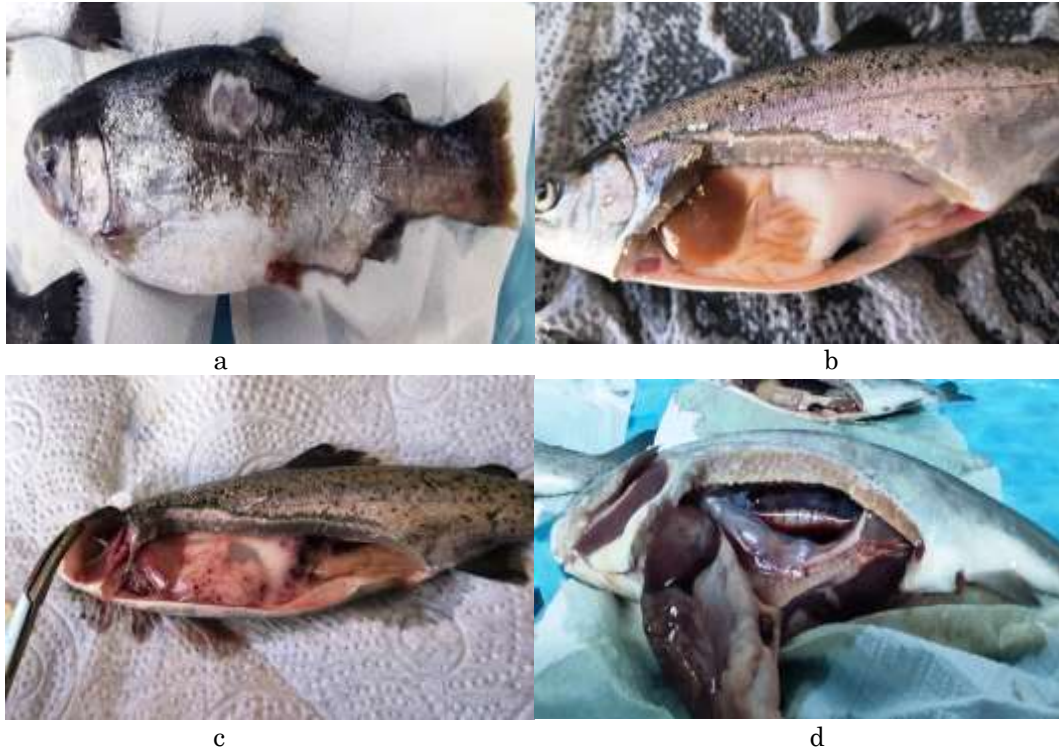
İşletmelerden farklı zamanlarda gelen örnekler tartılarak ağırlıkları kayıt altına alınmıştır. Yavru balık için toplam 27 numunenin ortalama ağırlığı $\bar{x} = 51,35$, $S_x = 0,017$ gr olarak bulunmuştur. Porsiyonluk olarak ayrılan grupta ise 43 numunenin ortalama ağırlığı $\bar{x} = 241,40$ $S_x = 0,038$ gr olarak bulunmuştur. İstatistiki olarak anlamlılık derecesi, $p < 0,05$ olarak belirlendi ve bulunan varyans (standart sapma) değerleri bundan düşük olduğundan çalışmaya alınan balık grupları arasında boy açısından anlamlı bir farklılık bulunamamıştır. İşletmeden alınan anamnez bilgilerinde yavru ve porsiyonluk balık arasında ölüm oranları arasında fark olmadığı ve %4 ila 20'ye varan ölümlerin gerçekleştiği beyan edildi. Çiftliklerdeki enfekte Gökkuşluğu alabalıklarında genel olarak yavaş hareket, yeme tepkisizlik, koyu vücut rengi, ekzoftalmi ve vücut yüzeyinde ülserler (Şekil 1a) görüldü. Diseksiyondan sonra, saha araştırmasında enfekte olmuş balıklarda soluk bir karaciğer (Şekil 1b), boş mide ve asidik mide sıvısı (Şekil 1c), böbreklerde kanama ve granülomlar (Şekil 1d) görüldü.

Patolojik incelemede mikroskopik bulgular ise; solungaç lamellerinde hiperemi, pankreas asiner hücrelerde dejenerasyon ve dağılmalar, kalpte yangı hücreleri ve kalınlaşmalar, karaciğerde ise yağlanma şeklinde gözlemlendi (Şekil 2a-d).

Bakteriyolojik Bulgular

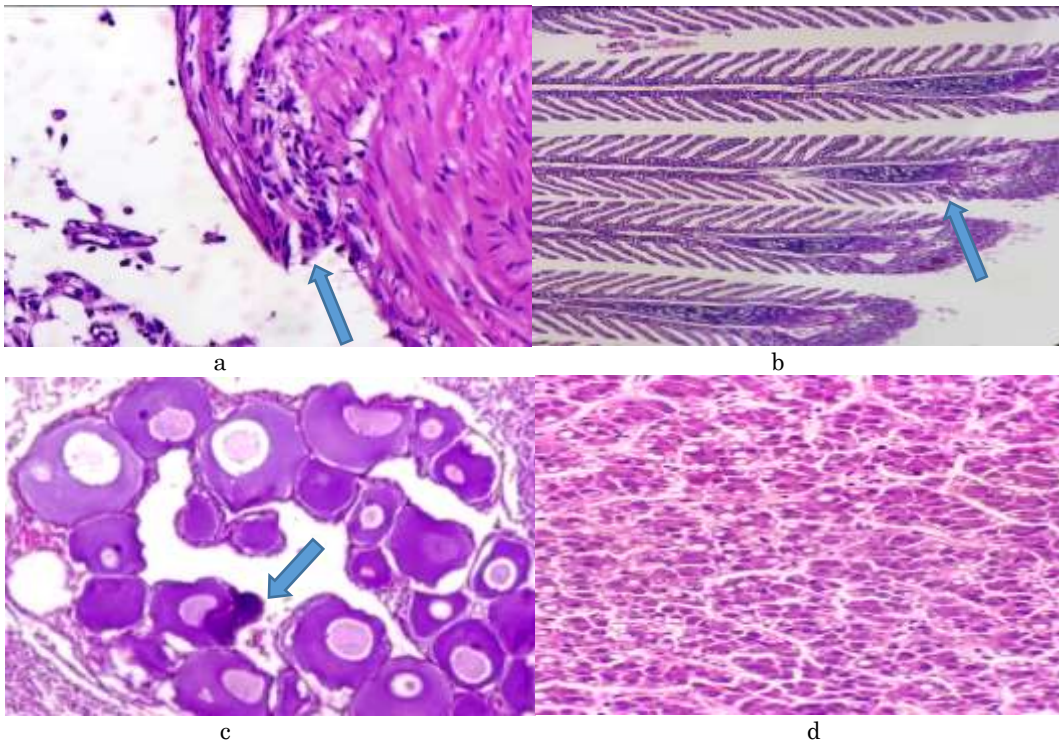
Toplamda 70 hasta balık örneğinden izole edilerek, identifikasyon için ayrılan bakteri suşlarının primer ile PCR'nın ardından jel elektroforez ile görüntülemesi yapıldı ve uygun bant görüntüsü gösteren örnekler sekans için ayrıldı. Sekansı yapılan suşların kromatogram görüntüleri Şekil 3'te verilmiştir. Sekansı yapılan 17 suşun fasta formatındaki sonuçları NCBI referans genleri ile kıyaslanmış ve 8 suş *Aeromonas sobria* (NCBI accession number: KC213891.1), 7 suş *Pseudomonas fluorescens* (NCBI accession number: KT447541.1), 2 suş ise *Hafnia*

alvei (NCBI accession number: CP050150.1) olarak tanımlandı. Tüm benzerlik oranları NCBI referans genler ile %99 üzeri olanlar arasından seçilmiştir. İdentifiye edilen suşların filogenetik ağaç görüntüleri ise Şekil 4' te verilmiştir.



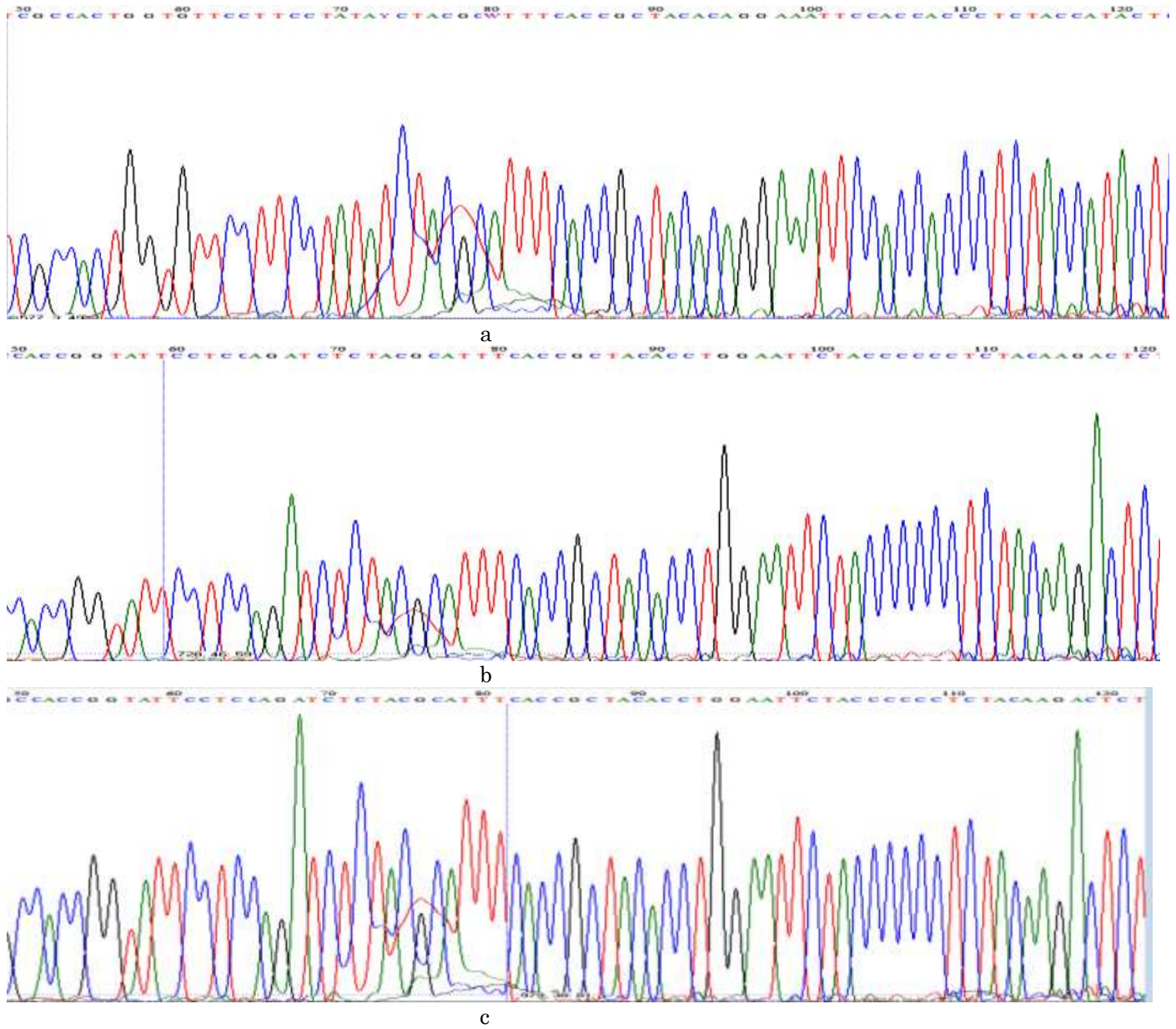
Şekil 1. (a) Hasta balıklarda deride ülseratif lezyonlar, (b) mide etrafında hemorajik ülserler, (c) soluk karaciğer, (d) Böbreklerde kanama ve granülomlar.

Figure 1. (a) Ulcerative lesions on the skin of sick fish, (b) hemorrhagic ulcers around the stomach, (c) pale liver, (d) hemorrhage and granulomas in the kidneys.



Şekil 2. (a) pankreasda dejenerasyon, (b) solungaç lamellerinde hiperemi, (c) pankreasda asiner hücrelerde dağılmalar, (d) karaciğerde yağlanma.

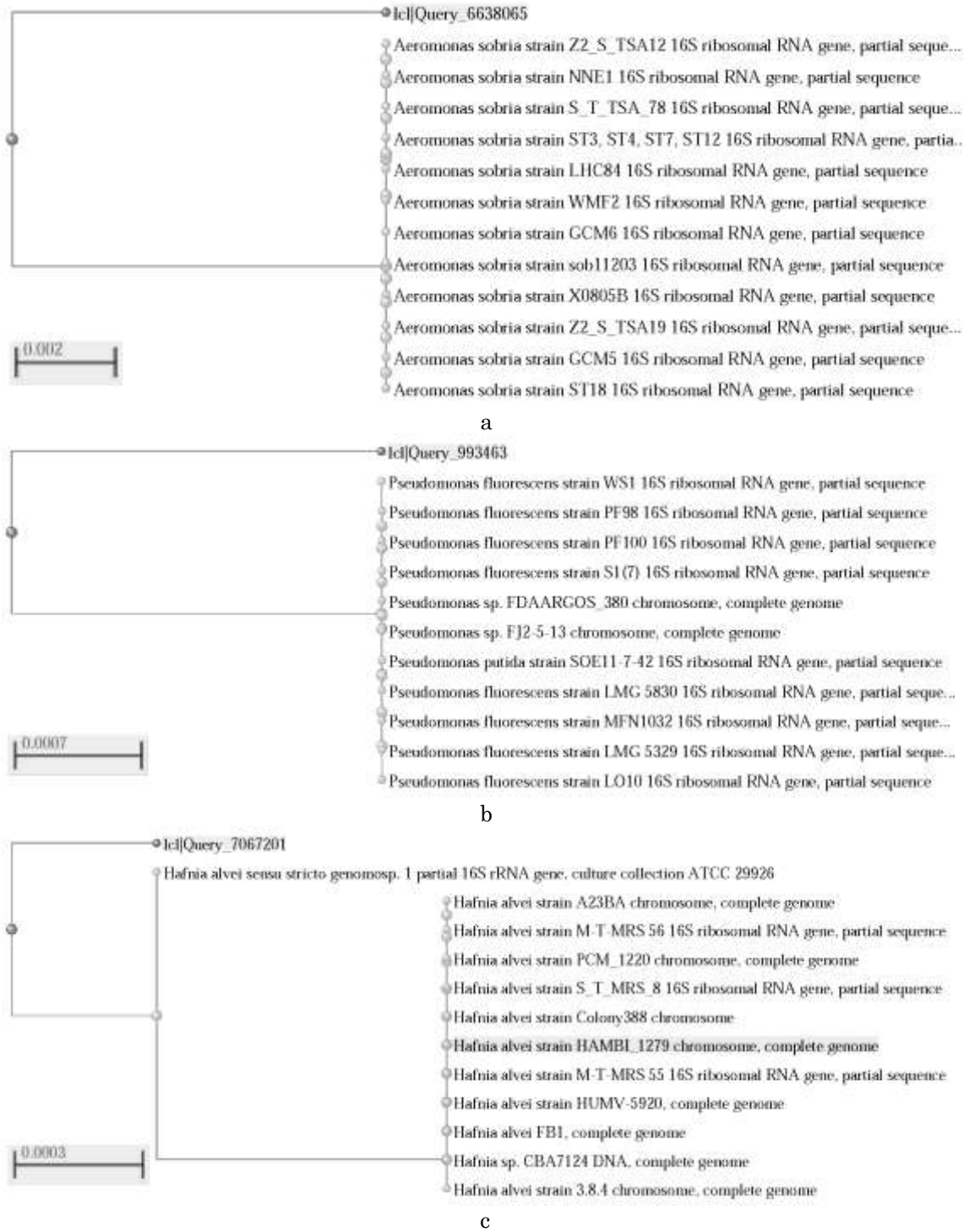
Figure 2. (a) degeneration of the pancreas, (b) hyperemia of the gill lamellae, (c) disintegration of acinar cells in the pancreas, (d) fatty liver.



Şekil 3. (a) *Aeromonas sobria* kromatogram grafiği, (b) *Pseudomonas fluorescens* kromatogram grafiği, (c) *Hafnia alvei* kromatogram grafiği.

Şekil 3. (a) *Aeromonas sobria* chromatogram graph, (b) *Pseudomonas fluorescens* chromatogram graph, (c) *Hafnia alvei* chromatogram graph.

Aeromonas suşlarının Kirby Bauer disk difüzyon testi sonrasında Penicilin, Amoksisilin, Enrofloksasin, Amoksisilin Clavulanic Acid, Oksitetrasiklin, Florfenicol ve Gentamicine duyarlı olduğu, Eritromosin, Chloramphenicol ve Colistin sulphate ise dirençli olduğu görüldü. *Pseudomonas* suşlarının disk difüzyon testi sonrasında Penicilin, Enrofloksasin, Amoksisilin clavulanic acid, Amoksisilin ve Gentamicine duyarlı olduğu, Eritromosin, Chloramphenicol, Oksitetrasiklin, Florfenicol ve Colistin sulphate dirençli olduğu görüldü. *Hafnia* suşlarının disk difüzyon testi sonrasında Penicilin, Enrofloksasin, Amoksisilin Clavulanic Acid, Amoksisilin, Florfenicol, Gentamicin, Chloramphenicol ve Oksitetrasikline duyarlı olduğu, Eritromosin ve Colistin sulphate ise dirençli olduğu görüldü (Tablo 1).



Şekil 4. (a) *Aeromonas sobria* filogenetik ağaç, (b) *Pseudomonas fluorescens* filogenetik ağaç, (c) *Hafnia alvei* filogenetik ağaç.
Figure 4. (a) *Aeromonas sobria* phylogenetic tree, (b) *Pseudomonas fluorescens* phylogenetic tree, (c) *Hafnia alvei* phylogenetic tree.

Tablo 1. Antibiyotik duyarlılık test sonuçları
Table 1. Antibiotic sensitivity test results

Bakteri suşu	Antibiyotik etken madde									
	P	AM	ENR	AMC	OX	FFC	GN	E	CL	CS
<i>Aeromonas</i>	S	S	S	S	S	S	S	S	R	R
<i>Pseudomonas</i>	S	S	S	S	R	R	S	R	R	R
<i>Hafnia Alvei</i>	S	S	S	S	S	S	S	R	S	R

P: Penicilin, AM: Amoksisilin, ENR: Enrofloksasin, AMC: Amoksisilin Clavulanic Acid, OX: Oksitetrasiklin, FFC: Florfenicol, GN: Gentamicin, E: Eritromisin, CL: Chloramphenicol, CS: Colistin sulphate.
S: Susceptibility (duyarlı), R: Resistance (Dirençli).

TARTIŞMA

Çalışma konusu olan baraj göllerinde yapılan su ürünleri yetiştiriciliğinin bakteriyel yönden incelenmesi ile ilgili Türkiye'de birçok çalışma yapılmış olup bunlardan bazıları; Elâzığ ilinde Kılıç ve ark. (2007), Erzurum'da Çarbaş ve ark., (2008), Malatya'da Şeker ve ark. (2006) tarafından il geneli örneklemeler ile bakteriyolojik inceleme şeklinde sıralabilir. Bu tip çalışmalar İl geneli yapılabildiği gibi baraj gölü düzeyinde de yapılabilmektedir. Deriner Baraj Gölünde su ürünleri üretiminin başlamasından önce doğal türler üzerinde bakteriyolojik ve parazitolojik bir çalışma yapılarak baraj gölünün balık patojenleri açısından risk haritası ortaya konmuştur (Düzgün, 2015). Trabzon ve Rize İllerinde alabalık işletmeleri üzerinde yapılan bir çalışmada ise işletmelerde görülen bakteriyel hastalıkların çoklu PCR yöntemi ile teşhisi yapılmıştır (Kayış, 2009). Çalışmaya konu baraj gölünde su ürünleri üretimi 2017 yılında başlamış olup alabalık türlerine yönelik herhangi bir bakteriyolojik inceleme çalışmasına rastlanmamıştır. Bu sebeple üretime yeni açılan baraj göllerinde bu tip survey çalışmaları hem üreticiler açısından hem de yapılacak akademik çalışmalar için örnek teşkil edilecektir.

Baraj gölünde su ürünleri yetiştiriciliği yapan işletmelerden izole edilerek identifikasyonu gerçekleştirilen; *Aeromonas* ve *Pseudomonas* genusuna ait bakteriler alabalık çiftliklerinde sıklıkla görülen ve mortaliteye neden olan patojen bakteriler olup, *Hafnia alvei* son yıllarda akuakültürde izole edilmeye başlamış ve mortaliteye neden olduğu bildirilmiştir (Kuzucu ve Özcan, 2023).

Aeromonas bakterileri; *Aeromonadaceae* familyası, *Gammaproteobacteria* ve *Aeromonadales* takımına aittir ve takımdaki tek ailedir. *Aeromonas* türlerinin taksonomisi, yeni türler tanımlandıkça ve mevcut taksonların tanımları iyileştirildikçe sürekli bir değişim halindedir. *Aeromonas* cinsine ait yeni türlerin tanımlaması, 57-63 mol% DNA G+C (guanin+sitozin) içeriğine ve 16S rDNA genine dayanmaktadır. *Aeromonas sobria* birçok canlıdan ve su ortamından izole edilebilmekte olup; balıktan, kurbağadan ve insandan izolasyonları bulunmaktadır (Popoff ve Véron, 1976; Smyrli ve Katharios, 2020). *Aeromonas sobria*, fakültatif anaerobik, Gram negatif, düz bir çubuk formundadır. Oksidaz pozitif ve katalaz negatiftir. İnkübasyonu, 25°C'de 24-48 saat sonra TSA ve BHIA'da gerçekleşir. Bu çalışmada da kullanılan 16S rRNA'nın dizi analizi *Aeromonas* türlerinin identifikasyonunda son yıllarda en güvenilir ve yaygın olarak kullanılan yöntem olarak bildirilmiştir (Martínez-Murcia ve ark., 2007). MAS (Motil *Aeromonas* Septisemi) enfeksiyonlarında genel olarak ülserler, abdominal distansiyon, kanama ve bazı durumlarda ekzoftalmi ve yüzgeç/kuyruk erimesi genel klinik bulgular olarak bildirilmektedir (Smyrli ve Katharios, 2020; Tanrıkul ve Dinçtürk, 2021). Bununla birlikte klinik bulgular genus içindeki türler arasında farklılıklar gösterebilmektedir. Bu çalışmada örneklerde gözlemlenen kuyruk erimesi, ekzoftalmi ve abdominal kanamalar bu bildirilen semptomlar ile eşleşmekte ve sekans sonuçları da bunu doğrulamaktadır.

Çalışma kapsamında *Pseudomonas fluorescens* izole edilen balıklarda, vücut yüzeyindeki peteşiyal hemorajiler, yüzgeç ve solungaç deformasyonlar, karaciğer ve böbrek dejenerasyonları ve de göz kanaması tespit edilmiştir. Bu bulgular Wiklund ve Bylund (1990), Austin ve Stobie, (1992), Lönnström ve ark. (1994), Markovic ve ark. (1996), Colquhoun ve ark. (1998), Austin ve Austin (1999) yaptığı çalışmalarda semptomlar ile benzerlik göstermiştir. *Pseudomonas* genusu bakterileri yaygın dağılım göstermekte olup; *Pseudomonadaceae* familyasından tamamı katalaz pozitif, gram negatif, aerobik, polar flagellası ile hareket edebilen bakterileri içerir. Bu bakteri türlerinin bazıları oksidaz pozitif, bazıları oksidaz negatiftir. *P. fluorescens*, *P. chlororaphis*, *P. anguilliseptica* ve *P. pseudoalcaligenes*'in alabalık türleri için patojen olduğu bilinmektedir (Austin ve Austin, 1999). *Pseudomonas* enfeksiyonunun klinik belirtilerinden, vücut yüzeyindeki peteşiyal hemorajiler, yüzgeç ve solungaç deformasyonlar bu çalışmadaki diseksiyon öncesi ve sonrası makroskobik muayenede gözlemlenmiştir. Son yıllarda bu genus ile ilgili yapılan çalışmalarda ise balıklarda patojenitesinin yanında antibiyotik dirençliliğinin de büyük bir sorun teşkil edebileceği bildirilmiştir (Algammal ve ark., 2020).

Çalışma kapsamında baraj gölünden gelen örneklerden izole ve identifiye edilen *Hafnia alvei*; *Enterobacteriaceae* familyasından, gram negatif, fakültatif ve anaerobik bir basildir. Uzun yıllardır insan bağırsak florasının doğal bir bakterisi olarak bilinmektedir. Doğal ortamda yaygın olarak bulunur ve birçok canlının bağırsaklarından izolasyonu bildirilmiştir (Okada, 2004). Son yıllarda bu bakterinin su ürünleri yetiştiriciliği alanında da izole edildiğini bildiren çalışmalar mevcuttur (Akaylı ve ark., 2019; Kuzucu ve Özcan, 2023). Akuakültürde antibiyotik duyarlılıkları ile ilgili birçok çalışma yapılmış olup farklı antibiyotik türlerine direnç ve duyarlılıklar bildirilmiştir. Bu çalışmada ise izole ve identifiye edilen üç bakterinin de eritromisin ve colistin sulphate antibiyotiklerine direnci belirlenmiştir.

SONUÇ ve ÖNERİLER

Bu çalışmada 2017 yılında su ürünleri üretimine açılan Hirfanlı baraj Gölünde faaliyet gösteren alabalık çiftliklerinin bakteriyel florası araştırılmış ve *Aeromonas sobria*, *Pseudomonas fluorescens* ve *Hafnia alvei* bakterileri izole ve identifiye edilmiştir. Baraj gölünde daha önce balık hastalıkları açısından tek çalışma yapılmış olup bu çalışmada da sadece göldeki sazan balıklarından *Aeromonas hydrophila* suşunun tanımlaması yapılmıştır.

Bu durum gölde üretimin başlaması ile alet-ekipman, yavru balık gibi yollarla farklı patojenlerin bölgeye taşınmış olabileceği düşüncesini ortaya koymak ile özellikle içsularda artan mikrobiyal yüke dikkat çekmektedir. Ayrıca su ürünleri üretimi açısından yeni bir baraj gölü olmasına rağmen bazı antibiyotik gruplarına oluşan direncin bilinçsiz antibiyotik kullanımından kaynaklanabileceği düşünülmektedir. Bu durumun önüne geçebilmek için üreticilere yönelik eğitim çalışmaları, yetiştiricilik ortamlarında oluşan mikrobiyal yük ile ilgili önlemler alınması gerekliliği ve yeni üretime başlanan su kaynaklarında bakteriyolojik survey çalışmaları havza yönetimi açısından önem arz etmektedir.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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

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Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

- Agriculture Organization of the United Nations. Fisheries Department. (2000). The state of world fisheries and aquaculture 2000. *The State of World Fisheries and Aquaculture, 2000 (Vol. 3)*. Food and Agriculture Organization of the United Nations.
- Akaylı, T., Ökmen, D., Yardımcı, R. E., Çanak, Ö., & Ürkü, Ç., (2019). Kültür gökkuşağı alabalıklarında (*O. mykiss*) *Hafnia alvei* enfeksiyonu. 20. Ulusal Su Ürünleri Sempozyumu, Mersin, Turkey.
- Algammal, A. M., Mabrok, M., Sivaramasamy, E., Youssef, F. M., Atwa, M. H., El-Kholy, A. W., Hozzein, W. N. (2020). Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor opr L and tox A virulence genes and bla TEM, bla CTX-M, and tet A antibiotic-resistance genes. *Scientific reports*, 10(1), 15961.
- Altun, S., Kubilay, A., & Diler, Ö. (2010). *Yersinia ruckeri* Suşlarının Fenotipik ve Serolojik Özelliklerinin İncelenmesi. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 16(B), 223-229. <https://doi.org/10.9775/kvfd.2009.1386>
- Anonim (2024). Kırşehir İl Tarım ve Orman Müdürlüğü verileri.
- Anonim. (2001) Statistic program SPSS for Windows, Version 11.00.263.
- Austin, B. ve D. A. Austin (1999). Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Third (Revised) Edition, London, UK, p. 457.
- Austin, B., & Stobie, M. (1992). Recovery of *Serratia plymuthica* and presumptive *Pseudomonas pseudoalcaligenes* from skin lesions in rainbow trout, *Oncorhynchus mykiss* (Walbaum), otherwise infected with enteric redmouth. *J. Fish Dis.*, 15, 541-543.
- Aydın, S., Erman, Z., & Bilgin, Ö. C. (2001). Investigations of *Serratia liquefaciens* in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Turkish Journal of Veterinary and Animal Sciences*, 25 (5), 643-650. <https://journals.tubitak.gov.tr/veterinary/vol25/iss5/2>
- Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single diffusion method. *American Journal of Clinical Pathology*, 45, 493-496.
- Çarbaş, A., Yanık, T., & Kaya, M. (2008). Erzurum ilindeki bazı ticari gökkuşağı alabalığı (*Oncorhynchus mykiss*) çiftliklerinin yetiştiricilik suyu, yem ve balıklarının mikrobiyolojik yönden incelenmesi. *Atatürk Üniv. Ziraat Fak. Derg.*, 39(1), 55-60.
- Çilli, E., Yeşilöz, H., Gökceci, Ö. F., Nuhay, Ç., Çağırman, A. A., Pekmez, K., & Kafa, B. (2023). Parasitological, bacteriological and virological research of carp (*Cyprinus carpio*) in a case in Hirfanlı Dam Lake in Türkiye. *Etlik Veteriner Mikrobiyoloji Dergisi*, 34(1), 108-113.
- Colquhoun, D. J., Skjerve, E., Poppa, T. T. (1998). *Pseudomonas fluorescens*, infectious pancreatic necrosis virus and environmental stress as potential factors in the development of vaccine related adhesions in Atlantic salmon, *Salmo salar* L. *J. Fish Dis.*, 21, 355-364.
- Defoirdt, T., Sorgeloos, P., Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology*, 14(3), 251-258. <https://doi.org/10.1016/j.mib.2011.03.004>
- Diler, Ö., Altun, S., Çalikuşu, F., & Diler, A. (2000b). Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın yaşadığı ortam ile ilişkili kalitatif ve kantitatif bakteriyel florası üzerine bir araştırma. *Turkish Journal of Veterinary and Animal Sciences*, 24(3), 251-259. <https://journals.tubitak.gov.tr/veterinary/vol24/iss3/10>

- Diler, Ö., Altun, S., Diler, A., Işıklı, B. I., & Gürcan, O. C. (2000a). Bazı balık çiftliklerinde gökkuşağı alabalıklarının (*Oncorhynchus mykiss*) mikroflorasının tespiti ve kontrolü üzerine bir çalışma. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 4(1), 58–69.
- Düzgün, A. (2015). *Deriner baraj gölü balık faunasının bakteriyel ve paraziter patojenler açısından araştırılması*. [Master's thesis, Recep Tayyip Erdoğan Üniversitesi/Fen Bilimleri Enstitüsü/Su Ürünleri Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- FAO. (2022). The state of world fisheries and aquaculture. In Towards blue transformation. <https://www.fao.org/3/cc0461en/cc0461en.pdf>
- Federation of European Aquaculture Producers (FEAP). (2022). *European aquaculture production report 2015–2021*. <https://feap.info/index.php/data/>
- İspir, Ü., Özcan, M., & Kırıcı, M. (2021). Fatty acid composition in *Yersinia ruckeri* strains isolated from Rainbow Trout farms. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 24(6), 1208-1212. <https://doi.org/10.18016/ksutarimdog.vi.838056>
- Karataş Düğenci, S., & Candan, A. (1997). Marmara bölgesindeki bir gökkuşağı alabalığı (*O. mykiss*) işletmesinde Yersiniosis vakası. IX. Ulusal Su Ürünleri Sempozyumu. 17 – 19 Eylül 1997, Eğirdir/Isparta. 641–648.
- Kayış, Ş. (2009). *Trabzon ve Rize illerinde bulunan bazı alabalık işletmelerinde görülen bakteriyel hastalıkların tespiti ve bazı etkenlerinin çoklu PCR ile teşhisi*. [Doktora Tezi, Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Kılıç, A., Şeker, E., Özcan, M., & İspir, Ü. (2007). Elâzığ'daki gökkuşağı alabalığı (*Oncorhynchus mykiss*) işletmelerinin bakteriyel yönden incelenmesi. *Fırat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, 19(2), 129–132.
- Kuzucu, O. (2023). *Karkamış Baraj Gölü'ndeki gökkuşağı alabalığı işletmelerinde bakteriyofajların izolasyonu, moleküler karakterizasyonu ve bakteriyofaj tedavisinin balıklara deneysel uygulaması*. [Doktora Tezi, Kahramanmaraş Sütçü İmam Üniversitesi Fen Bilimleri Enstitüsü Biyomühendislik Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Kuzucu, O., Özcan, M. (2023). Investigation of infection *Hafnia alvei* in Rainbow trout cage facilities in the Down Fırat Basin. *Hydrobiological Research*, 1(1), 38-46.
- Lönnström, L., T. Wiklund., G. Bylund. (1994). *Pseudomonas anguilliseptica* isolated from Baltic herring *Clupea harengus* membras with eye lesions. *Dis. Aquat. Org.*, 18, 143-147.
- Markovic, M., M. Radojicic, S. Cosic., D. Levnaic. (1996). Massive death of silver carp (*Hypophthalmichthys molitrix* Val.) and big head (*Aristichthys nobilis* Rich.) caused by *Pseudomonas fluorescens* bacteria. *Veterinarski Glasnik*, 50, 761-765.
- Martínez-Murcia, A.J., Figueras, M.J., Saavedra, M.J., Stackebrandt, E. (2007). The recently proposed species *Aeromonas sharmiana* sp. nov., isolate GPTSA-6^T, is not a member of the genus *Aeromonas*. *International Microbiology*, 10(1), 61.
- Medina, M., Fernandez-Espinel, C., Sotil, G., Yunis-Aguinaga, J., Flores-Dominick, V. (2020). First description of *Weissella ceti* associated with mortalities in farmed rainbow trout (*Oncorhynchus mykiss*) in Peru. *Aquaculture*, 529, 735608. <https://doi.org/10.1016/J.Aquaculture.2020.735608>.
- Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, 591(7851), 551–563.
- Okada, S. (2004). Ecology and population genetic structure of the genus *Hafnia* (*Enterobacteriaceae*). A thesis Doctor of Philosophy of the Australian National University.
- Özcan M. & Barış Y. 2019. Investigation of phenotypic and genotypic characteristics of isolation of strains *Vagococcus salmoninarum* cage business rainbow trouts (*Oncorhynchus mykiss*) of Kahramanmaraş province (Turkey). *Journal of Animal and Veterinary Advances*, 18(4),124-131. <https://doi.org/10.36478/javaa.2019.124.131>.
- Özcan M. Yılmaz Y. Donat E. & Kılavuz D. 2014. A study of *Vagococcus salmoninarum* infection in some cultured Rainbow Trout in cages in the Mediterranean and Southeast Anatolia regions. *Journal of Fisheries and Aquatic Sciences*, 29(1), 2130.
- Özcan, M., & Donat, E. (2022). Investigation of phenotypic and genotypic characteristics of isolated *Vibrio anguillarum* strains in rainbow trout cage farms in Kahramanmaraş city of Turkey. *Journal of the Hellenic Veterinary Medical Society*, 73(4), 4681-4688. <https://doi.org/10.12681/jhvms.22851>
- Özcan, M., & Gök, M.R. (2019). Determination of their antibiotic susceptibility and diagnosis of using convectional culture and molecular methods *Yersinia ruckeri* in the Rainbow Trout farms in Kahramanmaraş, Turkey. *Journal of Multidisciplinary Engineering Science Studies (JMESS)*, 5, 2, 2470-2476.
- Özcan, M., & Kuzucu, F. B. (2019). Investigation of Bacterial Diseases in Rainbow Trout Cages in the Down Fırat Basin. *World Journal of Engineering*, 5(6), 473-490.

- Özcan, M., & SarıeYYüpoğlu, M. (2013). Elazığ İlindeki Bazı Alabalık İşletmelerinde İzole Edilen *Flavobacterium psychrophilum*'un Antibakteriyel Duyarlılıklarının İncelenmesi. *Aquaculture Studies*, (2): 11-19. <https://doi.org/10.17693/yunusae.v2013i21905.235419>
- Özcan, M., & SarıeYYüpoğlu, M. (2014). Identification and investigation of phenotypic and genotypic characteristics of *Flavobacterium psychrophilum* in fry rainbow trouts (*Oncorhynchus mykiss*) in some trout. *International Journal of Sciences*, 3(3), 24-34.
- Popoff, M., Véron, M. (1976). A taxonomic study of the *Aeromonas hydrophila*–*Aeromonas punctata* group. *Journal of General Microbiology* 94(1), 11–22.
- Pridgeon, J.W., & Klesius, P.H. (2012). Major bacterial diseases in aquaculture and their vaccine development. *CAB Reviews* 7(048), 1–16. <https://doi.org/10.1079/PAVSNR201270>
- SarıeYYüpoğlu, M. (1984). Gökkuşığı Alabalıklarında (*S. gairdneri*) Mide-Bağırsak Bakteriyel Florasının Aerobik Yönden İncelenmesi. *Doğa Bilim Dergisi*, 8(3), 281-287.
- SarıeYYüpoğlu, M., Özcan, M., & Barata, S. (2017). Gökkuşığı alabalığı (*Oncorhynchus mykiss*)'nda deri ensizyonuyla operasyon uygulanması ve balığın canlılığının kontrolü üzerine bir araştırma. *Fırat Üniversitesi Fen Bilimleri Dergisi*, 29(1), 9-13.
- Şeker, E., Kılıç, A., Özcan, M., & İspir, Ü. (2006). Malatya'daki bazı gökkuşığı alabalık (*Oncorhynchus mykiss*) işletmelerinin bakteriyel florası. *Fırat Üniversitesi Doğu Araştırmaları Dergisi*, 5(1), 15-18.
- Smyrli M., Katharios P. (2020).12. *Aeromonas spp.*. In : Zrncic S. (ed.). *Diagnostic Manual for the main pathogens in European seabass and Gilthead seabream aquaculture*. Zaragoza : CIHEAM, p. 107-116. (Options Méditerranéennes : Série B. Etudes et Recherches; n. 75). <http://om.ciheam.org/om/pdf/b75/00007945.pdf>
- Sudheesh, P.S., Al-Ghabshi, A., Al-Mazrooei, N., & Al-Habsi, A. (2012). Comparative pathogenomics of bacteria causing infectious diseases in fish. *International Journal of Evolutionary Biology*, 2012(1), 457264. <https://doi.org/10.1155/2012/457264>
- Tanrıkul, T., Dinçtürk, E. (2021). A New Outbreak in Sea Bass Farming in Turkey: *Aeromonas veronii*. *Journal of the Hellenic Veterinary Medical Society*, 72(3), 3051-3058.
- Timur, G., Karataş, S., Çolak, S., & Akaylı, T. (1996). Gökkuşığı alabalık (*Oncorhynchus mykiss*, W, 1792) yavrularında görülen furunkulosis hastalığı üzerine bir çalışma. II. *International Symposium on Aquatic Products*. İstanbul, Türkiye.
- Wiklund, T., G. Bylund. (1990). *Pseudomonas anguilliseptica* as a pathogen of salmonid fish in Finland. *Aquaculture*, 8, 13-19.



Variability of Genetically Improved Farmed Tilapia (Gift Tilapia) Crossed With Upm Red Tilapia Hybrids By Mitochondrial DNA Conserved Region Sequence

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ABSTRACT

Mitochondrial DNA conserved region (mtDNA CR) segment of a cross between GIFT tilapia and UPM red tilapia, their F1-hybrid (F1) and Backcross (BcF1) was used to define their genetic variability. DNA was extracted from 25 randomly selected specimens of each of the test strains and mtDNA CR primer ORMT-F ORMT-R was used. The MEGA software using Maximum Parsimony (MP) was conducted for the evolutionary analysis of the strains using *Tilapia rendalli* (AF484717.1 accession numbers from NCBI database). A total of 441 polymorphic regions of 99 sequencing sites were observed, the sites with 12 alignment gaps were missing while the invariable monomorphic sites were 360 (81.6%) with 69 (15.6%) variable polymorphic sites. Parsimony informative sites were 68 with 1 singleton variable site. The conserved regions were effective in discriminating between the sampled fish because they contained important elements that aid replication and expression of the mitochondria genome. The clustering of the strain in the sub-tree of the phylogeny verifies a monophyletic relationship although some of the strains showed more relatedness by their consistent overlap and clustering. Similarly with 0.1 genetic variability conserve region could be used for discrimination between the strains.

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INTRODUCTION

Molecular studies of fish populations and examination of macromolecules are associated with the current development of starch gel electrophoresis with histochemical staining. This allows the detection of enzymes and a simple means of identification by the use of genetic markers. These markers provide genetic variants for population studies in fish. Genetic markers are of two types, protein and DNA. Protein markers are relatively cheaper, easy to analyze, and have a large database of different species while DNA requires a single tissue that could be preserved in ethanol for a scaly specimen, a small quantity for PCR analysis, and can detect changes in mutation that is not associated with protein electrophoretic mobility (Ferguson et al. 1995). Recent studies have shown the increasing rate at which mitochondria DNA (mtDNA) is used compared to nuclear DNA (nDNA) because of the formal high rate of mutation.

The use of mtDNA marker offers a better way for classifying related populations into strains because of its poor recombination ability and the inheritance through the maternal parent that allows placing into groups even after hybridization (Dasuki et al., 2023; Nwachi et al., 2020; Ogden 2008). Although much credit recently was given to the use of microsatellites for speciation, separate development per species due to the expensive and cumbersome transfer between species was needed when compared to the mitochondria gene that has traits that could be used to allocate strains to the parental population and could be transferred across species (Rastogi et al. 2007 & Esa et al. 2008). A report by (Brown et al. 2005 & Clayton 1982) infers that the conserved region is a parental template that is unique to the owner; it is used for H-strand synthesis and the monomeric mtDNA has a short H-strand with each conserved region attached to a molecule. This constraint made this region evolve faster than the mtDNA and a better target for any form of classification as described by Karamanlidis et al. (2016).



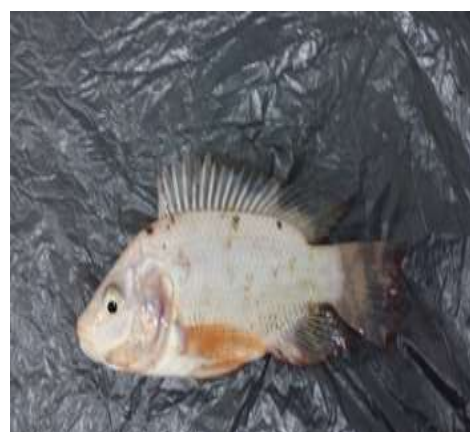
(a)



(b)



(c)



(d)

Plate 1 Strains of sampled fish (a) UPM red tilapia (b) GIFT tilapia (c) Backcross (BcF₁) and (d) Hybrid (F₁)

The sampled fish of UPM red tilapia, GIFT tilapia, F₁, and BcF₁ genomes (Plate 1) are the same but different at the level of DNA sequences and the protein-coding due to biological characteristics which largely depend on their amino acid content. The task of clarifying the strains based on their related taxa is connected to markers that are specific in action for intraspecific relationships. In the past, phenotypic markers (a direct means) were used to take measurements of dimension, size, and pigmentation as a means of discriminating between species. The genetic diversity method on the other hand is indirect and mostly based on markers with low inheritability even though they were genetically inherited, and the genetic basis of these markers measures genetic diversity.

Tilapia culture witnessed growth worldwide because of the increasing demand in local and international markets and is the second most cultured fish after Carp (FAO 2009). Tilapia is also an important fish in aquaculture because of its useful model in population genetics (Chakrabarty 2006; Nagl et al. 2001; Nwachi & Esa 2016). The phylogenetic relationship among *Oreochromis niloticus*, *Oreochromis mossambicus*, and *Oreochromis aureus* was studied by Esa et al (2008) using RAPD markers with the assertion that the highest genetic similarity occurred between *O. niloticus* and *O. mossambicus*. A report by Nagl et al (2001) on the mtDNA control region of *O. niloticus*, *O. mossambicus* and *O. aureus* was unable to explain the differences in their phylogenetic relationship although there is a relationship between the ND2 gene sequences of *O. Niloticus* and *O. mossambicus* (Klett & Meyer 2002). Studies by Betsen et al. (2016) and Ansah et al. (2014) show that Gift exhibited more potential of being productive than the normal tilapia. Hence, this study will examine the complete mtDNA conserve region of UPM red tilapia, GIFT tilapia, their hybrid (F₁), and backcross (BcF₁) assessing their phylogenetic and genetic relationship.

MATERIAL and METHOD

Laboratory work

The procedures for this experiment were considered and approved by the Board of Studies; Department of Aquaculture, University Putra Malaysia.

Total DNA extraction.

A total of 104 fish consisting of 26 specimens each per strain of (UPM red tilapia, GIFT tilapia, F₁, and BcF₁) was

used. DNA samples were extracted from the muscular part of the caudal region, at a prior preservation workstation to avoid cross contamination. The samples were properly labeled, preserved in 95 % ethanol, and stored in a -20-degree freezer for further use. Total DNA extraction from the sampled tissue was obtained with the aid of ReliaPrep™ gDNA Tissue Miniprep System (Promega, USA) according to the manufacturer's instructions. The quality and concentration of the DNA were assessed using agarose gel electrophoresis, the resulting sample was diluted to 100ng while the resulting genomic DNA was stored in a -20°C until further analysis.

Polymerase chain reaction (PCR) amplification of mitochondria conserve region (mtDNA)

Polymerase chain reaction (PCR) for mitochondria conserve region (mtDNA CR) was carried out using a set of primers in Table 1, the total PCR reaction volume for the mtDNA CR was 25µL cocktail which includes 2× power Taq PCR master mix from Biotek technology (Beijing China), dH₂O, primer (forward and reverse) and DNA template (Table 2).

Table 1. The primer sequence of ORMT-F and ORMT-R

No	Region	Sequence
1	ORMT-F (forward primer)	5'-CTAACTCCCAAAGCTAGGAATTCT-3'
2	ORMT-R (reverse primer)	5'-CTTATGCAAGCGTCGATGAAA-3'

(Gu et al. 2016)

Table 2. Reagents final volume and Concentration for PCR reactions

Reagent	Final concentration	Final volume (µl)
Power Taq PCR master mix	2×	12.5
ddH ₂ O	Mm	9.5
Forward primer	25 pmol/µl	0.5
Reverse primer	25 pmol/µl	0.5
DNA template	-	2.0
Total	-	25

The PCR conditions were adopted and involved initial denaturation at 94 °C for 3 minutes in a Thermocycler followed by 35 cycles for 94 °C for 30 seconds, 54 °C for 40 seconds, and 72 °C for 40 seconds and a final extension step of 72 °C for 10min. The negative control was used in all the reactions for contamination detection during the reaction system. The PCR products were separated on 1 % agarose gels and ran at approximately 45min at 75 V at a current of 300 amps, the size was quantified using a digested (Promega, USA) DNA ladder (GeneRuler™50-bp DNA Ladder) with a visualized product size of 413 - 450bp expected. The products were purified using DNA Wizard® SV Gel and PCR clean-up kit (Promega, USA) according to the manufacturer's instructions. The purified products were sequenced using the BigDye® Terminator v3.1 Cycle sequencing kit (ACGT) on an ABI 377 automated sequencer (PE Applied Biosystems) using only the forward primer ORMT-F.

The multiple sequence was aligned with ClustalW in BioEdit software (Hall 2011). Sequencing reaction using the reverse primer ORMT-R was subsequently carried out on some of the samples to verify the polymorphism in the DNA sequences detected using the forward primer. The Evolutionary analysis of the strains was conducted with MEGA software as described by Tamura et al. (2013) using neighbor-joining (NJ) tree, maximum parsimony, and maximum likelihood with conserve region sequence and using *Tilapia rendalli* as an out-group (AF328854.1; GenBank accession number) was constructed with the Kimura 2 parameter distance model (Saitou & Nei 1987 and Kimura 1980). The branching order of the tree was tested by bootstrapping at 550 replicate data.

Data analysis

To validate the similarity of the current study, sequences were compared with data from the National Centre for Biotechnology Information (NCBI) database with the aid of Basic Local Alignment Search Tools (BLAST). Sequences with higher ident values were considered and the sequences were corrected manually to reduce errors of mismatch with the actual sequencing report. Sequencing was aligned with clustalW of BioEdit version 7.2.5 (Chakrabarty, 2006). The genetic distance (D) of GIFT tilapia, UPM red tilapia, hybrid (F1), and backcross (BcF1) were carried out using distance-related statistical tools in MEGA version 6.06 software. The genetic diversity index was computed using DnaSP version 5.10 including nucleotide diversity (π), haplotype diversity (H), and the average number of nucleotide differences (K).

RESULTS

Phylogenetic analysis

A total of 104 tilapia tissue samples were collected from 4 populations of parent, hybrids, and backcross. Genomic DNA was extracted from 100 tissue samples and used for PCR amplification of mtDNA CR (conserve region) out of which 99 samples were sequenced. The fluorescent-based DNA sequencing results were displayed with DnaSp (version 5) referring to (Librado & Rozas 2009). A total of 441 polymorphic regions of 99 sequencing sites were observed, the sites with alignment gaps missing were 12 while the invariable monomorphic sites were 360 (81.6%) with 69(15.6%) variable polymorphic sites. Parsimony informative sites were 68 with 1 singleton variable site.

Table 3. Distribution of observed nucleotide diversity, haplotypes and diversity and segregation sites among the samples.

Haplotype	UPM red tilapia	GIFT tilapia	F ₁ -Hybrid	BcF ₁ backcross
1	1.00	-	0.96	1.00
2	-	0.84	-	-
3	-	0.08	-	-
4	-	0.08	-	-
5	-	-	-	-
Parameters				
Nucleotide diversity	0.0000	0.0302	0.0003	0.0000
Number of haplotypes	1	3	2	1
Nucleotide (P _{ijc})	0.0000	0.0000	0.0003	0.0326
Haplotype diversity	0.000	0.2933	0.0800	0.080
Number of segregation sites	0	66	2	0

In Table 3 GIFT tilapia recorded the highest number of observed haplotypes at 3 with a haplotype diversity (H) of 0.2933, a segregation sites value of 66, and a nucleotide diversity (Ω) of 0.0302. F₁ recorded nucleotide diversity (Ω) of 0.0003 with 1 haplotype. A total of 2 haplotypes and 0.0000 nucleotide diversity (Ω) was recorded by UPM red tilapia. UPM tilapia, F₁, and BcF₁ have one haplotype in common. In Table 5 GIFT tilapia recorded 4 conserved regions at conservation values of 0.942, 0.944, 0.938, and 0.955 while UPM red tilapia, F₁, and BcF₁ have only 1 respectively.

The Tajima's D of UPM red tilapia was -0.3543 but none significant while F₁ has -1.9617 but significant. Both GIFT tilapia and BcF₁ have -1.0851 and -1.1575. The Fu's fs were -0.289 for UPM red tilapia. GIFT tilapia recorded the highest mutation rate of 82 (Table 4).

Table 4. Hierarchical Analysis of Molecular Variance showing amount of population genetics

Parameters	UPM red tilapia	GIFT tilapia	F ₁	BcF ₁
Population	24	25	25	25
Mutation sites	2	82	12	1
Tajima's D	-0.3543	-1.0851	-1.9617	-1.1575
P-Value	p>0.10	p>0.10	P<0.05	p>0.10
Fu's fs statistic	-0.289	-0.148	-1.035	-1.061

The pairwise population frequencies in Figures 1a to 1d reveal that there are discrepancies between the expected and observed frequency of occurrence for the sampled fish.

The backcross (BcF₁) and UPM red tilapia have frequencies that correlate to the observed pairwise values at 0 - 0.8 at 20 pairwise distances and 0 - 0.6 at 20 pairwise distances while the hybrid (F₁) has a slight variation between the observed and expected value (0 - 0.05 at 20). GIFT tilapia recorded a variation of 0 - 0.2 at 60 pairwise distances between the observed and expected pairwise frequency.

Phylogenetic tree

A phylogenetic tree based on mtDNA CR conserved region was constructed. The sequences from the sample fish haplotype and fish of the same family *Tilapia rendalli* (accession number AF484717.1 as obtained from NCBI database) were used as our group and a root for the tree. The evolutionary history that was inferred by the maximum parsimony (MP) method in Figure 2 reveals that 1 out of every 3 trees was parsimonious. The length observed was 86, with a consistency index of 0.870968, and a retention index of 0.870968 while the composite index and parsimony-informative sites are 0.830458 and 0.758585 respectively.

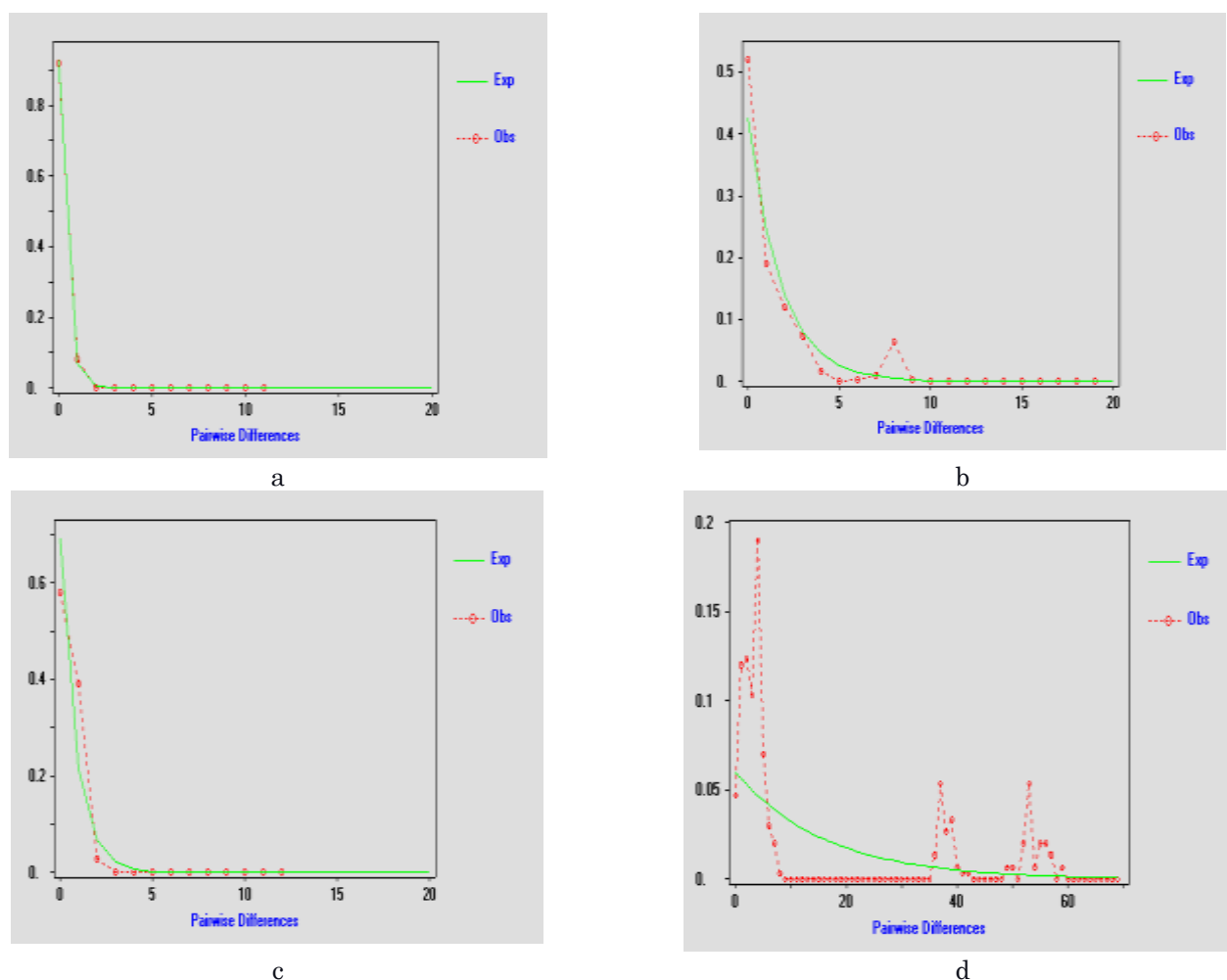


Figure 1 Pairwise distribution of sampled fish (a) (BcF₁) (b) F₁ (c) GIFT tilapia and (d) UPM red tilapia

Table 5. Conserve region of mtDNA analysis

Parameters	Region	UPM red tilapia	GIFT tilapia	F1	BcF1
Start to end	1	13-448	28-96	38-447	2-436
	2		45-98		
	3		284-380		
	4		382-448		
Conservation	1	1.00	0.942	1.00	1.00
	2		0.944		
	3		0.938		
	4		0.955		
Homozygosity	1	1.00	0.987	1.00	1.00
	2		0.989		
	3		0.985		
	4		0.993		
P-value	1	0.000	0.0051	0.000	0.000
	2		0.0127		
	3		0.0010		
	4		0.0018		

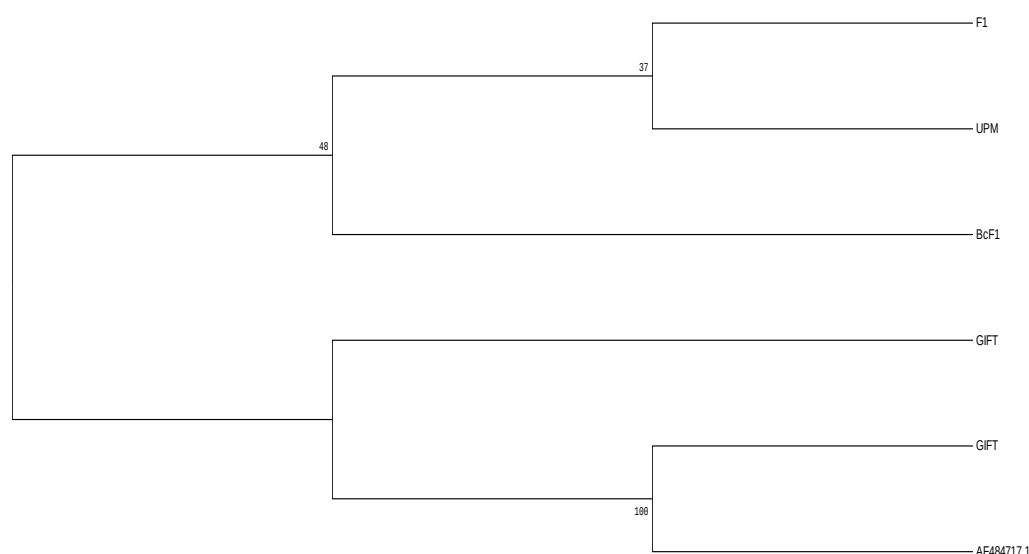


Figure 2: Maximum Parsimony (MP) tree showing relationships among Mitochondria conserve region (mtDNA) of UPM red tilapia, GIFT tilapia, F₁, and BcF₁ with *Tilapia rendalli* (AF484717.1) out-group. The bootstrap value (%) based on 50 pseudo replications for the MP analysis

The clustered percentage for the bootstrap test is at 1050 replicates while the MP tree was obtained with the aid of Subtree-Pruning-Regrafting (SPR) with 1 search level which the initial tree was obtained by the addition of 10 replicates sequences. A total of 55 nucleotide sequences were analysed at 1st+2nd + 3rd+ non-coding at 420 positions.

DISCUSSION

Tilapia is an important food fish can be identified using molecular techniques like; microsatellites DNA restriction enzymes, barcoding, Snp, analysis of the nuclear fragment of rDNA, and the first internal transcribed spacer. Morphological techniques; Length, weight relationship, morphometric, and meristic traits analysis following (Oladimeji et al. 2015; Hsieh et al. 2010; Ward et al. 2005). Over time, the use of molecular techniques proved to be more accurate and practical compared to the use of morphological methods (Lim et al. 2015 and McManus & Katz 2009). The use of biomarkers as opined by (Huang et al. 2004) in both evolutionary and gene regulatory activities improves the accuracy level in species identification and evolutionary trend of a strain.

Phylogenetic analysis of the main coding region (Conserve Region) of the mitochondria (mt) DNA in the parents, hybrid (F₁), and backcross (BcF₁) of UPM red tilapia and GIFT tilapia were examined. The conserved regions were found to be effective in discriminating between the sampled fish because they contained important elements that aid replication and expression of the mitochondria genome (Zou et al., 2015; Kinaro et al., 2015 Xiao et al., 2016). In the present study, 4 conserved regions were identified in GIFT tilapia while UPM red tilapia, F₁, and BcF₁ have one conserved region each. The identified areas were able to serve as templates for differentiation. The mtDNA is maternally inherited thereby, making possible speciation through the identification of sib and half sib in general hence, parent stock from F₁ and BcF₁ respectively. The conserved region is more species-specific because it occurs as a template inherited as part of mtDNA with special features that enhance speciation (Jiang et al., 2004).

The sequenced conserved region of 99 sampled fish produced 5 haplotypes three populations (UPM red tilapia, F₁, and BcF₁) have one haplotype in common which agrees with the opinion of (Zou et al., 2015) with assertions that the sampled fish shared common ancestors of *O. niloticus*. Similar pairwise distance population frequencies were observed in the pair of BcF₁ and UPM red tilapia compared to F₁ with slight variations to the large fluctuation that is observed in GIFT tilapia. This could be explained by the fact that the GIFT tilapia projects involved a large population of stock with varying transfer of maternal DNA and multiple control regions compared to the other sampled fish.

Examining the genetic diversity of the population in Table 3 with the aid of their haplotype diversity (H) and nucleotide diversity (π) reveals that Gift tilapia has the highest value at 0.2933 haplotype diversity (H) in the

population that was examined. It also recorded the highest nucleotide diversity (π) at 0.0302. He et al (2015) opined that haplotype diversity (H) of a strain depends on the number of populations that hybridized, indicating that the higher (H and π) value recorded by GIFT tilapia is a result of the high number of introgressions. The genetic distance (D) average values as inferred by Zou et al (2015) were 0.90, 0.30, and 0.05 for individuals. In this study (0.1) was recorded for all the population, which is in-between the recommended score, this is an indication that the strains examined were very near to each other. We can infer that; all the sampled fish could be differentiated at their conserved region and the genetic sustainability of the BcF1 is possible because of the hybrid vigor of the F1 male parent. However, the test fish despite coming from different strains were genetically linked together.

Researchers like Wu & Yang (2012), Kinaro et al. (2015), and He et al. (2015) analyzed the differences between strains of interest. While this study uses a Maximum parsimony (MP) phylogenetic tree to examine the relationship between sampled fish, a monophyletic relationship was observed between the strains due to the relatedness of the haplotype; it can be seen that 2 of the haplotypes are shared. The phylogenetic tree produced was validated based on the value of consistency index (CI) and retention index (RI); a high value of CI tends to decrease the homoplasy index (HI) and an RI that is close to 1 is an indication that the characters that form the tree fit (Hidayat et al., 2005; Zou et al., 2015). The CI and RI values were 0.870968 and 0.870968 respectively in this study. The phylogenetic analysis verifies the monophyletic relationship although some of the strains showed more relatedness by their consistence overlap and clustering. It is of note that despite their positions on the Sub-tree each strain maintains a level of uniqueness that could be used for proper identification.

CONCLUSION

The genetic variability of parents (Gift Tilapia and UPM red Tilapia), hybrids (F1), and backcross (BcF1) were examined, their distances were (0.1), an indication that they were closely related. Some of the populations shared one haplotype on close examination. The phylogenetic tree shows a close relationship to each other and to an out-group signifying similarity in their genetic makeup which can be related to the fish coming from the same genus.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

- Ansah, Y. B., Frimpong, E. A., & Hallerman, E. M. (2014). Genetically-improved tilapia strains in Africa: Potential benefits and negative impacts. *Sustainability (Switzerland)*, 6(6), 3697-3721. <https://doi.org/10.3390/su6063697>
- Bentsen, H. B., Gjerde, B., Eknath, A. E., de Vera, M. S. P., Velasco, R. R., Danting, J. C., Dionisio, E. E., Longalong, F. M., Reyes, R. A., Abella, T. A., Tayame, M. M., Ponzoni, R. W., et al. (2017). Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in *Oreochromis niloticus* and the further impact of the project. *Aquaculture*, 468, 206-217. <https://doi.org/10.1016/j.aquaculture.2016.10.018>
- Brown, T. A., Cecconi, C., Tkachuk, A. N., Bustamante, C., & Clayton, D. A. (2005). Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. *Genes & Development*, 19, 2466-2476. <https://doi.org/10.1101/gad.1352105>
- Chakrabarty, P. (2006). Systematics and historical biogeography of Greater Antillean Cichlidae. *Molecular Phylogenetics and Evolution*, 39, 619-627. <https://doi.org/10.1016/j.ympev.2006.01.014>
- Clayton, D. A. (1982). Replication of animal mitochondrial DNA. *Cell*, 28, 693-705. [https://doi.org/10.1016/0092-8674\(82\)90049-6](https://doi.org/10.1016/0092-8674(82)90049-6)
- Dasuki, A., Esa, Y., Christianus, A., & Ismail, M. F. S. (2023). Genetic variations of Malaysian and Golden Thai strains of climbing perch and their hybrids based on the partial mitochondrial cytochrome oxidase subunit 1 gene. *Malaysian Applied Biology*, 52(2), 139-146.
- Esa, Y., Shapor, S. S., Daud, S. K., Jeffrine, J., Ryan, R., Adha, K. A., Rahim1, & Soon Guan, T. (2008). Molecular systematics of Mahseers (Cyprinidae) in Malaysia inferred from sequencing of a mitochondrial cytochrome C oxidase I (COI) gene. *Pertanika Journal of Tropical Agric. Sci*, 31, 263-269.
- FAO (Food and Agriculture Organization of the United Nations). (2009). FishStat Plus Version 2.32. Universal software for fishery statistics time series. Available from <http://www.fao.org/fishery/statistics/software/fishstat/en>
- Ferguson, A. J. B., Taggart, P. A., Prodohl, O., Mcmeel, C., Thompson, C., Stone, P., McGinnity, A. H., et al. (1995). Population and conservation. *Journal of Fish Biology*, 47, 103-126. <https://doi.org/10.1111/j.1095-8649.1995.tb06048.x>
- Gu, D. E., Mu, X. D., Xu, M., Luo, D., Wei, H., Li, Y. Y., Zhu, Y. J., Luo, J. R., Hu, Y. C., et al. (2016). Identification

- of wild tilapia species in the main rivers of south China using mitochondrial control region sequence and morphology. *Biochemical Systematics and Ecology*, 65, 100-107. <https://doi.org/10.1016/j.bse.2016.02.007>
- Hall, A. T. (2011). BioEdit: An important software for molecular biology. *GERF Bulletin of Biosciences*, 2, 60-61.
- He, A., Luo, Y., Yang, H., Liu, L., Li, S., Wang, C., et al. (2011). Complete mitochondrial DNA sequences of the Nile tilapia (*Oreochromis niloticus*) and Blue tilapia (*Oreochromis aureus*): Genome characterization and phylogeny applications. *Molecular Biology Reports*, 38(3), 2015-2021. <https://doi.org/10.1007/s11033-010-0324-7>
- Hidayat, T., Tomohisa, Y., & Motomi, I. (2005). Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): Insights from plastid marker and nuclear ribosomal ITS sequences. *Journal of Plant Research*, 271-284. <https://doi.org/10.1007/s10265-005-0217-3>
- Hsieh, C. H., Chang, W. T., Chang, H. C., Hsieh, H. S., Chung, Y. L., & Hwang, D. F. (2010). Puffer fish-based commercial fraud identification in a segment of cytochrome b region by PCR-RFLP analysis. *Food Chemistry*, 121(4), 1305-1311. <https://doi.org/10.1016/j.foodchem.2010.02.004>
- Huang, J. L., Chang, H. T., Cheng, R., Hsu, H. H., Pai, T. W., et al. (2004). Identification of simple sequence repeat biomarkers through cross-species comparison in a tag cloud representation. *BioMed Research International*, 2014(1), 678971. <https://doi.org/10.1155/2014/678971>
- Jiang, M., Yang, C., & Wen, H. (2004). The complete mitochondrial genome of *Aspiorhynchus laticeps* and its phylogenetic analysis. *Meta Gene*, 2(1), 218-225. <https://doi.org/10.1016/j.mgene.2014.01.006>
- Karamanlidis, A. A., Gaughran, S., Aguilar, A., Dendrinis, P., Huber, D., Pires, R., Schultz, J., & Amato, G. (2016). Shaping species conservation strategies using mtDNA analysis: The case of the elusive Mediterranean monk seal (*Monachus monachus*). *Journal of Molecular Evolution*, 193, 71-79. <https://doi.org/10.1016/j.biocon.2015.11.014>
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120. <https://doi.org/10.1007/BF01731581>
- Kinaro, Z. O., Xue, L., Nyaundi, K. J., Shen, J., et al. (2015). The mitochondrial genome of an endangered native Singidia tilapia, *Oreochromis esculentus*: Genome organization and control region polymorphism. *Mitochondrial DNA*, 1736 (March 2016). <https://doi.org/10.3109/19401736.2015.1089493>
- Klett, V., & Meyer, A. (2002). What if anything, is a tilapia: Mitochondrial ND2 phylogeny of tilapiines and the evolution of parental care systems in the African cichlid fishes. *Molecular Biology and Evolution*, 865-883. <https://doi.org/10.1093/oxfordjournals.molbev.a004144>
- Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451-1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Lim, K. C., Lim, P. E., Chong, V. C., & Lee, K. H. (2015). Molecular and morphological analyses reveal phylogenetic relationships of stingrays focusing on the family Dasyatidae (Myliobatiformes). *PLoS ONE*, 10(5), e0129411. <https://doi.org/10.1371/journal.pone.0129411>
- McManus, G. B., & Katz, L. A. (2009). Molecular and morphological methods for identifying plankton: What makes a successful marriage? *Journal of Plankton Research*, 31(10), 1119-1129. <https://doi.org/10.1093/plankt/fbp061>
- Nagl, S., Tichy, H., Mayer, W. E., Samonte, I. E., McAndrew, B. J., & Klein, J. (2001). Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 20(3), 361-374. <https://doi.org/10.1006/mpev.2001.0979>
- Nwachi, O. F., Esa, Y., Christianus, A., & Kamarudin, S. M. (2020). Sexual restiveness and colouration between partial diallel cross of genetically improved farmed tilapia 'GIFT' and UPM red tilapia. *Jordan Journal of Biological Sciences*, 13(2), 219-222
- Nwachi, O. F., & Esa, Y. (2016). A review of production protocols used in producing economically viable monosex tilapia. *Journal of Fisheries and Aquatic Science*, 11 (January), 1-11. <https://doi.org/10.3923/jfas.2016.1.11>
- Nyingi, D., De Vos, L., Aman, R., & Agnès, J. F. (2009). Genetic characterization of an unknown and endangered native population of the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae; Teleostei) in the Loboi Swamp (Kenya). *Aquaculture*, 297(1-4), 57-63. <https://doi.org/10.1016/j.aquaculture.2009.09.017>
- Ogden, R. (2008). Fisheries forensics: The use of DNA tools for improving compliance, traceability and enforcement in the fishing industry. *Fish and Fisheries*, 462-472. <https://doi.org/10.1111/j.1467-2979.2008.00305.x>
- Oladimeji, T. E., Awodiran, M. O., & Komolafe, O. O. (2015). Genetic differentiation studies among natural populations of *Tilapia zillii*. *Notulae Scientia Biologicae*, 7(4), 423-429. <https://doi.org/10.15835/nsb749649>
- Rastogi, G., Dharne, M. S., Walujkar, S., Kumar, A., Patole, M. S., Shouche, Y. S., et al. (2007). Species identification and authentication of tissues of animal origin using mitochondrial and nuclear markers. *Meat Science*, 76, 666-674. <https://doi.org/10.1016/j.meatsci.2007.02.006>
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.
- Tamura, K., Stecher, G., Peterson, D., & Filipski, A. (2013). MEGA6: Molecular evolutionary genetics analysis

- version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729. <https://doi.org/10.1093/molbev/mst197>
- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360, 1847-1857. <https://doi.org/10.1098/rstb.2005.1716>
- Wu, L., & Yang, J. (2012). Identifications of captive and wild tilapia species existing in Hawaii by mitochondrial DNA control region sequence. *PLoS ONE*, 7(12), 1-9. <https://doi.org/10.1371/journal.pone.0051731>
- Xiaowei, L., Dayu, L., Zhiying, Z., Jing-Ling, Z., & Jue, Y. H. (2016). Genetic variation of mitochondrial DNA D-loop sequences from different strains of Nile tilapia (*Oreochromis niloticus*). *Southwest China Journal of Agricultural Science*, 8, 2-7.
- Zou, Z., Li, D., Zhu, J., Han, J., Xiao, W., & Yang, H. (2015). Genetic variation among four bred populations of two tilapia strains, based on mitochondrial D-loop sequences. *Mitochondrial DNA*, 26(3), 426-430. <https://doi.org/10.3109/19401736.2013.855735>



Determination and Comparison of Macro, Trace and Toxic Element Content of Some Roughages by ICP OES Technique

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ABSTRACT

It is critical to determine the amount of mineral and toxic elements in roughages, which play an important role in animal nutrition. In this study, macro (Ca, K, Na, Mg, P), trace (Fe, Mn, Zn, Cu, Ni, Co, Cr, Sn, V, Si, Se, Sr, Sb, B), and toxic element (Al, As, Be, Cd, Pb) analysis of 14 roughages were determined by the Inductively Coupled Plasma Optik Emission Spectrometry (ICP OES) method. CRM (NCS DC 73350, poplar leaves) confirmed the accuracy of the method. The samples were solubilized according to the microwave wet digestion method. The macro, trace and toxic element contents of 14 roughages were compared and evaluated according to the results of the analyses. Furthermore, the hierarchical clustering analysis (HCA) displayed the groups as dendrograms. Toxic elements like As, Be, Cd, Co, and Sb were not found in any of the samples. The only one that was found was Pb, which was 0.181±0.12, 0.45±0.07, and 0.40±0.13 mg kg⁻¹ in grain corn leaf, sorghum leaf, and lentil straw, in that order. On the other hand, Sn was found as 0.01±0.005 mg kg⁻¹ only in fodder pea stalk and Si was identified as 1015±140 mg kg⁻¹ only in chickpea straw. In the study, it can be suggested that different roughages contain macro and trace elements at varying rates, and these roughages can be included in the rations according to these element ratios.

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Bazı Kaba Yemlerin Makro, İz ve Toksik Element İçeriklerinin ICP OES Tekniği Kullanılarak Belirlenmesi ve Karşılaştırılması

ÖZET

Ruminant hayvanların beslenmesinde önemli bir yere sahip olan kaba yemlerde mineral ve toksik elementlerin miktarının belirlenmesi oldukça önemlidir. Bu çalışmada, 14 kaba yemin makro (Ca, K, Na, Mg, P), iz (Fe, Mn, Zn, Cu, Ni, Co, Cr, Sn, V, Si, Se, Sr, Sb ve B) ve toksik element (Al, As, Be, Cd, Pb) miktarları İndüktif Eşleşmiş Plazma Optik Emisyon Spektrometresi (ICP OES) yöntemi ile belirlenmiştir. Yöntemin doğruluğu CRM (NCS DC 73350, kavak yaprakları) ile teyit edilmiştir. Örnekler mikrodalga yaş yakma yöntemine göre çözündürülmüştür. Analiz sonuçları çerçevesinde 14 kaba yemin makro, iz ve toksik element içerikleri karşılaştırılmış ve değerlendirilmiştir. Ayrıca, hiyerarşik kümeleme analizi (HCA) ile gruplar dendrogram olarak gösterilmiştir. Çalışma sonucunda, tane mısır yaprağı, sorgum yaprağı ve mercimek samanında sırasıyla 0,181±0,12, 0,45±0,07, 0,40±0,13 mg kg⁻¹ Pb tespit edilirken, toksik elementler (As, Be, Cd, Co ve Sb) hiçbir örnekte tespit edilmemiştir. Öte yandan, Sn sadece yem bezelyesi sapında (0.01±0.005 mg kg⁻¹) ve Si sadece nohut samanında (1015±140 mg kg⁻¹) tespit edilmiştir. Çalışmada farklı kaba yemlerin değişen oranlarda makro ve eser element ihtiva ettiği ve bu kaba yemlerin içerdikleri bu element oranlarına göre rasyonlara dahil edilebileceği önerisi yapılabilir.

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INTRODUCTION

In addition to using high-yield breeds, countries must feed animals with rations that meet their nutritional requirements in an adequate and balanced manner to develop livestock farming. In the feeding of ruminants, various industrial wastes, pulps, and some cereal residues and straws are also used to meet the need for roughage due to insufficient quality roughage resources (Mohamoud & Kılıç, 2018). Ruminants rely heavily on the nutrients in the roughages they add to their rations, their microbial digestion in the rumen, and the conversion of these nutrients into metabolic energy (Selçuk et al., 2023). The contribution of breeding studies to the increase in efficiency in animal production was found to be 30–35%, and the contribution of improvements in the environment, care, nutrition, feed, feed processing, and technologies was found to be 60–65% (Kutlu & Özen, 2009). Sources defined as active substances in animal nutrition are basically examined in two basic groups: minerals and vitamins. The mineral requirements of animals are met by forming feed mixtures in certain ratios. While the needs of farm animals for macrominerals such as sodium, calcium, and phosphorus are met by the direct use of natural resources rich in these minerals, the need for trace minerals and vitamins is met by the addition of premixes prepared based on the relevant standards to the feed. Developments in this field include innovations in production technologies, especially in order to increase the usefulness of minerals and the stability and effectiveness of vitamins, as well as the use of preserved amino acid sources in the rumen for high-yielding dairy cows (Kutlu & Özen, 2009). The most significant advancement in mineral nutrition in recent years has been the discovery of newly defined functions for certain trace minerals and their use as feed supplements. Especially studies on Se are gaining importance with its antioxidant power (Surai, 2002a, 2005; Yu et al., 2008) and it improves reproductive quality in animals. (Surai, 2002b; Juniper et al., 2009). Despite ongoing detailed discussions on the essential properties of Cr, a trace element, experts agree that this element plays a crucial role in energy, fat, and protein metabolism (Pechova & Pavlata, 2007; Wang et al., 2007; Wang & Xu, 2008). This element, which forms the core of the glucose tolerance factor, aids insulin and optimizes glucose metabolism, prevents fat synthesis from carbohydrates, encourages the burning of fat for energy in the cell (mitochondria), and improves performance while reducing blood glucose and cholesterol concentrations. Within the scope of trace minerals and their importance in animal nutrition, a significant number of studies have been conducted on the B element in recent years, showing that boron is related to immune function (Hunt, 2003), reduces cholesterol and triglyceride levels, reduces lipid peroxidation, and increases the durability of antioxidant enzymes (Bakken & Hunt, 2003), improves mineral balance, and affects calcium metabolism and bone development (Qin & Klandorf, 1991; Kurtoğlu et al., 2001; Kurtoğlu et al., 2002; Eren et al., 2004; Bozkurt et al., 2007). It has been stated that it improves egg shell quality (Yeşilbağ & Eren, 2007) and increases the synthesis of some steroid hormones (Small et al., 1997). On the other hand, under field conditions, V phytotoxicity (chlorosis and dwarfing) is extremely rare. Intake of feed with 10 to 300 mg kg⁻¹ feed dry matter animals induced black diarrhea, weakness, spontaneous abortions, decreased milk production, and high mortality in animals.

Minerals are essential because they cannot be synthesized in the organism, that is, they are necessary for the continuation of vital activities (Ergül, 1993; Kutlu et al., 2005); therefore, a significant part of these substances that animals need are met by feed (Gökkuş et al., 2013; Eğritaş & Önal Aşçı, 2015). Plants, on the other hand, can obtain the nutrients they need from the soil as long as they are in an available form (Özyazıcı, 2019). Animals need to receive minerals with their rations, as their bodies cannot synthesize them biologically. These inorganic substances are essential for their healthy survival and optimal genetic structure. In addition to vitamins, the effective utilization of both vitamins and other nutrients is crucial. Macro elements, especially phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), which are among the mineral substances, are as important as other nutrients in the nutrition of animals (Ergül, 1993; Gülümser et al., 2017). The skeletal system of animals, the structure of teeth and bones, the development of the muscle and nervous system, and certain vital elements in animal physiology all depend on these minerals. It is well known that these minerals play a crucial role in various activities; Minerals with more than 50 milligrams per kilogram of body weight are called macrominerals (Kutlu et al., 2005). Calcium is essential for milk yield as well as for the skeleton, eggshell, and teeth. It enters the structure and takes part in the transmission of nerve impulses; its deficiency resulted in rickets, osteomalacia, and hypocalcaemia (Gürsoy & Macit, 2017). A deficiency in Ca can lead to bone softening, particularly in young animals, and deformation of bones in older animals. Acid-base balance involves K, and a deficiency leads to tetany (Gürsoy & Macit, 2017). Moreover, Mg functions as an enzyme activator, causing meadow tetany in cases of deficiency (Aksoy et al., 2000; Türkmen et al., 2011; Gürsoy & Macit, 2017). Magnesium deficiency is associated in decreased functions of some elements such as Ca, Mg, vitamin D, methionine, and arginine (Aksoy et al., 2000; Demir, 2005; Türkmen et al., 2011; Çimrin & Demirel, 2012). In addition, in Mg deficiency, various diseases may occur in

animals, and even death may occur if necessary precautions are not taken. (Ensminger et al., 1990; Ergül, 1993; Sabah & Çelik, 2001; Tekeli et al., 2003). In general, phosphorus (P) is essential for fertility, bone, and it functions in the formation of teeth, in case of deficiency, especially pica (abnormal labour) syndrome is observed. Additionally, P deficiency causes bone development to stop and teeth to become damaged. It causes fractures, loss of appetite, and weakness. On the other hand, minerals that contain less than 50 milligrams per kilogram of body weight, known as microminerals, include Fe, Cu, Zn, Mn, B, I, F, Se, Mo, Cr, V, Sn, Si, and Ni (Kutlu et al., 2005). Boron is involved in sugar transport in plants, cell wall formation, lignin formation, carbohydrate, respiration, and phenol metabolism, as well as the structural and functional properties of biological membranes, the endocrine system, the immune system, and the brain. Boron is involved in Ca and Mg metabolism. Iron is involved in the structure of hemoglobin in milk-fed animals, pregnant animals, and ovulation. Animals during the period have a high need for Fe, and a deficiency can lead to anemia (Gürsoy & Macit, 2017). In this regard, adding it to rations can effectively address anemia problems in animals. Copper forms the coenzyme moiety, or essential moiety, of many enzymes, participates in the structure of feather pigments, and contributes to hemoglobin synthesis in cases of deficiency, nervous ailments, anemia, and discoloration of hair and fleece (Gürsoy & Macit, 2017). Manganese is involved in bone development and cholesterol synthesis; deficiency, perosis (deformities of foot and leg skeletons in chickens), eggshell formation, deterioration, and reproductive impairment are observed (Gürsoy & Macit, 2017). Zinc has an antioxidant effect and takes part in the structure of enzymes in deficiency, decreased food intake, decreased feed utilization, impaired reproductive functions, growth retardation, and abnormalities in feathers, skin, hair, and wool (Gürsoy & Macit, 2017). On the other hand toxic elements; Cadmium (Cd) is a mineral element highly toxic to animals and humans, and not essential to physiological and biochemical functions (Reis et al., 2010). Cadmium, when more than 15 µg is taken daily, it accumulates in the kidneys, disrupts renal arterioles and glomeruli. In excess, it can be fatal. For cattle, diet containing 5 to 30 mg of Cd kg⁻¹ and, for sheep, diet containing > 40 mg of Cd kg⁻¹ of DM (dry matter) it would be toxic effect. In animal diets, the maximum concentration of Cd tolerated is 0.5 mg kg⁻¹ (McDowell, 1992; NRC, 2001). When Cd is ingested, it is absorbed by intestinal cells and transported by blood flow to the liver. In the liver, this mineral element induces metallothionein synthesis, a protein that is involved in detoxification of heavy metals. Lead (Pb) is also a ubiquitous heavy metal. Lead has biological functions in the animal body but is highly toxic to animals and humans (NRC, 2001). Being one of the most dangerous minerals to animal health, has worldwide distribution and is accumulated in the environment by industrial pollution (Patra et al., 2006; Patra et al., 2007; Swarup et al., 2007). Lead poisoning in animals can be considered a risk to public health, since there is an accumulation of this mineral in meat and milk (when animals have blood concentration ≥ 0,20 µg of Pb mL⁻¹), for human consumption, it may intoxicate them (Swarup et al., 2005). For cattle, 400 to 600 mg of Pb kg⁻¹ of BW (body weight), for sheep 4,5 mg of Pb kg⁻¹ of BW, for goats 400 mg of Pb kg⁻¹ is considered toxic. Arsenic (As) causes poisoning and even deaths in animals and humans. Efforts are being made to combat arsenic, which is widely found in nature, but is frequently brought to the agenda due to its use in the production of various industrial, medical and agricultural chemicals (Kaya et al., 1990). Conversely, elemental As is not toxic, although some arsenical compounds are highly toxic. There are no reports of animal toxicity attributed to naturally occurring Al in the environment. Bentonite, kaolin, Al₂(SO₄)₃, Al₂O₃, and Al(OH)₃ have been used as growth stimulants for rabbits, chickens, sheep, cattle, and hogs without adverse effects as long as they composed no more than 1-2 percent of the diet (Sorenson et al., 1974). Under natural dietary conditions because of the low concentrations of Sb believed generally to occur in plants, toxicity to animals from plant sources is unlikely. Sittig (1976) reported, however, that marine animals concentrate Sb in their muscle tissue, and that studies had indicated certain levels in this tissue that were toxic to some fish. Moreover, many reports do not specify the chemical form of an element when discussing its toxicity. It can be assumed for most of these reports that, under the specified natural or experimental conditions, the element existed in an undetermined compound and that only the total concentration of the element was known. For most reports in which the basis for expressing concentrations of an element in plant or animal tissues is not specified, a dry-weight basis can be assumed. Some reports define the minimum lethal dose (MLD); that is, the amount of a substance that kills the organism in one dose. In reports that relate toxicity to body weight of the experimental animals, "live" or "wet" weight of the animal is commonly used. Selenium (Se) is an essential micromineral to animals (Kommisrud et al., 2005; Haddad and Alves, 2006; Carroll and Forsberg, 2007) and humans. This mineral element is part of several selenoproteins, including glutathione peroxidase (GSH-Px). On the other hand, when Se is ingested on high concentrations above the maximum tolerable dose of Se in diet (2 ppm) (McDowell, 1992) may cause poisoning in animals and humans, and is also known as selenosis (Papp et al., 2007). Furthermore, foods containing ≥ 200 mg Se kg⁻¹ for cattle and more than 0.8 mg Se kg⁻¹ BW for sheep are considered toxic. The toxic doses have not been known yet because there is disagreement between results of published studies, and still the physiological mechanisms involved in mineral poisoning are also not yet understood. For some mineral elements, the published papers that have evaluated these mechanisms are lacking probably due to the difficulty of describing the

physiological mechanisms, changes involved in the process of poisoning and also difficulty of establishing toxic dose of a mineral element due to the dependence of each mineral element, its form, specie and age of animals and the interaction between minerals in metabolism (Reis et al., 2010)

Graphite furnace atomic absorption spectrometry (GFAAS) (Rusin et al., 2021), flame atomic absorption spectrometry (FAAS) (Ahmadi & Ziraati, 2015), inductively coupled plasma optic emission spectrometry (ICP OES) (Tokay & Bağdat, 2022), mass spectrometry (ICP MS) (Kowalska et al., 2020; Bağdat et al., 2019), and polarography and voltammetry (Palisoc et al., 2018) are some of the methods used to find elements in substances. Among these techniques, ICP OES offers a wide dynamic linear range.

The purpose of this study was to compare the macro, trace, and potential toxic elements of 14 roughages, which play a significant role in animal nutrition, and to make a valuable contribution to the literature and research on this topic.

MATERIAL and METHOD

After harvesting the crops, 14 roughages were collected from the field in sufficient quantities and placed in polythene bags. Coarse impurities were removed from the samples, and then the samples were kept in an oven at 65 °C for 24 hours.

Reagents and Digestion Procedure

The samples were thoroughly crumbled in a blender, and 0.3 g of each sample was weighed into TFM containers for microwave (Milestone Start D) wet digestion and 2 ml of 30% H₂O₂, then 8 ml of 65% HNO₃ was added to the samples, and solubilization was carried out according to the digestion program in Table 1.

Table 1. Microwave digestion program for roughages samples

Çizelge 1. Kaba yemler için mikrodalga yakma programı

Step (plant samples)	Time (min)	T	Power (W) (°C)
1	20:00	180	1200
2	15:00	180	1200

Laboratory Analyses

After solubilization, the samples were made up to 50 ml with ultrapure water (Millipore Corporation, USA) and analyzed in ICP OES (Thermo ICAP 6300, England). The operating conditions of the ICP OES device are shown in Table 2. In addition, the element content of certified reference materials (NCS DC 73350, poplar leaves) and recovery of elements by method are shown in Table 3, and the amount of elements contained in the roughage is shown in Table 4.

Table 2. Instrumental operating conditions for Thermo ICAP 6300 ICP-OES

Çizelge 2. Thermo ICAP 6300 ICP-OES için enstrümantal çalışma koşulları

Parameter	Normal	Hydride System
RF Power	1100 W	1300 W
Flush Pump rate	100 rpm	-
Pump speed	50 rpm	30 rpm
Purge gas	Argon	Argon
Pump tubing type	Tygon Orange/white	-
Coolant Gas		
Flow	12 L min. ⁻¹	16 L min. ⁻¹
Auxiliary gas		
Nebulizer flow	0.6 L min. ⁻¹	0.3 L min. ⁻¹
Auxiliary gas flow	0.5 L min. ⁻¹	0.5 L min. ⁻¹
View Mode	Axial, Radial	Axial
Auto sampler	Cetac ASX-260	-

Table 3. Element content of certified reference materials (NCS DC 73350, poplar leaves) and recovery of elements by method

Çizelge 3. Sertifikalı referans maddenin (NCS DC 73350, kavak yaprakları) element içeriği ve elementlerin metotla geri kazanımı

Elements	Found value (mg kg ⁻¹)	Certified value (mg kg ⁻¹)	Recovery (%)
Al (Aluminium)	842.4±11	1040±60	81
As (Arsenic)	0.344±0.2	0.37±0.09	93
B (Boron)	51.4±0.6	53±5	97
Ca (Calcium)	11615±78	13506±161	86
Cd (Cadmium)	0.316±0.006	0.32±0.07	99
Co (Cobalt)	0.428±0.006	0.42±0.03	102
Cr (Chromium)	0.555±0.46	0.55±0.007	101
Cu (Copper)	7.22±0.07	9.3±1	77.6
Fe (Iron)	275±2.4	274±17	100.4
K (Potassium)	102.9±93	13800±70	74.6
Mg (Magnesium)	4855±15	6500±50	74.7
Mn (Manganese)	36.9±0.25	45±4	82
Ni (Nickel)	2.01±0.2	1.9±0.3	106
P (Phosphorus)	2569±6.8	2435±15	105.5
Pb (Lead)	1.635±0.76	1.5±0.3	109
Sr (Strontium)	113.9±1	154±9	74
Zn (Zinc)	32.5±0.25	37±3	88

Statistical Analyses

Hierarchical clustering analysis (HCA) was performed according to the between-group linkage method in the SPSS-22 statistical program, and results are shown as a dendrogram in Figure 4.

RESULTS and DISCUSSION

Macro elements: In terms of Ca, lentil straw had the highest value (11711±33 mg kg⁻¹) followed by camelina stalk (7343±5.8 mg kg⁻¹), while the lowest value was in the grain corn cob (309±4.1 mg kg⁻¹). The study found lentil straw to be a very good source of calcium for animals. Furthermore, in terms of K, sweet corn stalk (30685±120 mg kg⁻¹) and fodder pea stalk (27251±48 mg kg⁻¹) had the highest value, while sorghum straw (4054±33 mg kg⁻¹) had the lowest value. Mg was highest in sweet corn leaf (3437±91 mg kg⁻¹) and lowest in grain corn cob (351±2 mg kg⁻¹). On the other hand, wheat straw had the highest Na value (369±6.7 mg kg⁻¹), while grain corn leaf (102±9.4 mg kg⁻¹) had the lowest value. In terms of P, sorghum stalk had the highest value (497±3.5 mg kg⁻¹), followed by sweet corn leaf (435±14 mg kg⁻¹), while grain corn cob (107±0.07 mg kg⁻¹) and soybean stalk (107±0.4 mg kg⁻¹) had the lowest value. Table 4 and Figure 1 display the results. When compared with the study conducted by Gürsoy & Macit, (2017), P, K, Ca, and Mg ratios of macronutrients of legume forage crops samples were changed 1.16-1.28%, 0.70-2.69%, 0.56-1.61%, and 0.11-0.51%, respectively. While the macronutrients of wheat forage crops, P ranged between 1.10-1.19%, K content 1.99-3.25%, Ca content 0.09-1.15%, and Mg content 0.07-0.26%. On the other hand, the P values obtained in this study were found to be considerably lower than those of both wheat forage crops and legume forage crops. While Ca values were in the same range in Camelina stalk, rice straw, and wheat straw with wheat forage crops, but lower than legume forage crops. Mg values of rice straw were in the same range of both wheat forage crops and legume forage crops, but Mg values of wheat straw and camelina stalk were lower than the others. On the other hand, K values were in the same range as legume forage crops but lower than wheat forage crops. Emmanuel et al. (2022) used AAS method in the analysis of sweet sorghum stalk samples and reported that the elemental concentrations were 25.02-22.87 for Ca, 27.14-45.40 for K, 25.02-22.52 for Na, 8.60-8.50 for Mg, 12.81-14.98 for Fe, 3.71-2.19 for Mn, 0.96-0.52 for Cu, and 18.15-17.90 mg/kg for P.

Figure 1 presents the radar graph of macro elements in roughages. This graph displays the prominent elements in each roughage.

Table 4. The amount of elements contained in some roughages (mg kg⁻¹, n=3, dry weight)

Çizelge 4. Kaba yemlerin içerdiği element miktarı (mg kg⁻¹, n=3, kuru ağırlık)

Elements	Al	As	B	Be	Ca	Cd	Co	Cr	Cu	Fe
Grain corn stalk	14.6±1	nd	6.9±0.2	nd	4503±0.2	nd	nd	0.52±0.5	4.1±0.35	39.5±1.6
Grain corn leaf	10±2.5	nd	10±0.2	nd	5281±270	nd	nd	0.8±0.8	3.9±1.3	38.5±0.5
Grain corn cob	32.9±0.9	nd	4.4±0.04	nd	309±4.1	nd	nd	nd	2±0.5	20.1±0.7
Sweet corn stalk	13.4±0.6	nd	8.6±0.14	nd	2774±21	nd	nd	0.38±0.16	1.3±0.5	31±1.5
Sweet corn leaf	29±2	nd	11.4±0.5	nd	6963±181	nd	nd	0.75±0.6	3.48±0.5	26±0.3
Sorghum straw	nd	nd	5.7±0.13	nd	1473±27	nd	nd	1.1±0.25	4.14±0.26	9.25±0.7
Sorghum leaf	41±1.3	nd	4.1±0.02	nd	4952±150	nd	nd	1.69±0.4	7.6±0.44	90±2.7
Wheat straw	21±2	nd	5.8±	nd	2679±53	nd	nd	1.7±1.2	4.2±0.9	51±0.9
Lentil straw	278±3	nd	20±0.02	nd	11711±33	nd	nd	1.4±0.4	7±0.5	334±5
Fodder pea stalk	22.8±0.12	nd	9.2±0.13	nd	5626±103	nd	nd	0.48±0.34	3.9±0.54	56±0.8
Soybean stalk	7±1.7	nd	14.6±0.36	nd	6381±310	nd	nd	0.85±0.07	4.8±1.4	28.8±0.4
Camelina stalk	64.9±3	nd	15±0.01	nd	7343±5.8	nd	nd	0.56±0.07	4.5±0.45	96±1.1
Rice straw	nd	nd	4.5±0.1	nd	2019±1.9	nd	nd	0.61±0.19	3.9±0.2	19.8±0.6
Chickpea straw	4.4±1.2	nd	9.7±0.35	nd	4874±168	nd	nd	0.50±1	2.7±0.13	10.5±0.34
Elements	K	Mg	Mn	Na	Ni	P	Pb	Sb		
Grain corn stalk	10319±23.1	2378±6.3	89±0.4	122±0.1	0.03±0.06	227±1.4	-	nd	-	nd
Grain corn leaf	11780±467	2614±127	109±4.7	102±9.4	nd	181±1.5	0.18±0.12	nd	-	nd
Grain corn cob	5154±26	351±2	6.6±0.01	132±3.2	0.60±0.05	107±0.07	nd	nd	-	nd
Sweet corn stalk	30685±120	1301±1.2	24.9±0.2	171±2.8	0.55±0.05	223±2.5	nd	nd	-	nd
Sweet corn leaf	18866±532	3437±91	90±2.5	138±5	1±0.2	435±14	nd	nd	-	nd
Sorghum straw	4054±33	490±3.6	16±0.08	229±6	0.18±0.04	215±5.8	nd	nd	-	nd
Sorghum leaf	12238±269	1548±35	42±1	117±4.5	0.46±0.004	497±3.5	0.45±0.07	nd	-	nd
Wheat straw	19182±241	680±10	14±0.2	369±6.7	1.6±0.008	162±0.8	-	nd	-	nd
Lentil straw	18933±127	1936±5	43±0.3	157±0.5	2.8±0.1	225±2	0.40±0.13	nd	-	nd
Fodder pea stalk	27251±48	989±2	7.3±0.13	180±0.1	0.30±0.005	216±3	nd	nd	-	nd
Soybean stalk	9043±200	2507±51	17±0.1	133±3.5	1±0.005	107±0.4	nd	nd	-	nd
Camelina stalk	11934±101	698±1.6	10±0.18	197±0.9	0.97±0.03	309±3	nd	nd	-	nd
Rice straw	14213±23	1962±12	60.3±0.5	320±3.9	0.80±0.1	118±0.3	nd	nd	-	nd
Chickpea straw	19244±565	1001±38	4.9±0.06	115±10	nd	144±3.75	nd	nd	-	nd
Elements	Se	Si	Sn	Sr	V	Zn				
Grain corn stalk	0.8±0.3	nd	nd	41.2±0.09	3.8±0.9	20.1±0.05				
Grain corn leaf	0.52±0.5	nd	nd	39.3±1.7	3.2±0.6	15.9±0.06				
Grain corn cob	1.2±0.2	nd	nd	6.2±0.03	0.20±0.04	74±0.05				
Sweet corn stalk	nd	nd	nd	79.4±0.35	1.8±0.2	25±0.2				
Sweet corn leaf	0.73±0.3	nd	nd	53.6±1.7	4.7±0.7	22.6±0.7				
Sorghum straw	0.51±0.02	nd	nd	9±0.1	0.53±0.14	5.8±0.2				
Sorghum leaf	0.62±0.08	nd	nd	35±0.8	1.4±0.2	14.6±0.1				
Wheat straw	0.92±0.06	nd	nd	43±0.6	0.34±0.48	14.6±0.07				
Lentil straw	0.8±0.9	nd	nd	33.5±0.09	nd	16±0.12				
Fodder pea stalk	1.6±0.4	nd	0.01±0.005	71.3±0.25	1.2±0.04	3.9±0.07				
Soybean stalk	1.4±0.002	nd	nd	64±1.1	3.5±0.3	5.9±0.1				
Camelina stalk	0.96±0.79	nd	nd	65±0.5	nd	8.6±0.08				
Rice straw	1.75±0.2	nd	nd	30±0.1	2.5±0.01	15±0.14				
Chickpea straw	0.55±0.1	1015±140	nd	46.5±1.3	1.25±0.5	8±0.19				

nd: not detected

Trace Elements

Lentil straw had the highest B content (20±0.02 mg kg⁻¹), Fe (334±5 mg kg⁻¹) and Ni (2.8±0.1 mg kg⁻¹) (Table 4, Figure 2). Furthermore, when it came to Fe content, lentil straw outperformed other roughages by a significant margin. Wheat straw had the highest value in Cr (1.7±1.2 mg kg⁻¹), followed by sorghum leaf (1.69±0.4 mg kg⁻¹). On the other hand, Cu was highest in sorghum leaf (7.6±0.44 mg kg⁻¹) and lowest in sweet corn stalk (1.3±0.5 mg kg⁻¹). Grain corn leaf (109±4.7 mg kg⁻¹) and sweet corn leaf (90±2.5 mg kg⁻¹) were found to be high in Mn, but, chickpea straw (4.9±0.06 mg kg⁻¹) had the lowest Mn content. In terms of Ni, in the grain corn leaf and the chickpea straw was below the LOD. The highest value for Se was found in rice straw (1.75±0.2 mg kg⁻¹). The stalk of sweet

corn was also below the LOD. In terms of Sr, sweet corn stalk ($79.4 \pm 0.35 \text{ mg kg}^{-1}$) had the highest value, followed by fodder pea stalk ($71.3 \pm 0.25 \text{ mg kg}^{-1}$) and the lowest value was found in grain corn cob ($6.2 \pm 0.03 \text{ mg kg}^{-1}$). The highest V value was observed in sweet corn leaf ($4.7 \pm 0.7 \text{ mg kg}^{-1}$), while lentil straw and camelina stalk were below the LOD, and Zn was highest in grain corn cob ($74 \pm 0.05 \text{ mg kg}^{-1}$), and lowest in fodder pea stalk ($3.9 \pm 0.07 \text{ mg kg}^{-1}$).

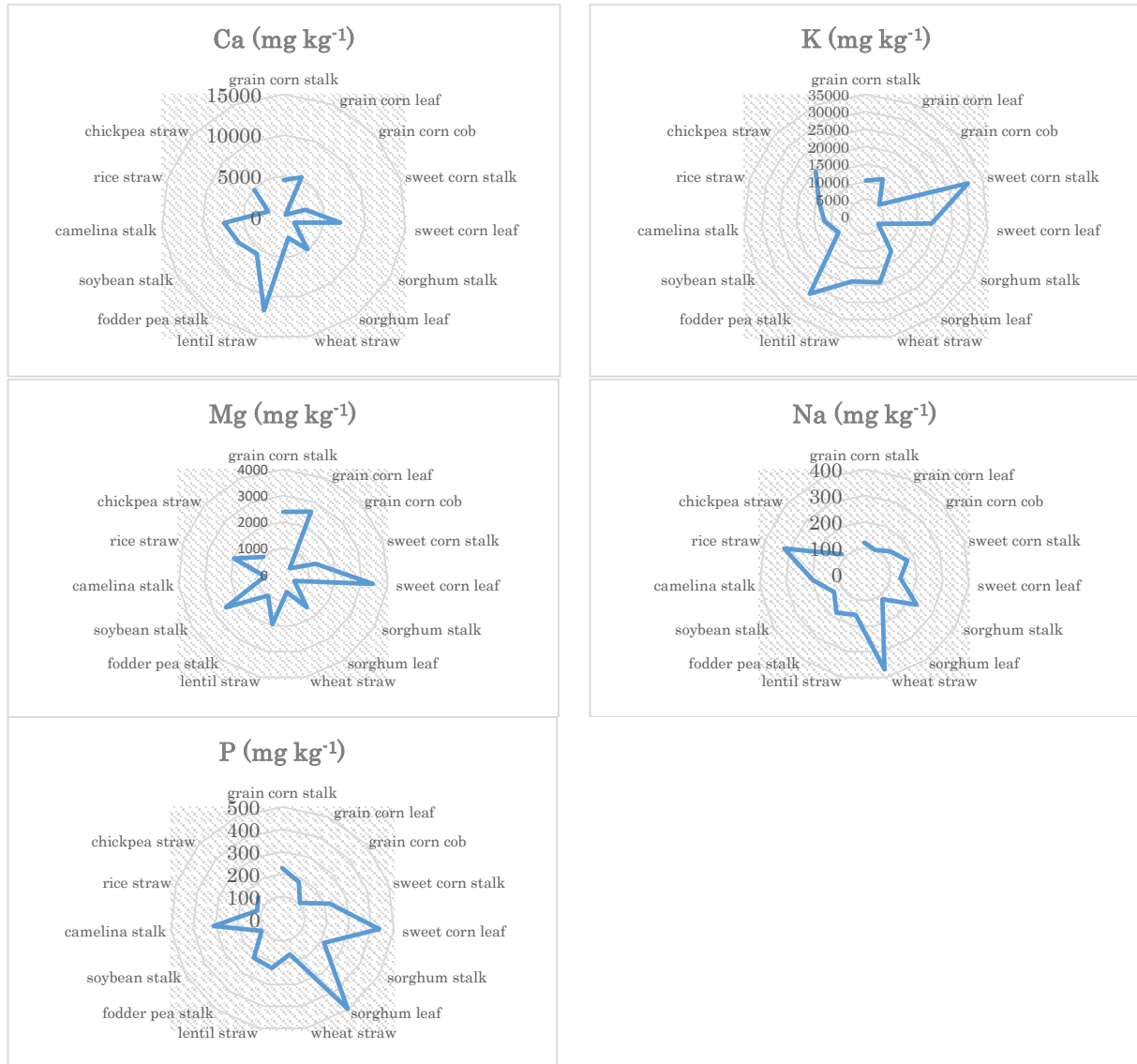
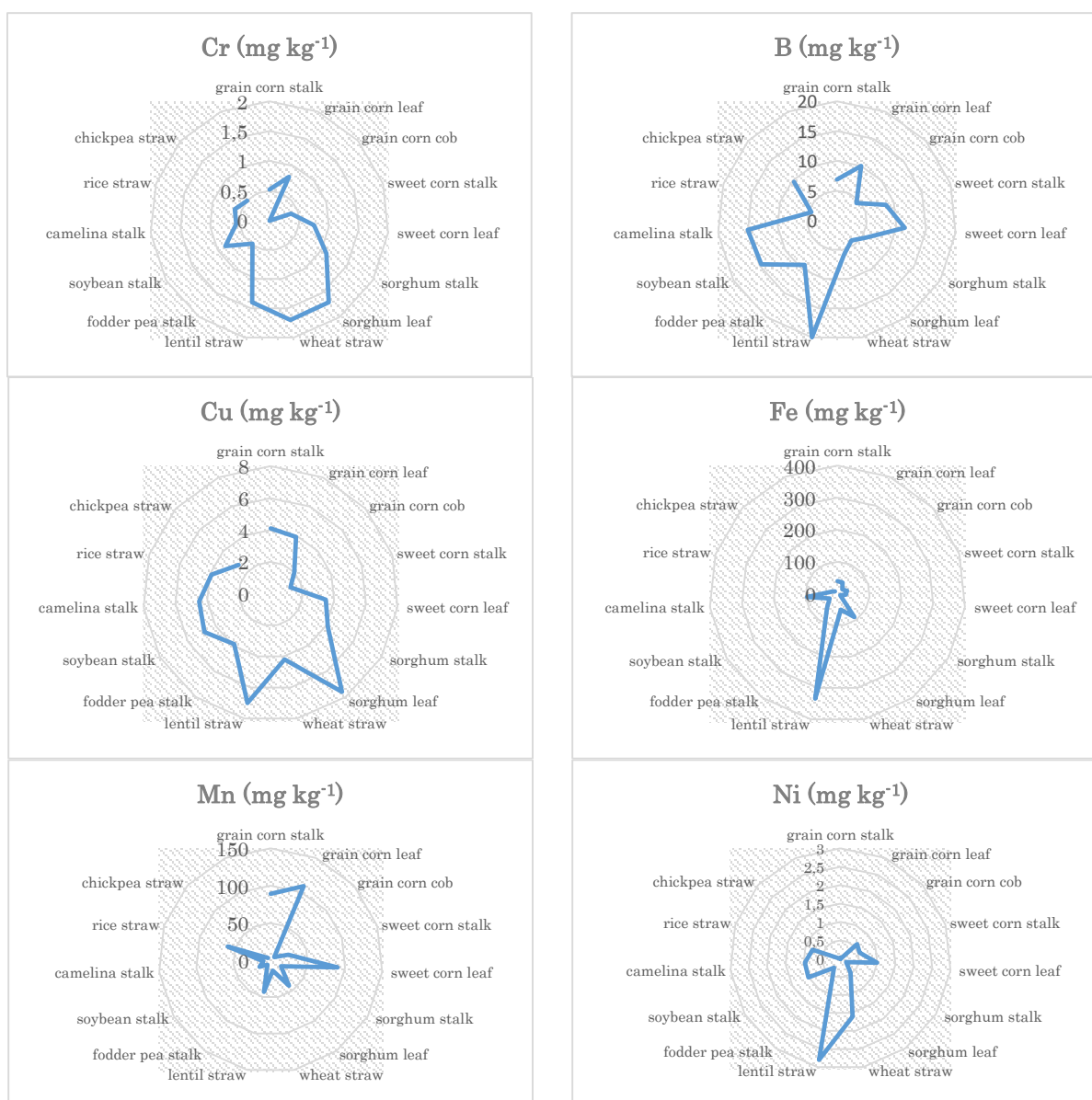


Figure 1. Graphical representation of macro element concentrations in roughages
Şekil 1. Kaba yemlerdeki makro element konsantrasyonlarının grafiksel gösterimi

Sn was found only in fodder pea stalk ($0.01 \pm 0.005 \text{ mg kg}^{-1}$), while Si was found only in chickpea straw ($1015 \pm 140 \text{ mg kg}^{-1}$). All samples contained Co and Sb below the limit of detection. According to Gürsoy & Macit, (2017), micronutrient amounts of legume forage crops were changed 105.9-893.7 ppm, 2.22-12.36 ppm, 14.11-195 ppm, 18.18-66.58 ppm and 5.91-40.39 ppm for Fe, Cu, Zn, Mn and B, respectively. While micronutrient amounts determined for Fe, Cu, Zn, Mn and B in wheat forage crops varied between 74.90-630.6 ppm, 4-9.84 ppm, 31.49-335.6 ppm, 24.63-94.51 ppm and 0.35-26.64 ppm, respectively. In this study, the trace elements of lentil straw, chickpea straw, and soybean stalk, which were used as legume forage, were compared with those of legume and wheat forage crops in Gürsoy and Macit (2017) study. The current study found that lentil straw had about the same Fe content as wheat and legume forage crops. On the other hand, the amount of Fe in chickpea straw and soybean stalk was less than that in wheat and legume forage crops. On the other hand, Cu was in the same range with both wheat and legume forage crops. In lentil straw, Zn was only in the same range as legume forage crops, but in chickpea straw and soybean stalk, it was considerably lower than the others. On the other hand, Mn in lentil straw was in the same range as both wheat and legume forage crops. However, chickpea straw and soybean stalk

had lower values. B was in the same range across all crops. On the other hand, according to Baloda et al. (2018), in a study conducted in India, elemental contents in green and dry roughages was reported to be average 0.49% for Ca in sorghum as green roughage with a range of 0.46-0.55% and ranged 0.28-0.52% for P with an average of 0.42%, and the critical levels for Ca and P were reported to be 0.30 and 0.25%. It was reported that the range for Zn was 19.78-29.55 ppm, the average value for Zn was 27.16 ppm and the critical level was 30 ppm. It was reported that the Cu range was around 27.3-50.2 ppm. It was reported that the range of Fe was between 147.3-203.6 ppm and the average was 179 ppm. It was stated that the highest Fe value was above 500 ppm in feeds. Mn concentration in sorghum was 37.9-64.3 ppm and the average value was 50.98. It was reported that the critical value was around 40 ppm. In dry roughage, Ca in wheat straw averaged 0.37% and the range was 0.28-0.45 ppm. Some samples were 0.30 % below the critical level. The range of P concentration was 0.14-0.25 % with an average of 0.20 % and below the limit of 0.25 %. Zn content averaged 19.6 ppm and the range was found to be between 12.6-27.7 ppm with values below the critical level (30 ppm). Cu content in wheat straw was 7.70 ppm on average and the range was 5.8-9.5 ppm. The average Fe content was 227.2 ppm and the range was 207.3-247.2 ppm and the values were 50 ppm above the critical level. Mn content in wheat straw averaged 43.8 ppm with a range of 36.9-54.04 ppm.

Figure 2 presents the radar graph of trace elements in roughages. This graph displays the prominent element in each roughage.



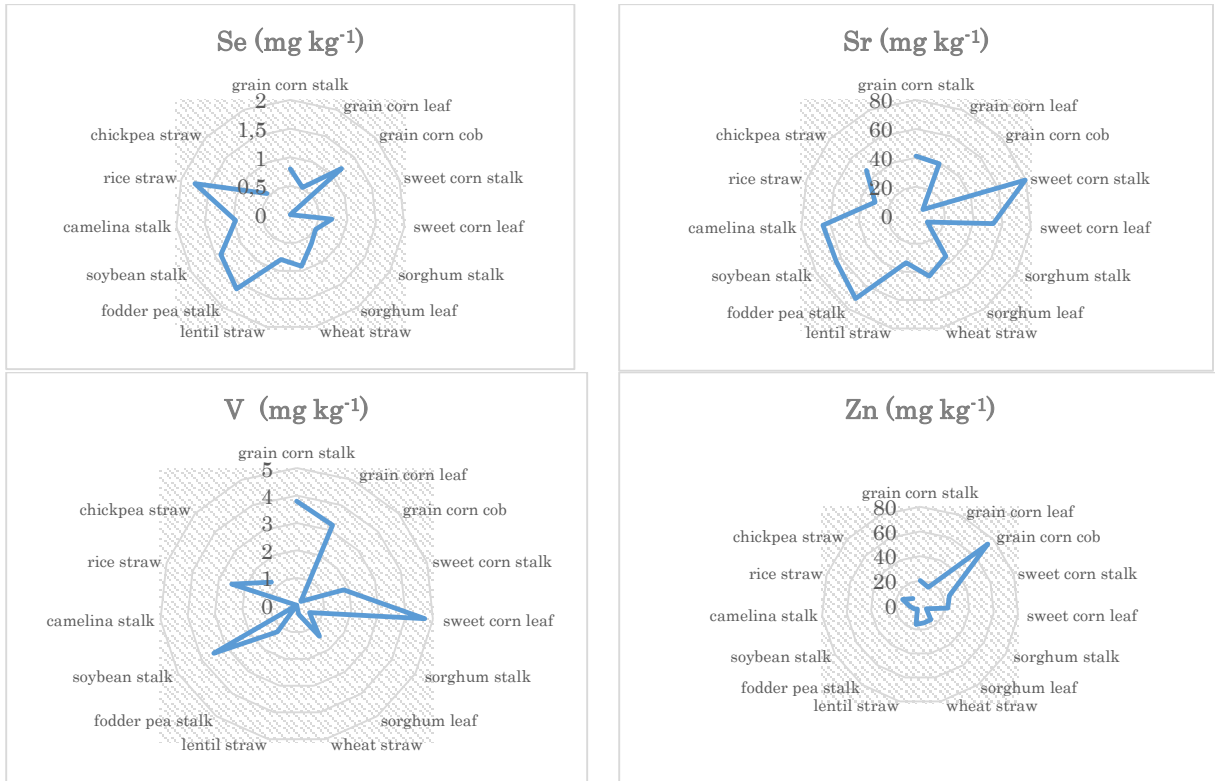


Figure 2. Graphical representation of trace element concentrations in roughages
Şekil 2. Kaba yemlerdeki eser element konsantrasyonlarının grafiksel gösterimi

Toxic Elements

Al concentration was highest in lentil straw ($278 \pm 3 \text{ mg kg}^{-1}$) and below the LOD in rise straw and sorghum straw. Pb was detected in grain corn leaf ($0.18 \pm 0.12 \text{ mg kg}^{-1}$), sorghum leaf ($0.45 \pm 0.07 \text{ mg kg}^{-1}$), and lentil straw ($0.40 \pm 0.13 \text{ mg kg}^{-1}$), whereas it was below LOD in other roughages (Table 4, Figure 3). Other toxic elements, all samples (Table 4) showed that Be, As, and Cd were below the limit of detection (LOD).

Figure 3, the radar graph of toxic elements in roughages, is given below. This graph displays the dominant element in each roughage. Al, As, Cd, Pb, Hg, and Be are toxic to all animals.

The SPSS-22 program evaluated the hierarchical cluster analysis (HCA) of the samples, dividing the roughages into two groups and five clusters. The first cluster includes grain corn leaves, sorghum leaves, grain corn stalks, soybean stalks, camelina stalks, and rice straw. The second cluster includes grain corn cob and sorghum straw. The third cluster includes wheat straw, chickpea straw, and sweet corn leaves. The fourth cluster includes lentil straw. The fifth cluster includes sweet corn stalks and fodder pea stalks. Clusters that are close to each other in the dendrogram can be characterised as close to each other in terms of elemental composition.

Figure 4 shows the dendrogram of 14 roughages as a result of HCA analysis.

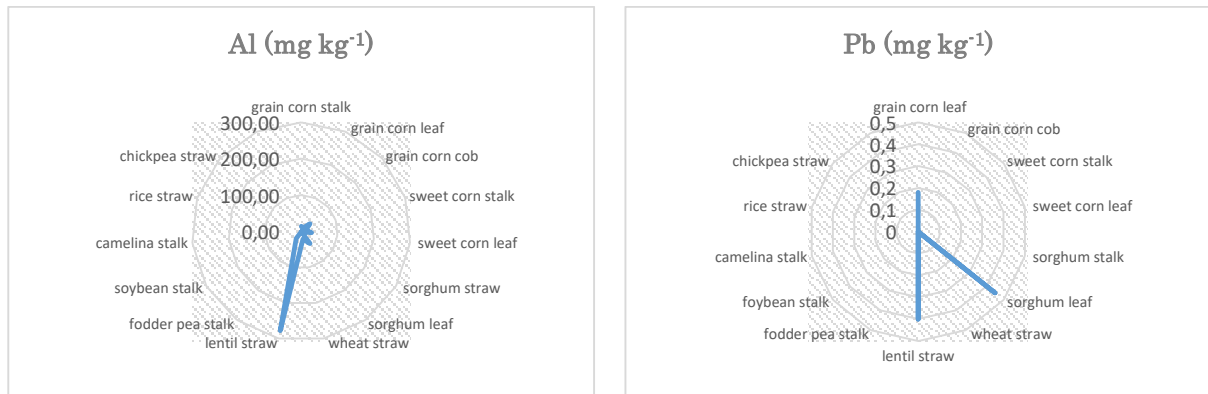


Figure 3. Graphical representation of toxic element concentrations in roughages
Şekil 3. Kaba yemlerdeki toksik element konsantrasyonlarının grafiksel gösterimi

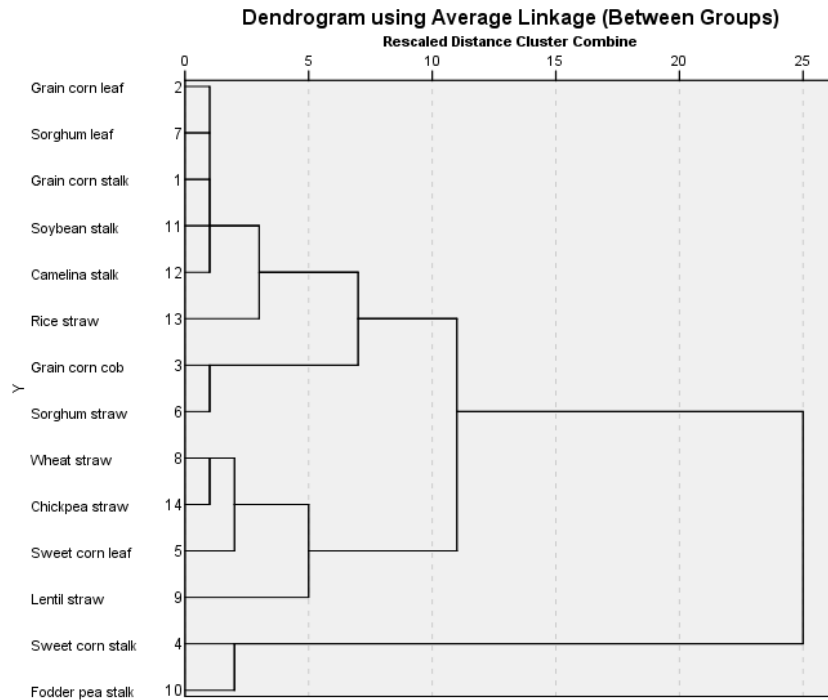


Figure 4. Dendrogram consisting of cluster analysis of roughages
Şekil 4. Kaba yemlerin küme analizinden oluşan dendrogram

CONCLUSION

Macro, trace, and potentially toxic element analyses of 14 roughages used in ruminant rations were carried out, and comparisons were made in terms of these elements. Each element has certain functions in the organism. Animals that consume these elements in high doses will undoubtedly experience health disturbances. Some elements will help in the treatment of various disorders, among which Zn, Fe, Ca, and Se are the most important ones. The analyses revealed that each roughage possesses prominent elements; lentil straw, for instance, exhibited high concentrations of Ca, Fe, and B, while Al and Cr exceeded acceptable levels. Sweet corn stalks were rich in K, while maize cobs were rich in zinc. Only chickpea straw exhibited a high Si content. None of the samples contained the toxic elements (As, Be, Cd, Co, and Sb), but samples of grain corn leaves, sorghum leaves, and lentil straw showed acceptable levels of Pb. However, the experiments were conducted only in the laboratory; these results need to be verified in the field. It is clear that such studies will benefit researchers and the feed industry.

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.

REFERENCES

- Aksoy, A., Macit, M., & Karaoğlu, M. (2000). *Hayvan besleme*, Atatürk Üniversitesi, Ziraat Fakültesi, Ofset Tesisi, Erzurum, 179-199.
- Ahmadi, A., & Ziarati, P. (2015). Chemical composition profile of canned and frozen sweet corn (*Zea mays* L.) in Iran. *Oriental Journal of Chemistry*, 31(2), 1065–1070. <https://doi.org/10.13005/ojc/310256>
- Bakken, N. A., & Hunt, C. D. (2003). Dietary boron decreases peak pancreatic *in Situ* insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *Journal of Nutrition*, 133(11), 3577–3583. <https://doi.org/10.1093/jn/133.11.3577>
- Baloda, S., Sihag, S., Promila, S., & Yadav, K.K. (2018). Mineral Composition of Green and Dry Roughage in Gurgaon District of Haryana, India, *Int.J.Curr.Microbiol.App.Sci.* 7(4), 1411-1416. <https://doi.org/10.20546/ijemas.2018.704.159>
- Bozkurt, Z., Görgülü, M., & Çelik, L. (2007). Kekik (*Oregano vulgare*) ve çörekotu (*Nigella sativa*) esansiyel yağı ile propolisin buğday samanının *in vitro* gerçek kuru madde, organik madde ve NDF sindirilebilirliğine etkileri.

- IV. Ulusal Hayvan Besleme Kongresi, 24 – 28 Haziran 2007, Uludağ, Bursa, *Tam Metinler Kitabı*, 94-97.
- Carroll, J.A., & Forsberg, N.E. (2007). Influence of stress and nutrition on cattle immunity. *Vet. Clin. North Am. Food Anim. Pract.* 23(1), 105-149. <https://doi.org/10.1016/j.cvfa.2007.01.003>
- Çimrin, T., & Demirel, M. (2012). Kanatlı karma yemlerinde bor elementlerinin kullanımı. Yüzüncü Yıl Üniversitesi, *Fen Bilimleri Enstitüsü Dergisi*, 17 (1), 46-56. <https://dergipark.org.tr/tr/pub/yyufbed/issue/21966/235853>
- Demir, B. S. (2005). *Borun insan ve bitki için önemi ve bazı üzüm çeşitlerinde bor tayini (Tez No: 198126)*. [Yüksek Lisans Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Kimya Anabilim Dalı] Yükseköğretim Kurulu Ulusal Tez Merkezi.
- Eğritaş, Ö., & Aşçi, Ö. Ö. (2015). Yaygın fiğ - tahıl karışımlarının bazı mineral madde içeriğinin belirlenmesi. *Akademik Ziraat Dergisi* 4(1), 13-18, <https://dergipark.org.tr/tr/pub/azd/issue/32193/358127>
- Emmanuel, S.A., Mika, S.R., Alabi, F.M., Olajide, O.O., Sallau, A.A., Adedirin, O., Fadeyi, A.E., Akiode, S.O., Uzama, D., & Orishabide, A.T. (2022). Nutritional analysis of sweet sorghum stalk as main excipient of compounded dairy and beef cattle feed, *Natural Products Chemistry & Research*, 10 (1), 1-4. <https://www.researchgate.net/publication/358721955>
- Ensminger, M. E., Oldfield, J. E., & Heinemann, W.W. (1990). *Feeds & Nutrition*, second ed., The Ensminger Publishing Company, California, U.S.A., pp. 890.
- Eren, M., Uyanik, F., & Kucukersan, S. (2004). The influence of dietary boron supplementation on egg quality and serum calcium, inorganic phosphorus, magnesium levels and alkaline phosphatase activity in laying hens. *Research in Veterinary Science*, 76 (3), 203-210. <https://doi.org/10.1016/j.rvsc.2003.11.004>
- Ergül, M. (1993). *Yemler bilgisi ve teknolojisi*. Ege Üniversitesi Ziraat Fakültesi Yayınları No: 487,
- Gökkuş, A., Parlak, A. Z., Baytekin, H., & Hakyemez, B. H. (2013). Akdeniz kuşağı çalılı meralarında otsu türlerin mineral içeriklerinin değişimi. *Tekirdağ Ziraat Fakültesi Dergisi*, 10(1), 1-10, <https://dergipark.org.tr/tr/pub/jotaf/issue/19037/201336>
- Gülümser, E., Mut, H., Doğrusöz, M. Ç., & Başaran, U. (2017). Baklagil yem bitkisi tahıl karışımların ot kalitesi üzerinde ekim oranlarının etkisi. *Selçuk Tarım ve Gıda Bilimleri Dergisi*, 31(3), 43-51. <https://doi.org/10.15316/SJAFS.2017.33>
- Gürsoy, E., & Macit, M. (2017). Determination of mineral contents of some legume and cereal forages grown as naturally in pastures of Erzurum Province. *Alinteri Zirai Bilimler Dergisi*, 32(1), 1-9. <https://doi.org/10.28955/alinterizbd.279756>
- Haddad, C.M., & Alves, F.V. (2006). *Minerais para gado de corte*. In: Bittar C, Moura JC, Faria VP, Mattos WRS, editores. Minerais e Aditivos Para Bovinos. Piracicaba: Fundação de Estudos Agrários Luiz de Queiroz – FEALQ, 63-76.
- Hunt, C. D. (2003). Dietary boron: An overview of the evidence for its role in immune function. *The Journal of Trace Elements in Experimental Medicine*, 16(4), 291-306. <https://doi.org/10.1002/jtra.10041>
- Juniper, D.T., Phipps, R.H., Ramos-Morales, E., & Bertin, G. (2009). Effects of dietary supplementation with selenium enriched yeast or sodium selenite on selenium tissue distribution and meat quality in lambs. *Animal Feed Science and Technology*, 149(3-4), 228-239. <https://doi.org/10.1016/j.anifeedsci.2008.06.009>
- Kaya, S., Bilgili, A., Doğan, A., Liman, B.C. (1990). Mezbahada kesilen sığırların et ve bazı organlarında arsenik kalıntıları, *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 37(2), 359-363. https://doi.org/10.1501/Vetfak_0000001345
- Kommisrud, E., Osteras, O., & Vatn, T. (2005). Blood selenium associated with health and fertility in Norwegian dairy herds. *Acta Vet. Scand.* 46 (4), 229-240. <https://doi.org/10.1186/1751-0147-46-229>
- Kowalska, G., Pankiewicz, U., & Kowalski, R. (2020b). Determination of the level of selected elements in canned meat and fish and risk assessment for consumer health. *Journal of Analytical Methods in Chemistry*, 1-13. <https://doi.org/10.1155/2020/2148794>
- Kurtoglu, V., Kurtoglu, F., & Coskun, B. (2001). Effects of boron supplementation of adequate and inadequate vitamin D-3-containing diet on performance and serum biochemical characters of broiler chickens. *Research in Veterinary Science*, 71 (3), 183-187. <https://doi.org/10.1053/rvsc.2001.0517>
- Kurtoglu, V., Kurtoglu, F., Coşkun, B., Şeker, E., Balevi, T., & Cetingül, I.S. (2002). Effects of boron supplementation on performance and some serum biochemical parameters in laying hens. Conference: XII. European Poultry Conference At: Verona Italy, *Revue de Medecine Veterinaire*, 153(12), 823-828.
- Kutlu, H. R., Görgülü, M., & Baykal Çelik, L. (2005). *Genel hayvan besleme ders notu*. (<http://www.muratgorgulu.com.tr/ckfinder/userfiles/files/GENEL%20HAYVAN%20BESLEME.pdf>), Erişim tarihi: 15.10.2019.
- Kutlu, H.R. (2009). *Tavukların beslenmesi*. In: M.Türkoğlu ve M. Sarıca (Editör), *Tavukçuluk Bilimi (Yetiştirme, Besleme ve Hastalıklar)*, 3. Baskı, Bey Ofset Matbacılık, Ankara, pp.353-498.

- Kutlu, H.R., & Özen, N. (2009). Hayvan beslemede son gelişmeler, VI. Ulusal Zootekni Bilim Kongresi, 24-27 Haziran 2009, Atatürk Üniversitesi, Erzurum.
- Mohamoud, A., & Kılıç, Ü. (2018). Farklı samanlarda lignin peroksidaz enzimi kullanımının yem değeri üzerine etkisi. *KSÜ Tarım ve Doğa Derg isi*, 21(3), 374-384, <https://doi.org/10.18016/ksudobil.346585>
- McDowell, L.R. (1992). *Cadmium*. In: McDowell LR, (ed.). Minerals in animal and human nutrition. London: Academic Press, 359-61.
- National Research Council (NRC) (2001). *Minerals* In: National Research Council, editors. Nutrient requirements of dairy cattle. Washington: National Academy Press; 2001. p.105-61.
- Özyazıcı, M.A. (2019). Determination of mineral contents of sorghum (*Sorghum sp.*) and corn (*Zea mays L.*) varieties grown for roughage, *International Journal of Scientific and Technological Research*, 5(12), 227-237, <https://doi.org/10.7176/JSTR/5-12-24>
- Palisoc, S. T., Bentulan, J. M. O., & Natividad, M. T. (2018). Determination of trace heavy metals in canned food using Graphene/AuNPs/[Ru(NH₃)₆]³⁺/Nafion modified glassy carbon electrodes. *Journal of Food Measurement & Characterization*, 13(1), 169-176. <https://doi.org/10.1007/s11694-018-9930-1>
- Patra, R.C., Swarup, D., Sharma, M.C., & Naresh, R. (2006). Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. *J. Vet. Med. A* 53 (10), 511-517. <https://doi.org/10.1111/j.1439-0442.2006.00868.x>
- Patra, R.C., Swarup, D., Naresh, R., Kumar, P., Nandi, D., Shekhar, P., Roy, S., & Ali, S.L. (2007). Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. *Ecotoxicol. Environ. Saf.* 66 (1), 127-131. <https://doi.org/10.1016/j.ecoenv.2006.01.005>
- Papp, L.V., Lu, J., Holmgren, A., & Khanna, K.K. (2007). From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxid. Redox Signal* 9 (7), 775-806. <https://doi.org/10.1089/ars.2007.1528>
- Pechova, A., & Pavlata, L. (2007). Chromium as an essential nutrient. *Veterinary Medicina*. 52(1), 1-18. <https://doi.org/10.17221/2010-VETMED>
- Qin, X., & Klandorf, H. (1991). Effekt der diätetischen borergränzung auf eiproduktion, oberteilqualität und kalziummetabolismus in gealterten bratrostbrüterhennen. *Junges Geflügeltier Science.*, 70(10), 2131-2138.
- Reis, L.S., Pardo, P. E, Camargos, A. S. & Oba, E. (2010). Mineral element and heavy metal poisoning in animals December 2010 Special issues, *Journal of Medicine and Medical Sciences*, 1(12), 560-579.
- Rusin, M., Domagalska, J., Rogala, D., Razzaghi, M., & Szymala, I. (2021). Concentration of cadmium and lead in vegetables and fruits. *Scientific Reports*, 11(1), 11913, <https://doi.org/10.1038/s41598-021-91554-z>
- Sabah, E. & Çelik, M.Y. (2001). İşçehisar (Afyon) mermer artıklarının hayvan yemi katkı maddesi olarak kullanılabilirliğinin araştırılması. Türkiye III. Mermer Sempozyumu (Mersem '2001), 3-5 Mayıs, Afyon, Bildiriler Kitabı,
- Selçuk, B., Bakır, T., Beycioğlu, T., Kamalak, A., & Kılı, F. (2023). Comparison of feed value characteristics of safflower straw varieties. *KSU J. Agric Nat* 26 (2), 424-429. <https://doi.org/10.18016/ksutarimdog.vi.1136792>
- Sittig, M., (1976). *Toxic metals pollution control and worker protection*: Park Ridge, N. J., Noyes Data Corporation, 350 p.
- Small, J. A., Charmley, E., Rodd, A. V., & Fredeen, A. H. (1997). Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. *Canadian Journal of Animal Science*, 77(1), 55-62. <https://doi.org/10.4141/a96-042>
- Sorenson, J. R. J., Campbell, I. R., Tepper, L. B., & Lingg, R. D., (1974). Aluminum in the environment and human health, *Environ. Health Persp.*, 8, 3-95, <https://doi.org/10.1289/ehp.7483>
- Surai, P. (2002). Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. *World S Poultry Science Journal*, 58(3), 333-347. <https://doi.org/10.1079/wps20020026>
- Surai, P. (2002b). Selenium in poultry nutrition 2. reproduction, egg and meat quality and practical applications. *World S Poultry Science Journal*, 58(4), 431-450. <https://doi.org/10.1079/wps20020032>
- Swarup, D., Patra, R.C., Naresh, R., Kumar, P., & Shekhar, P. (2005). Blood lead levels in lactating cows reared around polluted localities of lead into milk. *Sci. Total Environ.* 347 (1-3), 106-110. <https://doi.org/10.1016/j.scitotenv.2004.12.055>
- Tekeli, S.A. (2003). İran Üçgülü (*Trifolium resupinatum L.*)'nde Bazı Morfolojik ve Kimyasal Özelliklerin Zamana ve Toprak Üstü Biomasına Bağlı Olarak Değişimi. *Tarım Bilimleri Dergisi*, 9(3), 352-360. https://doi.org/10.1501/tarimbil_00000000836
- Tokay, F., & Bağdat, S. (2022). A novel and simple approach to element fractionation analysis: Single step fractionation of milk. *Food Chemistry*, 379, 132162. <https://doi.org/10.1016/j.foodchem.2022.132162>
- Türkmen, İ., Biricik, H., Deniz, G., Gezen, Ş.Ş., Tuncer, D., Çolpan, İ., Küçükarsan, K., Küçükarsan, S., Yalçın, S., Şehu, A., Saçaklı, P., Ergün, A., & Yıldız, G. (2011). *Temel yem bilgisi ve hayvan besleme*, Anadolu Üniversitesi, Web Ofset, Eskişehir, 16-18.

- Wang, M.Q., Xu, Z.R., Zha, L.Y., & Lindemann, M.D. (2007). Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. *Animal Feed Science and Technology*, 139 (1-2), 69-80. <https://doi.org/10.1016/j.anifeedsci.2006.12.004>
- Wang, Y.B., & Xu, B.H. (2008). Effect of different selenium source (sodium selenite and selenium yeast) on broiler chickens. *Animal Feed Science and Technology*, 144 (3-4), 306-314. <https://doi.org/10.1016/j.anifeedsci.2007.10.012>
- Yeşilbağ, D., Eren, M., & Agel, E. (2009). *Biberiye (Rosmarinus officinalis) aromatik bitki ve uçucu yağının broyler rasyonlarında kullanımı*, Tübitak Araştırma Projesi 107O682 Nolu Sonuç Raporu.
- Yu, L.L., Wnag, R.L., Zhang, Y.Z., Kleeman, D.O., Zhu, X.P., & Jia, Z.H. (2008). Effects of selenium supplementation on polyunsaturated fatty acid concentrations and antioxidant status in plasma and liver of lambs fed linseed oil or sunflower oil diets. *Animal Feed Science and Technology*, 140 (1), 39-51. <https://doi.org/10.1016/j.anifeedsci.2007.02.003>

Effect of Cage Environmental Enrichment on Egg Quality in Two Laying Hen Strains

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ABSTRACT

This study investigated the effects of environmental enrichment in cage systems on egg quality, focusing on two laying hen strains, Hyline Brown (HB) and Isa Tinted (IT). Conventional cage systems (CC) were compared with environmentally enriched cages (EEC) that included nest boxes, perches, and pecking stones. Egg quality was evaluated every eight weeks from 24 to 72 weeks of age using one randomly selected egg per cage (4 groups × 14 eggs = 56 eggs). Measured parameters included egg weight, shape index, breaking strength, shell thickness, yolk color, Haugh unit, albumen index, yolk index, and the presence of blood and meat spots. Results revealed no statistically significant differences in egg quality parameters between CC and EEC systems, suggesting that environmental enrichments alone do not influence egg quality. However, strain-specific differences were identified: HB hens produced heavier eggs with thicker and stronger shells, whereas IT hens laid eggs with darker yolks. Age-related changes in egg quality were observed in both strains, with declines in shell thickness, breaking strength, and Haugh unit over time. These findings emphasize the limited impact of cage enrichments on egg quality and highlight the significant roles of genotype and age. Further research is warranted to explore the broader implications of environmental enrichments on poultry production.

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Kafeste Çevresel Zenginleştirmenin İki Farklı Yumurtacı Hibritte Yumurta Kalitesi Üzerine Etkisi

ÖZET

Bu çalışmada, kafes sistemlerindeki çevresel zenginleştirmenin yumurta kalitesi üzerindeki etkileri araştırılmış ve iki yumurtacı hibrit olan Hyline Brown (HB) ve Isa Tinted (IT) ait yumurtalar incelenmiştir. Geleneksel kafes sistemleri (CC), folluk, tünük ve gagalama taşları içeren çevresel olarak zenginleştirilmiş kafeslerle (EEC) karşılaştırılmıştır. Yumurta kalitesi, 24 ila 72 haftalık yaşlar arasında her sekiz haftada bir, her kafesten rastgele seçilen bir yumurta kullanılarak değerlendirilmiştir (4 grup × 14 yumurta = 56 yumurta). Yumurta kalitesi parametreleri olarak yumurta ağırlığı, şekil indeksi, kırılma mukavemeti, kabuk kalınlığı, sarısı rengi, Haugh birimi, albümin indeksi, sarısı indeksi ve kan ve et lekelerinin varlığı belirlenmiştir. Araştırma bulgularında, CC ve EEC sistemleri arasında yumurta kalitesi parametrelerinde istatistiksel olarak anlamlı bir fark olmadığı ve çevresel zenginleştirmelerin tek başına yumurta kalitesini etkilemediği tespit edilmiştir. Ancak, hibrite özgü farklılıklar belirlenmiştir: HB tavukları kırılma mukavemeti yüksek, daha kalın kabuklu daha ağır yumurtalar üretirken, IT tavukları daha koyu sarılı yumurtalar vermiştir. Her iki hibritte de yumurta kalitesinde yaşa bağlı değişiklikler gözlemlendi ve zamanla kabuk kalınlığında, kırılma mukavemetinde ve Haugh biriminde düşüşler belirlendi. Bu bulgular, kafes içi zenginleştirmelerin yumurta kalitesi üzerindeki sınırlı etkisini ve genotip ile yaşın önemli etkisini vurgulamaktadır. Kanatlı üretiminde çevresel zenginleştirmenin etkilerini daha iyi anlamak için ek araştırmalara ihtiyaç vardır.

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INTRODUCTION

Eggs are an affordable and widely consumed source of animal protein, prized for their exceptional nutritional content and contributions to human health (Hisasaga et al., 2020; Özentürk & Yıldız, 2020). As consumer preferences evolve, there is a growing demand for healthier, higher-quality egg products. In the food industry, egg quality encompasses both physical and nutritional attributes that significantly influence consumer acceptance (Özentürk & Yıldız, 2020; Rakonjac et al., 2021; Çınar & Arslan Duru, 2022). The primary objective of the egg industry is to produce high-quality eggs and deliver them to consumers efficiently. Several factors, including genotype, age, and housing systems, play critical roles in determining egg quality (Göger, 2019; Lordelo et al., 2020; Özentürk & Yıldız, 2020; Rakonjac et al., 2021).

Among these, housing systems are of particular importance for poultry, which are highly sensitive to environmental conditions. Globally, conventional cage systems are the predominant housing method for laying hens (Orihuela et al., 2019; Tainika & Şekeroğlu, 2020). However, increasing public concern regarding animal welfare has spurred efforts to improve these systems. In Europe, conventional cages have largely been replaced by enriched cages equipped with perches and nest boxes, reflecting advancements aimed at better accommodating the natural behavioral needs of poultry (Da Silva Pires et al., 2021; Rakonjac et al., 2021; Tainika & Şekeroğlu, 2021; Arulnathan et al., 2024; Majewski et al., 2024; Rodríguez-Hernández et al., 2024). Research on new cage enrichment designs is ongoing. Environmental enrichment refers to changes in the chicken's environment that provide practical and economic benefits, aimed at positively affecting the chicken's physiological development, natural behavior expression, reduction of abnormal and harmful behaviors, stress reduction, improved health, and increased use of available environmental resources (Riber et al., 2018; Campbell et al., 2019). While studies on cage enrichment typically focus on productivity and animal welfare, a key question in various production systems is whether egg quality can be influenced by environmental enrichment. Therefore, it is crucial to assess how these environmental changes impact egg quality (Ghanima et al., 2020; Philippe et al., 2020; Popova et al., 2020). Bari et al. (2020) reported significant effects of environmental enrichment on both internal and external egg quality traits. While studies suggest that providing hens with more freedom in their environment does not significantly increase egg defects, physical quality traits are still affected (Nannoni et al., 2022). In contrast, some studies show that hens in traditional cages tend to lay eggs with stronger eggshells (Englmaierová et al., 2014; Philippe et al., 2020). However, several studies indicate that the housing environment has no effect on egg quality (Dikmen et al., 2017; Ghanima et al., 2020; Ketta et al., 2020; Alig et al., 2023a). The effect of egg quality in newly designed systems with enriched environments remains a topic of interest, as previous studies have yielded conflicting results. Recent research has explored the use of materials such as hanging CDs, ropes, and toy balls for enrichment purposes, with ongoing investigations into various enrichment materials (Moroki & Tanaka, 2016; Campbell et al., 2019; Tainika & Şekeroğlu, 2021). The current study introduces the innovative use of pecking stones as an enrichment feature in the cage system. Previous studies have observed that egg albumen consistency decreased during the laying period when hens were reared in environments enriched with pecking stones and alfalfa bales (Schreiter et al., 2020a). Other studies have found that environmental enrichment with pecking stones and alfalfa bales during rearing resulted in a higher percentage of cracked eggs in different laying hen genotypes, and that environmental enrichment during the laying period increased egg weights (Schreiter et al., 2020b). These studies were conducted in litter housing systems (Schreiter et al 2020a; 2020b), whereas the present study introduces pecking stones into the cage system. Understanding how egg quality is affected in such new systems is crucial, as innovative designs and enrichments could influence consumer preferences.

Genotype is another key factor influencing egg quality (Özentürk & Yıldız, 2020; Krawczyk et al., 2023). Differences in egg quality traits between genotypes have been well-documented (Hisasaga et al., 2020; Özentürk & Yıldız, 2020; Sharma et al., 2022; Akintunde & Teye, 2023; Alig et al., 2023a; Alig et al., 2023b). However, the majority of research examining the effects of rearing systems on egg quality has focused on brown-egg-laying hens, as highlighted in the review by Pires et al. (2021). Therefore, further research is required to understand how housing environments influence egg quality in both brown and white layer hens. Some studies suggest that housing systems have no impact on white layers (Philippe et al., 2020; Dalle Zotte et al., 2021; Sharma et al., 2022), while others indicate that different strains may respond differently to various environmental conditions (Rakonjac et al., 2021; Sharma et al., 2022). Additionally, certain strains may benefit more from additional space and enrichment features (Campbell et al., 2019; Ross et al., 2020). Therefore, it is crucial to investigate the egg quality of different strains under enriched environmental conditions.

This study aims to evaluate the effects of environmental enrichment in cage systems on egg quality, focusing on two laying hen strains: Hyline Brown (HB) and Isa Tinted (IT). We compared conventional cage systems (CC) with environmentally enriched cage systems (EEC), which include nest boxes, perches, and pecking stones. By examining both internal and external egg quality traits, this study provides insights into how enrichment influences egg quality and explores the role of genotype in mediating these effects. The innovative use of pecking stones in cage systems underscores the potential of novel enrichment strategies to enhance egg quality.

MATERIAL and METHOD

The research was ethically approved by the Atatürk University Faculty of Veterinary Medicine Unit Ethics Committee (Protocol no: 2024/22, dated 27.09.2024).

Animals and Housing

The study was conducted at the Poultry Unit of Atatürk University's Food and Livestock Research and Application Centre, using Hyline Brown (HB) and Isa Tinted (IT) hybrids, which were brown and white layer hens, respectively. At 20 weeks of age, 280 hens (140 of each strain) were randomly allocated to cages, with uniformity in body weight considered. The experiment spanned from 24 to 72 weeks of age.

Two hen strains (HB and IT) and two cage designs—conventional cages (CC) and environmentally enriched cages (EEC)—were used. For EECs, a nest area enclosed on three sides with a curtain was set up inside the existing cage. Wooden perches were installed at a height of 10 cm across the middle of the cage, providing each bird with a 15 cm-long perch. Additionally, plastic boxes (10 × 6 × 6 cm) containing pecking stones were mounted 25 cm above the cage floor. The pecking stones were commercially produced mineral stones composed of calcium, magnesium, sodium, trace elements, oyster shell flour, carob flour, and calcium carbonate.

The study utilized 56 cage compartments, with 28 compartments assigned to each system (CC and EEC). Within each system, 14 cages housed HB hens, and 14 housed IT hens, with five hens per cage, providing each bird 750 cm² of space.

The poultry house was ventilated using side wall windows, ceiling chimneys, and a 140 × 140 cm fan operating under negative pressure, keeping indoor temperatures between 16°C and 24°C. Lighting was maintained for 16 hours daily using fluorescent lamps emitting white light. During the productive period, hens were provided water and granulated feed ad libitum, formulated specifically for each production stage: first-stage layer feed from 21-45 weeks (2750 ME, 16.26% CP), second stage from 46-60 weeks (2720 ME, 15.83% CP), and third stage from 61-72 weeks (2720 ME, 15.65% CP).

Determination of Egg Quality Parameters

For egg quality analysis, a total of 56 eggs (one egg from each cage in 4 groups × 14 cages) were randomly selected every 8 weeks, beginning at the start of egg production (24 weeks) and continuing through 72 weeks. The eggs were kept at room temperature for 24 hours prior to evaluation. The shape index, representing the ratio of egg width to length, was measured using a Rauch index device. Egg weight (g) and breaking strength (kg/cm²) were assessed with a Digital Egg Tester (DET-6000®). The eggs were then broken, and the yolk diameter, yolk height, albumen height, Haugh unit, yolk index, and yolk color were measured using the same device. The albumen index was manually calculated by measuring the albumen's length and width with a caliper. Blood and meat spot occurrence (%) and shell thickness were also assessed. Shell thickness (mm) was determined by taking samples from the blunt, middle, and pointed ends of each egg, removing the membranes, and measuring with a micrometer. The average of the three measurements was recorded as the final shell thickness value (Özentürk & Yıldız 2020).

Statistical Analysis

The data collected in this study were analyzed using IBM® SPSS version 20 for both descriptive and analytical purposes. A General Linear Model (GLM) was applied to evaluate egg quality parameters across different age periods. The Chi-square test (X²) was used as a non-parametric method for assessing the presence of blood and meat spots in the eggs. Additionally, logistic regression analysis was conducted to further analyze the blood and meat spot data.

RESULTS

Data on egg external quality parameters are presented in Table 1, while data on egg internal quality parameters are presented in Table 2. Furthermore, egg blood and meat spot data are shown in Table 3. The egg weight, shell thickness, and shell fracture strength of eggs belonging to the HB strain were higher ($p < .05$). More blood and

meat stains were observed in the eggs of the HB hybrid ($p < .01$). Egg yolk color differed between strains ($p < .01$). There was no significant difference between strains in terms of shape index, haugh unit, albumen, and yolk index values ($p > .05$). No statistical difference was observed in terms of egg quality parameters in eggs taken from both cage systems ($p > .05$). All egg internal and external quality characteristics, except for blood and meat stain data, varied over time. The effect of age on egg quality was found to be significant ($p < .01$).

Table 1. Effects of genotype, cage system and age on external egg quality parameters ($X \pm S_x$)
Çizelge 1. Genotip, kafes sistemi ve yaşın yumurta dış kalite parametreleri üzerine etkisi ($X \pm S_x$)

	Group (Grup)	Egg weight (Yumurta ağırlığı)	Shape index (Şekil indeksi)	Shell fracture strength (Kabuk kırılma mukavemeti)	Shell thickness (Kabuk kalınlığı)
Mean values (Ortalama değerler)	Hyline Brown	63.70±0.32 ^x	77.37±0.16	4.43±0.06 ^x	0.428±0.003 ^x
	Isa Tinted	62.58±0.32 ^y	77.67±0.16	4.08±0.06 ^y	0.415±0.003 ^y
	Conventional cage	63.22±0.32	77.70±0.16	4.24±0.06	0.419±0.003
	Environmental enrichment	63.07±0.32	77.34±0.16	4.28±0.06	0.424±0.003
	w24	54.01±0.60 ^d	72.05±0.30 ^e	4.55±0.12 ^a	0.364±0.006 ^e
	w32	62.01±0.60 ^c	79.91±0.30 ^a	4.87±0.12 ^a	0.423±0.006 ^{bed}
	w40	63.51±0.60 ^{bc}	79.13±0.30 ^{ab}	4.58±0.12 ^a	0.439±0.006 ^{abc}
	w48	65.67±0.60 ^a	78.70±0.30 ^b	4.55±0.12 ^a	0.441±0.006 ^{ab}
	w56	65.14±0.60 ^{ab}	78.84±0.30 ^b	4.04±0.12 ^b	0.447±0.006 ^a
	w64	65.55±0.60 ^a	77.54±0.30 ^c	3.76±0.12 ^{bc}	0.422±0.006 ^{cd}
	w72	66.11±0.60 ^a	76.48±0.30 ^d	3.46±0.12 ^c	0.413±0.006 ^d
p values (p değerleri)	Genotype	.014	.179	<.001	.009
	Cage system	.740	.113	.693	.308
	Genotype x Cage system	.937	.005	.331	.674
	Age*	<.001	<.001	<.001	<.001

HB: Hyline Brown; IT: Isa Tinted; CC: Conventional Cage System; EEC: Environmentally Enriched Cage; w: Week of age; ^{a-e}: Differences between means with different letters on the same column are significant; (P<.001); ^{x-y}: Different letters within one column are significantly different.(P<.05); *Repeated-measures analysis of variance results based on time in the interval of 24-72 weeks.

Table 2. Effects of genotype, cage system and age on internal egg quality parameters ($X \pm S_x$)
Çizelge 2. Genotip, kafes sistemi ve yaşın yumurta iç kalite parametreleri üzerine etkisi ($X \pm S_x$)

	Group (Grup)	Yolk color (Sarı renk)	Haugh unit (Haugh birimi)	Albumen index (Ak indeksi)	Yolk index (Sarı indeksi)
Mean values (Ortalama değerler)	Hyline Brown	9.71±0.06 ^y	85.65±0.76	10.11±0.18	44.93±0.24
	Isa Tinted	10.33±0.06 ^x	84.82±0.76	10.14±0.18	45.19±0.24
	Conventional cage	10.04±0.06	85.50±0.76	10.24±0.18	45.12±0.24
	Environmental enrichment	10.00±0.06	84.98±0.76	10.00±0.18	44.99±0.24
	w24	8.80±0.11 ^d	88.16±1.41 ^{ab}	11.96±0.33 ^a	49.95±0.44 ^a
	w32	10.66±0.11 ^a	89.97±1.41 ^a	11.64±0.33 ^a	48.70±0.44 ^b
	w40	10.59±0.11 ^a	84.68±1.41 ^{bc}	9.84±0.33 ^{bc}	48.31±0.44 ^b
	w48	10.38±0.11 ^a	85.45±1.41 ^{bc}	10.27±0.33 ^b	44.45±0.44 ^c
	w56	9.95±0.11 ^{bc}	82.46±1.41 ^c	9.26±0.33 ^c	41.58±0.44 ^d
	w64	10.04±0.11 ^b	83.01±1.41 ^c	8.92±0.33 ^c	41.35±0.44 ^d
	w72	9.74±0.11 ^c	82.93±1.41 ^c	8.98±0.33 ^c	41.06±0.44 ^d
p values (p değerleri)	Genotype	<.001	.439	.919	.440
	Cage system	.643	.622	.337	.704
	Genotype x Cage system	.037	.034	.095	<.001
	Age*	<.001	.001	<.001	<.001

HB: Hyline Brown; IT: Isa Tinted, CC: Conventional Cage System; EEC: Environmentally Enriched Cage; ^{a-d}: Differences between means with different letters on the same column are significant (P<.001); ^{x-y}: Different letters within one column are significantly different (P<.05); *Repeated-measures analysis of variance results based on time in the interval of 24-72 weeks.

Table 3. Analysis of factors influencing blood and meat spots

Çizelge 3. Kan ve et lekelerini etkileyen faktörlerin analizi

Factors	Count in positive (Pozitif değerler)		Count in negative (Negatif değerler)		Total count (Toplam değer)	X ² (df)	p
	N	(%)	N	(%)			
Genotype (Genotip)						28.638(1)	<.001
Hyline Brown	69	35.20	127	64.80	196		
Isa Tinted	23	12.04	168	87.96	191		
Cage System (Kafes sistemi)						0.643(1)	.423
CC	49	25.52	143	74.48	192		
EEC	43	22.05	152	77.95	195		
Age (Weeks) (Yaş-haftalar)						11.558(6)	.073
24	8	14.29	48	85.71	56		
32	14	25.00	42	75.00	56		
40	11	19.64	45	80.36	56		
48	8	14.29	48	85.71	56		
56	17	30.36	39	69.64	56		
64	19	33.93	37	66.07	56		
72	15	29.41	36	70.59	51		

HB: Hyline Brown; IT: Isa Tinted; CC: Conventional Cage System; EEC: Environmentally Enriched Cage.

DISCUSSION

Enrichments like perches, nest boxes, and pecking stones aim to fulfill these behavioral needs, which can potentially improve both welfare and production outcomes (Nicol, 2015; Nicol et al., 2017). Understanding the interplay between the ability of laying hens to perform natural behavior and egg quality is therefore a significant focus in poultry research (Tainika & Şekeroğlu, 2021). Behavioral enrichment contributes to the welfare of hens by reducing stress and encouraging physical activity. Lower stress levels could theoretically improve egg production and quality by mitigating the adverse effects of chronic stress on physiological processes (Henriksen et al., 2011; De Haas et al., 2013; Bari et al., 2020b; Hemsworth & Edwards, 2020). Some studies report that egg quality is positively affected by rearing systems designed to enhance welfare (Bhanja and Bhadauria, 2018; Dedousi et al., 2020). However, the results of this study challenge the assumption that enrichments inherently improve egg quality in cage systems. Results indicate no statistically significant differences in egg quality between hens housed in environmentally enriched cages (EEC) and conventional cages (CC). This suggests that enrichments alone do not substantially influence egg quality, as eggs produced in both systems showed minimal, non-significant differences. This outcome underscores the complexity of the physiological mechanisms regulating egg formation, which are predominantly driven by hormonal and metabolic factors (Gil, 2008; Zinca et al., 2024). Previous research on egg quality and environmental enrichment has often focused on cage-free systems (Bari et al., 2020b; Dedousi et al., 2020). The observed differences in these studies may stem not only from environmental enrichments, such as perches and nest boxes, but also from the larger space and increased mobility provided by cage-free systems. In contrast, our study examines enrichment within cage systems, where stocking density remains unchanged. This restricted space may limit the potential welfare benefits of enrichment and, consequently, its impact on egg quality. These findings align with earlier studies reporting minimal or no effects of enrichment on internal and external egg quality traits in cage systems (Dikmen et al., 2017; Onbaşlar et al., 2020; Philippe et al., 2020). For instance, Alig et al. (2023a) found no significant differences in egg weight, albumen quality, Haugh unit, yolk color, shell breaking strength, or shell quality in brown layers housed in enriched and conventional cages. Similarly, Alig et al. (2023b) reported no significant differences in egg quality parameters in white eggs from enriched and conventional cage systems.

One consideration is the potential energy allocation trade-offs associated with enriched environments. While these environments encourage natural behaviors like perching and foraging, the additional energy expenditure required for these activities might offset any welfare-related benefits in terms of egg quality (Jacobs et al., 2023; Herrera-Alcaino et al., 2024). Another explanation for the lack of significant differences could be that egg quality is primarily influenced by intrinsic factors such as genetics, nutrition, and the age of the hens rather than by cage enrichments alone (Sarica et al. 2018; Franco et al. 2020; Zinca et al. 2024). In this study, both cage design groups were fed diets with identical nutritional content. While enriched cages provide more opportunities for hens to

express natural behaviors, these behaviors may not directly translate into measurable improvements in egg quality traits.

The HB hybrid exhibited higher egg weight, shell thickness, and breaking strength compared to the IT hybrid. Differences in egg quality between strains are well-documented (Ketta et al. 2020; Lordelo et al. 2020). The higher egg weight observed in brown layer hybrids compared to white layer hybrids aligns with previous studies (Özentürk & Yıldız 2020; Özentürk & Yıldız 2021). Egg weight heritability is high (0.4-0.6), which helps explain the differences observed between hybrids (Sarica et al. 2018; Göger 2019). The lower egg weight of white layer hybrids can be attributed to their lighter breed class compared to brown layer hybrids in terms of live weight (Özentürk & Yıldız 2020). The egg shape index values for HB and IT hybrids were within ideal ranges for commercial egg production (Sarica et al. 2018; Mahawar et al., 2023). The higher shell thickness and breaking resistance of HB hybrid eggs indicate superior shell quality, possibly related to physiological differences between hybrids (Özentürk & Yıldız 2020).

We found that IT hybrid produced darker egg yolks. Darker yellow yolks are preferred by consumers in many countries and thus hold higher market value (Samiullah et al. 2017; Rondoni et al. 2020). Although egg yolk color is particularly influenced by the amount of synthetic xanthophyll in the diet (Nour et al. 2017), all hens in this study were fed the same diet. Philippe et al. (2020) suggest that yolk color intensity could also be affected by egg production levels, with paler yolks related to higher productivity. Reflecting this dilution effect, this study found that the HB hybrid, recognized for its higher laying rates in catalog results, produced lighter yolks. Additionally, it has also been reported that higher stress levels result in darker yolks, as higher levels of blood corticosterone lead to darker yolks (Alig et al. 2023a). The differences observed among strains could be attributed to studies indicating that different hybrids exhibit varying stress levels under cage conditions (Özentürk & Yıldız, 2021). Therefore, the darker egg yolk color in the IT hybrid in this study may be related to its high stress levels. Both HB and IT hybrid eggs had high Haugh unit values, indicating high quality. However, eggs from the HB hybrid exhibited a higher incidence of blood and meat stains, potentially due to physiological differences (Sarica et al. 2018; Özentürk & Yıldız 2020). Supporting this, studies have shown that brown layer hens generally produce eggs with more blood and meat stains compared to white layer hens (Yardım & Akşit, 2021; Uysal & Laçin, 2024).

Egg weight typically increased with time, while shell breaking strength and thickness peaked around the onset of peaked production and then declined with age. This decrease in shell thickness over time may result from reduced calcium deposition during shell formation in the uterus or from the same amount of calcium being distributed over a larger surface area as the size of the egg increases (Samiullah et al. 2017). A decrease in shell breaking resistance can also be attributed to these factors. As eggs age, the Haugh unit, a reliable indicator of freshness based on the relationship between egg weight and albumen height, decreases due to moisture loss and protein breakdown in the albumen (Da Silva Pires et al. 2021). This change in the Haugh unit over time is due to the fact that egg whites are heavier than yolks, causing the relative value to decrease as egg weight increases with age. The aging process may affect the egg white production function in the oviduct, leading to a reduction in albumen and yolk height by impacting the bonds holding the egg white and yolk (Özentürk & Yıldız 2020). Similarly, the white index and yolk index also decreased over time. While the shape index of eggs was higher at the beginning of the production period, it decreased towards the end. This change may be explained by alterations in the activity of oviduct muscles and changes in pelvic bone anatomy with age. Furthermore, feeding programs that evolve over time based on the egg production period and feed nutritional value, as well as variations in climatic conditions such as indoor temperature according to seasons, may also have affected the shape index. These results corroborate previous studies reporting similar findings for both cage systems (Dikmen et al. 2017; Samiullah et al. 2017; Özentürk & Yıldız 2020).

CONCLUSION

As a conclusion, this study provides comprehensive insights into the effects of cage design and strain on the egg quality. There were no significant differences in overall egg quality between the two rearing systems, indicating a need for further research to understand the specific impact of enrichment tools on egg quality. Breed selection played a crucial role in egg quality. In both CC and EEC, HB had higher egg weight, and better shell quality than IT, making them a preferred choice in both rearing systems. Results also indicated that while egg weight increased over time, eggshell quality and Haugh unit values decreased over time.

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Contribution Rate Statement Summary of Researchers

ÜÖ: Conception and design of study, Acquisition of data, Drafting the manuscript, Analysis and/or interpretation of data. AU: Acquisition of data, Drafting the manuscript, Critical review/revision.

Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

- Akintunde, A. O., & Teye, A. A. (2023). Comparative study on egg characteristics of Yoruba ecotype Nigerian local chickens and Isa Brown chickens fed graded levels of moringa oleifera seed meal. *Agricultural Science Digest*, 43, 877–882. <https://doi.org/10.18805/ag.DF-430>.
- Alig, B. N., Malheiros, R. D., & Anderson, K. E. (2023a). Evaluation of physical egg quality parameters of commercial brown laying hens housed in five production systems. *Animals*, 13(4), 716. <https://doi.org/10.3390/ani13040716>.
- Alig, B. N., Malheiros, R. D., & Anderson, K. E. (2023b). The effect of housing environment on physical egg quality of white egg layers. *Poultry*, 2(2), 222–234. <https://doi.org/10.3390/poultry2020018>.
- Arulnathan, V., Turner, I., Bamber, N., Ferdous, J., Grassauer, F., Doyon, M., & Pelletier, N. (2024). A systematic review of potential productivity, egg quality, and animal welfare implications of extended lay cycles in commercial laying hens in Canada. *Poultry Science*, 103475. <https://doi.org/10.1016/j.psj.2024.103475>.
- Bari, M. S., Cohen-Barnhouse, A. M., & Campbell, D. L. M. (2020a). Early rearing enrichments influenced nest use and egg quality in free-range laying hens. *Animal*, 14, 1249–1257. <https://doi.org/10.1017/S1751731119003094>.
- Bari, M. S., Downing, J. A., Dyal, T. R., Lee, C., & Campbell, D. L. (2020b). Relationships between rearing enrichments, range use, and an environmental stressor for free-range laying hen welfare. *Frontiers in Veterinary Science*, 7, 480. <https://doi.org/10.3389/fvets.2020.00480>.
- Bhanja, S. K., & Bhadauria, P. (2018). Behaviour and welfare concepts in laying hens and their association with housing systems. *Indian Journal of Poultry Science*, 53(1), 1–10. <https://doi.org/10.5958/0974-8180.2018.00009.0>.
- Çınar, E., & Arslan Duru, A. (2022). Anason Tohumu İlavesinin Yumurtacı Tavukların Performans, İç ve Dış Yumurta Kalite Özellikleri ve Yumurta Sarısı Kolesterol Konsantrasyonuna Etkisi. *KSÜ Tarım ve Doğa Dergisi*, 25(1), 198–205. <https://doi.org/10.18016/ksutarimdogavi.890836>.
- Campbell, D. L. M., de Haas, E. N., & Lee, C. (2019). A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. *Poultry Science*, 98(1), 9–28. <https://doi.org/10.3382/ps/pey319>.
- Da Silva Pires, P. G., Bavaresco, C., Prato, B. S., Wirth, M. L., & Moraes, P. O. (2021). The relationship between egg quality and hen housing systems-A systematic review. *Livestock Science*, 250, 104–159. <https://doi.org/10.1016/j.livsci.2021.104597>.
- Dalle Zotte, A., Cullere, M., Pellattiero, E., Sartori, A., Marangon, A., & Bondesan, V. (2021). Is the farming method (cage, barn, organic) a relevant factor for marketed egg quality traits? *Livestock Science*, 246, 104453. <https://doi.org/10.1016/j.livsci.2021.104453>.
- De Haas, E. N., Kemp, B., Bolhuis, J. E., Groothuis, T., & Rodenburg, T. B. (2013). Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. *Poultry Science*, 92(9), 2259–2269. <https://doi.org/10.3382/ps.2012-02996>.
- Dedousi, A., Stojčić, M. Đ., & Sossidou, E. (2020). Effects of housing systems on keel bone damage and egg quality of laying hens. *Veterinary Research Forum*, 11 (4), 299. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. <https://doi.org/10.30466/vrf.2019.99568.2375>.
- Dikmen, B. Y., Ipek, A., Şahan, Ü., Sözcü, A., & Baycan, S. C. (2017). Impact of different housing systems and age of layers on egg quality characteristics. *Turkish Journal of Veterinary & Animal Sciences*, 41(1), 77–84. <https://doi.org/10.3906/vet-1604-71>.
- Englmaierová, M., Tumová, E., Charvátová, V., & Skřivan, M. (2014). Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination Original Paper. *Czech Journal Animal Science*, 59. <https://doi.org/10.17221/7585-CJAS>.
- Franco, D., Rois, D., Arias, A., Justí, J. R., Martí-Qujal, F. J., Khubber, S., Barba, F. J., Pedrouso, M. L., & Lorenzo, J. M. (2020). Effect of breed and diet type on the freshness and quality of the eggs: A comparison between Mos (indigenous Galician breed) and Isa brown hens. *Foods*, 9(3), 342. <https://doi.org/10.3390/foods9030342>.
- Ghanima, M. M. A., Alagawany, M., Abd El-Hack, M. E., Taha, A., Elnesr, S. S., Ajarem, J., Allam, A. A., & Mahmoud, A. M. (2020). Consequences of various housing systems and dietary supplementation of thymol,

- carvacrol, and euganol on performance, egg quality, blood chemistry, and antioxidant parameters. *Poultry Science*, 99, 4384–4397. <https://doi.org/10.1016/j.psj.2020.05.028>.
- Gil, D. (2008). Hormones in avian eggs: physiology, ecology and behavior. *Advances in the Study of Behavior*, 38, 337-398. [https://doi.org/10.1016/S0065-3454\(08\)00007-7](https://doi.org/10.1016/S0065-3454(08)00007-7)
- Göger, H. (2019). Affecting factors of egg weight. *Tavukçuluk Araştırma Dergisi*, 39–47.
- Herrera-Alcaíno, S., Luna, D., González-Pavez, J., Cordero, P., & Guzmán-Pino, S. A. (2024). Social enrichment improves affective state and foraging behavior compared to physical enrichment, while maintaining growth performance in broiler chickens. *Animals*, 14(22), 3186. <https://doi.org/10.3390/ani14223186>
- Hemsworth, P. H., & Edwards, L. E. (2020). Natural behaviours, their drivers and their implications for laying hen welfare. *Animal Production Science*, 61(10), 915-930. <https://doi.org/10.1071/AN19630>.
- Henriksen, R., Groothuis, T. G., & Rettenbacher, S. (2011). Elevated plasma corticosterone decreases yolk testosterone and progesterone in chickens: linking maternal stress and hormone-mediated maternal effects. *PloS one*, 6(8), e23824. <https://doi.org/10.1371/journal.pone.0023824>.
- Hisasaga, C., Griffin, S. E., & Tarrant, K. J. (2020). Survey of egg quality in commercially available table eggs. *Poultry Science*, 99, 7202–7206. <https://doi.org/10.1016/j.psj.2020.09.049>.
- Jacobs, L., Blatchford, R. A., De Jong, I. C., Erasmus, M. A., Levensgood, M., Newberry, R. C., ... & Weimer, S. L. (2023). Enhancing their quality of life: environmental enrichment for poultry. *Poultry Science*, 102(1), 102233. <https://doi.org/10.1016/j.psj.2022.102233>.
- Ketta, M., Tumová, E., Englmaierová, M., & Chodová, D. (2020). Combined effect of genotype, housing system, and calcium on performance and eggshell quality of laying hens. *Animals*, 10(11), 21-20. <https://doi.org/10.3390/ani10112120>.
- Krawczyk, J., Lewko, L., Sokołowicz, Z., Koseniuk, A., & Kraus, A. (2023). Effect of hen genotype and laying time on egg quality and albumen lysozyme content and activity. *Animals*, 13, 1611. <https://doi.org/10.3390/ani13101611>.
- Lordelo, M., Cid, J., Cordovil, C. M., Alves, S. P., Bessa, R. J. B., & Carolino, I. (2020). A comparison between the quality of eggs from indigenous chicken breeds and that from commercial layers. *Poultry Science*, 99(3), 1768–1776. <https://doi.org/10.1016/j.psj.2019.11.023>.
- Mahawar, M., Altuntaş, E., & Gül, E. N. (2023). Mass Modelling of Eggs Based on Shape Index Using Regression Analysis. *KSÜ Tarım ve Doğa Dergisi*, 26(1), 132-139. <https://doi.org/10.18016/ksutarimdogavi.992588>.
- Majewski, E., Potori, N., Sulewski, P., Was, A., Morawska, M., Gebeska, M., Rawlikowska, A. M., Grontkowska, A., Szili, V., & Erdos, A. (2024). End of the Cage age? a study on the impacts of the transition from cages on the EU laying hen sector. *Agriculture*, 14(1), 111. <https://doi.org/10.3390/agriculture14010111>.
- Moroki, Y., & Tanaka, T (2016). A pecking device as an environmental enrichment for caged laying hens. *Animal Science Journal*, 87(8), 1055–1062. <https://doi.org/10.1111/asj.12525>.
- Nannoni, E., Buonaiuto, G., Martelli, G., Lizzi, G., Trevisani, G., Garavini, G., & Sardi, L. (2022). Influence of increased freedom of movement on welfare and egg laying pattern of hens kept in aviaries. *Animals*, 12, 2307. <https://doi.org/10.3390/ani12182307>.
- Nicol, C.J. (2015). *The Behavioural Biology of Chickens*; CABI: Wallingford, UK, p. 192.
- Nicol, C.J., Bouwsema, J., Caplen, G., Davies, A.C., Hockenhull, J., Lambton, S.L., Lines, J.A., Mullan, S., & Weeks, C.A. (2017). *Farmed Bird Welfare Science Review*; Department of Economic Development, Jobs, Transport and Resources: Melbourne, Australia, pp. 1–321
- Nour, V., Panaite, T. D., Corbu, A. R., & Vlaicu, P. A. (2017). Yolk colour and carotenoid composition of the eggs produced by laying hens fed diets containing tomato processing waste. *International Multidisciplinary Scientific GeoConference: SGEM*, 17, 429–436. <https://doi.org/10.5593/sgem2017H/63>.
- Onbaşılar, E. E., Kahraman, M., Güngör, Ö. F., Kocakaya, A., Karakan, T., Pirpanahi, P., Doğan, B., Metin, D., Akan, M., Şehu, A., Erbay Elibol, F. K., & Yalçın, S. (2020). Effects of cage type on performance, welfare, and microbiological properties of laying hens during the molting period and the second production cycle. *Tropical Animal Health Production*, 52(6), 3713–3724. <https://doi.org/10.1007/s11250-020-02409-0>.
- Orihuela, A., Mota-Rojas, D., Velarde, A., Strappini-Asteggiano, A., Thielo de la Vega, L., Borderas-Tordesillas, F., & Alonso-Spilsbury, M. (2019). Environmental enrichment to improve behaviour in farm animals. *CABI Reviews*, 1–25. <https://doi.org/10.1079/PAVSNNR201813059>.
- Özentürk, U., & Yıldız, A. (2020). Assessment of egg quality in native and foreign laying hybrids reared in different cage densities. *Brazilian Journal Poultry Science*, 22, eRBCA. <https://doi.org/10.1590/1806-9061-2020-1331>.
- Özentürk, U., & Yıldız, A. (2021). Comparison of performance parameters, stress, and immunity levels of native and commercial layers reared in different cage densities in Turkey. *Turkish Journal of Veterinary & Animal Sciences*, 45(6), 1052-1064. <https://doi.org/10.3906/vet-2012-91>.

- Philippe, F. X., Mahmoudi, Y., Cinq-Mars, D., Lefrançois, M., Moula, N., Palacios, J., Pelletier, F., & Godbout, S. (2020). Comparison of egg production, quality and composition in three production systems for laying hens. *Livestock Science*, 232, 103-917. <https://doi.org/10.1016/j.livsci.2020.103917>.
- Popova, T., Petkov, E., Ayasan, T., & Ignatova, M. (2020). Quality of eggs from layers reared under alternative and conventional system. *Brazilian Journal Poultry Science*, 22(01), 1-8. <https://doi.org/10.1590/1806-9061-2019-1172>.
- Rakonjac, S., Dosković, V., Bošković, S. B., Škrbić, Z. I., Lukić, M. I., Petričević, V. I., & Petrović, D. M. (2021). Production performance and egg quality of laying hens as influenced by genotype and rearing system. *Brazilian Journal Poultry Science*, 23(02), 1-8. <https://doi.org/10.1590/1806-9061-2019-1045>.
- Riber, A. B., Casey-Trott, T. M., & Herskin, M. S. (2018). The influence of keel bone damage on welfare of laying hens. *Frontiers in Veterinary Science*, 5(6), 1-12. <https://doi.org/10.3389/fvets.2018.00006>.
- Rodríguez-Hernández, R., Rondón-Barragán, I. S., & Oviedo-Rondón, E. O. (2024). Egg Quality, Yolk Fatty Acid Profiles from Laying Hens Housed in Conventional Cage and Cage-Free Production Systems in the Andean Tropics. *Animals*, 14, 168. <https://doi.org/10.3390/ani14010168>.
- Rondoni, A., Asioli, D., & Millan, E. (2020). Consumer behaviour, perceptions, and preferences towards eggs: A review of the literature and discussion of industry implications. *Trends in Food Science & Technology*, 106, 391–401. <https://doi.org/10.1016/j.tifs.2020.10.038>.
- Ross, M., Rausch, Q., Vandenberg, B., & Mason, G. (2020). Hens with benefits: Can environmental enrichment make chickens more resilient to stress? *Physiology & Behavior*, 226, 113077. <https://doi.org/10.1016/j.physbeh.2020.113077>.
- Samiullah, S., Omar, A. S., Roberts, J., & Chousalkar, K. (2017). Effect of production system and flock age on eggshell and egg internal quality measurements. *Poultry Science*, 96(1), 246–258. <https://doi.org/10.3382/ps/pew289>.
- Sarıca, M., Türkoğlu, M., & Yamak U. S. (2018). *Tavukçuluk Bilimi, Yetiştirme, Besleme, Hastalıklar: Tavukçuluktaki gelişmeler ve Türkiye tavukçuluğu*. Bey Ofset Matbaacılık, Ankara. Ed. Sarıca M, Türkoğlu M. pp.1-36.
- Schreiter, R., Damme, K., & Freick, M. (2020a). Edible Environmental Enrichments in Littered Housing Systems: Do Their Effects on Integument Condition Differ Between Commercial Laying Hen Strains? *Animals*, 10, 2434. <https://doi.org/10.3390/ani10122434>.
- Schreiter, R., Damme, K., Klunker, M., Raoult, C., Von Borell, E., & Freick, M. (2020b). Effects of edible environmental enrichments during the rearing and laying periods in a littered aviary—Part 1: integument condition in pullets and laying hens. *Poultry Science*, 99, 5184–5196. <https://doi.org/10.1016/j.psj.2020.07.013>.
- Sharma, M. K., McDaniel, C. D., Kiess, A. S., Loar II, R. E., & Adhikari, P. (2022). Effect of housing environment and hen strain on egg production and egg quality as well as cloacal and eggshell microbiology in laying hens. *Poultry Science*, 101(2), 101595. <https://doi.org/10.1016/j.psj.2021.101595>.
- Tainika, B., & Şekeroğlu, A. (2020). Effect of production systems for laying hens on hen welfare. *TURJAF*, 8(1), 239–245. <https://doi.org/10.24925/turjaf.v8i1.239-245.3251>.
- Tainika, B., & Şekeroğlu, A. (2021). Environmental enrichments in laying hen production systems with emphasis on welfare and egg quality. *TURJAF*, 9(8), 1398-1406. <https://doi.org/10.24925/turjaf.v9i8.1398-1406.4240>.
- Yardım, Z., & Akşit, M. (2012). The effect of cage system and stocking density on performance, egg quality and microbial load of eggshell of laying hens. *Turkish Journal of Agriculture-Food Science and Technology*, 9(11), 2004-2012. <https://doi.org/10.24925/turjaf.v9i11.2004-2012.4649>.
- Uysal, A., & Laçın, E. (2024). The effect of different cage densities on laying performance and egg quality in brown and white laying hens. *Brazilian Journal Poultry Science*, 26, eRBCA-2024. <https://doi.org/10.1590/1806-9061-2024-1920>.
- Zinca, A. I., Lungu, V. D., Moise, A. E., & Drăgotoiu, D. (2024). The influence of laying hen rearing systems and maintenance technologies on egg quality parameters: A review. *Scientific Papers Animal Science and Biotechnologies*, 57(1), 260-260.



Aydın İlindeki Veteriner Hekimlerin Sığır ve Küçükbaş Hayvanların Taşımacılığına Yönelik Refah Algısı

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ÖZET

Türkiye’de hayvan taşımacılığı oldukça yoğun olup hayvan refahını olumsuz etkileyen önemli faktörlerden biridir. Aydın ili kapsamında 139 veteriner hekim ile gerçekleştirilen anket çalışması üzerinden hayvan nakillerinde hayvan refahını incelemek, nakillere yönelik yasal düzenlemeleri de dikkate alarak konuyu değerlendirmek amacıyla 2020 yılında bu çalışma gerçekleştirilmiştir. Katılımcıların %81.3’ü nakillerin kısmen veya tamamen hayvanlar için travmatik bir dönem olduğunu bildirmiştir. Nakillerdeki önemli stres kaynaklarının sırasıyla araçta yeterli alanın olmaması (%96.4), nakil mesafe ve süresi (%89.9), nakil araçlarının özellikleri (%88.5), hayvanların araçlarına yüklenmesi-indirilmesi (%72.7) ile yol-iklim koşulları (%59) olduğu saptanmıştır. Nakilde refahın sağlanması, hayvanların gittikleri yere uyum (%96.4) yanı sıra gösterecekleri performans (%79.2) açısından da önemli bulunmuştur. Hayvan refahı konusunda yasal düzenlemelerin yürürlüğe konmasıyla birlikte yetiştiricilerin bu konuda daha duyarlı oldukları (%64.1), böylece kısmen de olsa refahın güvence altına alındığı (%69.1), nakliye sonrası duyulan olumsuzlukların azaldığı (%71.9) ifade edilmiştir. Ayrıca nakillerde kullanılan vasıtaların donanımlarının genellikle yeterli olduğu (%54.7) ama yetersiz bulanların da azımsanmayacak bir düzey gösterdiği (%45.3), taşımaların genellikle (%94) karayolu ile gerçekleştiği ve 4 saatten az sürdüğü (%59) saptanmıştır. Bununla birlikte özellikle 8 saati aşan taşımalarda ek mevzuatlar nedeniyle refah durumuna daha fazla özen gösterilmesi gerekliliği açıktır. Tüm bu nedenlerle hayvan nakillerinin refah standartlarında gerçekleşmesi ancak ilgili tüm tarafların bu konuda eğitilmeleri ve uygulamaların yetkililerce yeterli denetlenmesi ile mümkün olduğu ortaya çıkmıştır.

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Anahtar Kelimeler

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Nakil koşulları
Nakil mevzuatı

Welfare Perception of Veterinarians in Aydın Province Regarding the Transportation of Cattle and Small Ruminants

ABSTRACT

Animal transportation in Turkey is considerably high and one of the important factors negatively affecting animal welfare. This study was carried out to examine animal welfare in animal transportation and to evaluate the issue considering legal regulations regarding transportation through a survey conducted with 139 veterinarians in Aydın province in 2020. 81.3% of the participants reported that the transports were partially or completely traumatic periods for the animals. It was determined that important sources of stress in transportation are space allocated per animal in a vehicle (%96.4), transportation distance and duration (%89.9), characteristics of transportation vehicles (%88.5), loading-unloading of animals onto transportation vehicles (%72.7), and road-climate conditions (%59). Ensuring the welfare of animals during transport was found to be important in terms of their adaptation to the destination (96.4%) as well as their performance (79.2%). It has been observed that with the introduction of legal regulations on animal welfare, breeders have begun to be more sensitive about animal welfare

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(%64.1), partially secured welfare (69.1%), and reduced the negative effects felt after transportation (71.9%). It was also found that the equipment of the vehicles used in transportation was generally sufficient (54.7%), but that those who found them inadequate showed a considerable level (45.3%), that transportation was generally carried out by road (94%) and took less than 4 hours (59%). However, due to additional legislation, it has become necessary to pay more attention to the welfare situation, especially in transports exceeding 8 hours. For all these reasons, it has become clear that animal transports can only be carried out in accordance with welfare standards if all relevant parties are trained on this issue and the practices are adequately monitored and inspected by the authorities.

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GİRİŞ

Hayvan refahı, hayvanın ihtiyaçlarını ve mutluluğunu gözeten, olası kısıtlama, zorlama, eziyet, acı, endişe ve korkunun yarattığı stresin yanı sıra sağlık kavramı ile de doğrudan ilişkilidir (Özgür, 2010; İzci ve ark., 2021). Çiftlik hayvanlarının korunması ve refahının sağlanmasında, bakım-besleme, nakil ve kesim aşamaları artan bir öneme sahiptir. Özellikle nakil koşulları, hayvan refahını etkileyen önemli aşamalardan biridir.

Hayvanlar, çeşitli amaçlarla bir yerden bir başka yere nakledilmektedir. Bu nedenle hayvanların nakil araçlarına yüklenmesi ve boşaltılmaları, aracın yapısı, nakil süresi ve mesafesi, sürüş kalitesi, uzun nakillerde yem ve suyun hayvanlara sağlanıp sağlanamaması, dinlenme süresi ve tüm bunları içeren nakil planı oldukça önemlidir (Altınçekiç & Koyuncu, 2010; Kara & Koyuncu, 2011). Nitekim ülkeler bu amaçla çeşitli yasa ve yönetmelikler yayınlamaktadır. Avrupa Birliği de 1991'de hayvan taşımacılığına ilişkin ortak yasal bir çerçeve çizmiş, 2007 yılından itibaren yürürlüğe giren, taşıma sırasında hayvanların korunmasına ilişkin tüzük (1/2005/EC) ile taşımada hayvanlar için yeterli bir koruma sağlamayı amaçlamıştır. Türkiye'de de gerekli yasal düzenlemeler yapılmış, kontrol ve denetim mekanizmaları yerleştirilmeye başlanmıştır (Demirel & Çak, 2016; Birli ve ark., 2025).

Türkiye'de hayvanların taşınması sırasında refahlarının sağlanmasına yönelik yasal düzenlemelerden biri 1986 yılında kabul edilen ve 2010 yılında yürürlükten kaldırılan 'Hayvan Sağlığı ve Zabıtası Kanunu'na bağlı çıkartılan yönetmekte hayvanların nakli ve refahı ile ilgili bazı iyileştirmeler gerçekleştirilmiştir. Türkiye'de hayvan nakillerinde refahla ilgili önemli yasal düzenlemeler, AB mevzuatı ile uyum çerçevesinde oluşturulan, 5996 sayılı 'Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu' doğrultusunda 2011 yılında yayınlanan 'Yurt İçinde Canlı Hayvan ve Hayvansal Ürünlerin Nakilleri Hakkında Yönetmelik (28145)' (Anonim, 2011a) ile nakillerde refahla ilgili en önemli yasal düzenleme olan 'Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliği (28152)'dir (Anonim, 2011b). Bu yönetmeliklerle hayvanların nakillerindeki uygulamalar, kullanılacak araçlar ve görevli personellerle ilgili standartlar ortaya konmuştur.

'Yurt İçinde Canlı Hayvan ve Hayvansal Ürünlerin Nakilleri Hakkında Yönetmelik' beş kez (Anonim, 2013; Anonim, 2018; Anonim, 2020a; Anonim, 2020b; Anonim, 2022), 'Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliği' ise dokuz kez (Anonim, 2014; Anonim, 2015a; Anonim, 2016a; Anonim, 2017a; Anonim, 2019; Anonim, 2021a; Anonim, 2021b; Anonim, 2023; Anonim, 2024a) değişikliğe uğramış, bazı maddelerin yürürlük tarihleri 31 Aralık 2026'ya ertelenmiştir.

Konuyla ilgili bir diğer yasal düzenleme ise 30 Mayıs 2015 tarihinde yayınlanan ve 2016 yılında yürürlüğe girmesi beklenen 'Hayvan nakillerinde kontrol ve dinlendirme istasyonu yönetmeliğidir (Anonim, 2015a). Fakat bu yönetmelik de sonradan çıkartılan üç yönetmelik ile (Anonim, 2015c; Anonim, 2016b, Anonim, 2017b) yürürlük tarihi 31.12.2019 şeklinde değişikliğe uğramıştır. Avrupa Birliği'nin 'Nakil Sırasında Hayvanların Korunması (91/628/EEC)' direktifi ve 'Nakil ve Nakille İlgili Uygulamalar Sırasında Hayvanların Korunması (1/2005/EC)' başlıklı regülasyonları ile uyumlu olarak hazırlanmış bu yönetmelik, hayvan nakilleri sırasında kontrollerinin yapıldığı ve hayvanların dinlendirildiği istasyonlardaki koşulları, istasyonlarda yapılacak işlem ve denetimlerle ilgili kuralları ortaya koymuştur.

Türkiye'de her bölge et ihtiyacını kendi bölgesinde yetiştirilen hayvanlardan yeterince sağlayamamaktadır. Bu nedenle özellikle nüfusun yoğun olduğu batı bölgelerine ülkenin doğusundan giderek artan sayıda hayvan

nakilleri gerçekleşmektedir. Nitekim Türkiye’de son yıllarda gerçekleşen büyükbaş ve küçükbaş hayvan hareketleri incelendiğinde nakillerinin hayvan sayılarına oranı azımsanamayacak kadar yüksektir (Çizelge 1). Büyükbaş hayvan varlığının %5.8-17.9’u, küçükbaş hayvanların ise %8.6-20.5’inin yıllık olarak bir yerden başka bir yere taşındığı görülmektedir. 2013 yılından itibaren sevk sayılarıdaki oransal düşüşün nedeni, 14.05.2013 tarihinde yapılan yönetmelik değişikliği ile ilçeler arasındaki nakillerde hayvan sevk raporu düzenleme zorunluluğunun kaldırılmış olmasıdır. Fakat il içi hayvan hareketlerinin nakillerin önemli bir kısmını oluşturduğu unutulmamalıdır. Türkiye’de sadece 2023 yılında il içinde 9,843,619 adet büyükbaş ve 20,137,499 adet küçükbaş hareketliliğinin gerçekleştiği bildirilmiştir (Anonim, 2024b). Bu nedenle hayvan nakillerinin uygun şartlarda gerçekleştirilmediği ve hayvan refahından uzaklaşıldığı durumlarda büyük ekonomik kayıpların olacağı açıktır (İzci ve ark., 2021).

Bu çalışmanın amacı, artan hayvan nakilleri sırasında hayvan refahının durumunu Aydın ilinde kamuda görev yapan veteriner hekimlerin görüşleri üzerinden irdelemek, büyükbaş ve küçükbaş hayvan nakillerine yönelik yasal düzenlemeleri de dikkate alarak konuyu değerlendirmektir.

Çizelge 1. Türkiye’de yıllara göre hayvan hareketlerinin sayı (baş) ve oranı (%)

Table 1. Number (head) and rate (%) of animal transports in Türkiye by year

Yıl	Büyükbaş hayvan sayısı	Nakil olan büyükbaş hayvan sayısı	Nakil olan büyükbaş hayvan oranı	Küçükbaş hayvan sayısı	Nakil olan küçükbaş hayvan sayısı	Nakil olan küçükbaş hayvan oranı
2010	11,454,526	1,680,662	14.7	29,382,924	6,011,091	20.5
2011	12,483,969	1,724,350	13.8	32,309,518	5,997,033	18.6
2012	14,022,347	2,504,123	17.9	35,782,519	6,934,432	19.4
2013	14,532,848	2,304,612	15.9	38,509,795	6,926,641	18.0
2014	14,244,673	2,131,373	15.0	41,462,349	6,534,302	15.8
2015	14,127,837	1,776,847	12.6	41,924,100	6,395,434	15.3
2016	14,222,228	1,455,865	10.2	41,329,232	6,735,571	16.3
2017	16,105,025	1,709,506	10.6	44,312,308	6,994,212	15.8
2018	17,220,903	1,296,120	7.5	46,117,399	6,257,080	13.6
2019	17,872,331	1,339,200	7.5	48,481,479	6,866,729	14.2
2020	18,157,971	1,046,844	5.8	54,112,626	7,056,145	13.0
2021	18,036,117	1,280,040	7.1	57,519,204	6,051,515	10.5
2022	17,023,791	1,063,216	6.2	56,265,750	5,037,223	9.0
2023	16,583,005	962,994	5.8	52,363,410	4,523,059	8.6

(Anonim, 2024b)

MATERYAL ve METOD

Bu araştırma, Aydın ili sınırları içerisinde sığır, koyun ve keçi nakillerinde hayvan refahının değerlendirildiği 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, 2020 yılında Aydın ilinin 17 ilçesinde kamu personeli olarak görev yapan tüm veteriner hekimlere ulaştırılmış, biri hariç 139 veteriner hekime anket uygulanmıştır. Ankete katılan personelin tamamı hayvan hareketlerine yönelik veteriner sağlık raporları düzenlemiş, dönüşümlü görevlendirilenlerle aşılama, hayvan sevk, ruhsatlandırma, denetim kontrol ve mezbaha kesimleri gibi görev alanlarında sahada bulunmuşlar ve tüm nakil süreçlerinde görev almışlardır.

Ankete katılan veteriner hekimlere çiftlikte hayvan refahıyla ilgili durumu sorgulayan 25 soru ve bölgelerindeki büyükbaş ve küçükbaş hayvan nakillerini hayvan refahı yönünden değerlendirmeleri istenen 37 soru sorulmuştur. Çiftlikte hayvan refahına ait sonuçlar, Birli ve ark. (2025) tarafından ortaya konmuştur. Hayvan nakillerinde refah sorularına ait sonuçları ise bu çalışmada ele alınmıştır.

Taşımada hayvan refahıyla ilgili kapalı uçlu çoktan seçmeli sorular yanında beşli likert ölçeğinde olmak üzere toplam 37 soru ankette yer almıştır. Katılımcıların tutum ve eğilimini belirleyen beşli Likert ölçeğindeki sorularda ‘Katılıyorum’, ‘Kısmen katılıyorum’, ‘Kararsızım’, ‘Kısmen katılmıyorum’, ‘Katılmıyorum’ şeklinde seçenekler sunulmuştur. Nakledilen türlerin yoğunluğu, nakil amacı, nakilde kullanılan araçlar, taşıma mesafesi ve taşıma süresi hakkında veterinerlerin tahmini bu konulardaki düzeylere ‘En çok’, ‘Çok’, ‘Orta’, ‘Az’ ve ‘Olmadı’ gibi seçenekler üzerinden değerlendirilmiştir.

Anket internet ortamında 'Google Formlar' kullanılarak katılımcılara ulaştırılmış, doldurulan formlar otomatik olarak tek kaynakta toplanmıştır.

İstatistik Analizler

Her soru için elde edilen sonuçlar, sorudaki seçenekleri tercih edenlerin adet ve yüzdeleri üzerinden değerlendirilmiştir. Araştırmada kullanılan sorulara ait güvenirlik durumu Cronbach's Alpha değeri ile ortaya konmuştur. Çalışmada ele alınan kriterlerin ankete katılanların cinsiyeti, yaş ve mesleki tecrübe grubu ile olan ikili ilişkileri çapraz tablolar ve ki-kare analiziyle irdelenmiştir (İkiz ve ark., 2000). Söz konusu ilişkinin önem durumları ki-kare değeri (χ^2), serbestlik derecesi (sd) ve P-değerleri verilerek raporlanmıştır. Ancak alt gruplarda beklenen frekansı beşten az olan gözlerin oranının %20'den fazla olduğu durumunda Fisher'in Exact Test sonuçları üzerinden yorumlamaya gidilmiştir. Ele alınan kriterlerin cinsiyet, yaş ve tecrübe grubu ile olan ikili ilişkisi eğer önemsiz bulunmuş ise ele alınan kriterin sadece ana düzeylerine ait adet ve yüzde sonuçları verilmiştir. Fakat kriterin cinsiyet, yaş ve tecrübe grubu ile ilişkisi önemli bulunmuşsa ikili çapraz tablolarla alt gruplar dikkate alınarak değerlendirme yapılmıştır. Anket sorularının cinsiyet, yaş ve tecrübe grubuna 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

Taşımada hayvan refahı algısını belirlemeye çalışan soruların Cronbach's Alpha güvenirlik değeri 0.788 olarak saptanmıştır. Bu düzey, çalışma sonuçlarının yeterli güvenirliğe sahip olduğunu göstermektedir.

Ankete katılan 139 veteriner hekimin yaş, cinsiyet, eğitim ve mesleki tecrübe düzeyleri gibi demografik özellikleri Birli ve ark (2025) tarafından ayrıntılı açıklanmıştır.

Anket sonuçlarına göre hayvanların çiftlik şartlarındaki refahlarına gösterilen özenin nakil esnasında da gösterilmesi gerektiğini veteriner hekimlerin neredeyse tamamı (%84.2+%12.9=%97.1) onaylamıştır (Çizelge 2). Bununla birlikte nakliyenin hayvanları bir şekilde gidecekleri yerlere ulaştırmak olduğuna inanan katılımcılar da (%8.6+%28.8=%37.4) vardır. Halbuki bir diğer soruda nakliyenin hayvanların hayatları boyunca yaşadıkları en travmatik dönem olduğunu katılımcılar büyük çoğunlukla (%36.7+%44.6=%81.3) kabul etmiş, %10.8'i (%5.8+%5) bu görüşe katılmadığını, %7.9'u ise bu konuda kararsız kaldığını bildirmiştir (Çizelge 2). Çizelge 2'deki sorulara verilen cevaplar bir arada değerlendirildiğinde nakil sırasında refah olgusuna büyük önem verildiği, bununla birlikte nakliyenin sadece bir taşıma olmadığının %61.8 (%49.6+%12.2) yerine daha yüksek bir oranda kabul edilmesi gerektiği beklentisi oluşmaktadır. Bu düzeyin beklenenden düşük çıkması, katılımcıların bir kısmının bu soruyu değerlendirirken refah olgusuna fazla odaklanmadıklarını göstermektedir. Bununla birlikte genel olarak nakliyenin sadece hayvanların taşınmasından ibaret olmadığını, nakliye koşullarının fizyolojik ve psikolojik zorluklar içeren bir süreç olduğunu, nakil koşullarının iyileştirilmesinin hayvanların sağlıkları ve verimleri üzerinde olumlu katkılar sağlayacağı konusunda büyük oranda fikir birliği olduğu görülmektedir. Taşımada hayvan refahının sağlanması için ek yatırımlar gerekli olsa da sağlıklı bireyler ve kaliteli ürün yanında veteriner masraflarında da önemli azalmalar sağlayacağı muhakkaktır.

Çizelge 2. Nakilde hayvan refahı kavramı

Table 2. Concept of animal welfare in transport

Anket sorusu	Seçenekler	Adet	%
Hayvanların çiftlik şartlarındaki refahlarına gösterilen özenin nakil esnasında da gösterilmesi çok önemlidir	Katılıyorum	117	84.2
	Kısmen katılıyorum	18	12.9
	Kararsızım	2	1.4
	Kısmen katılmıyorum	1	0.7
	Katılmıyorum	1	0.7
Nakliye sadece hayvanları bir şekilde gidecekleri yerlere ulaştırmaktır	Katılıyorum	12	8.6
	Kısmen katılıyorum	40	28.8
	Kararsızım	1	0.7
	Kısmen katılmıyorum	17	12.2
	Katılmıyorum	69	49.6
Hayvanların nakliyesi, hayatları boyunca yaşadıkları en travmatik dönemlerdendir	Katılıyorum	51	36.7
	Kısmen katılıyorum	62	44.6
	Kararsızım	11	7.9
	Kısmen katılmıyorum	8	5.8
	Katılmıyorum	7	5.0

Nakilde en sık görülen refah sorununun %88.5 oranı ile stres olduğu saptanmıştır (Çizelge 3). Hayvan nakillerinde karşılaşılan stres kaynaklarına yönelik sorulan soruda katılımcılara çoklu seçim şansı verilmiş, önem sırasıyla araçta hayvan başına alan (%96.4), nakil süresi (%89.9), nakil aracının özellikleri (%88.5), hayvanların araçlara yüklenmesi (%72.7), rampaların durumu (%67.6) ile yol-iklim koşullarının (%59) hayvan refahı üzerine doğrudan etkili faktörler olduğu ortaya konmuştur (Çizelge 3).

Çizelge 3. Nakilde hayvan refahına etki eden faktörler

Table 3. Factors affecting animal welfare during transport

Anket sorusu	Seçenekler	Adet	%
Nakil sırasında en çok oluşabilen refah sorunu hangisidir?	Stres	123	88.5
	Yaralanma	11	7.9
	Hastalık	2	1.4
	Ölüm	3	2.2
Hayvan nakillerinde hangi faktörler hayvan refahı üzerine doğrudan etkili olmaktadır (çoklu seçim mümkün)	Araçta hayvan başına ayrılan alan	134	96.4
	Nakil süresi	125	89.9
	Nakil aracının özellikleri	123	88.5
	Araçlara yükleme	101	72.7
	Yükleme boşaltma rampaları	94	67.6
	Yol ve iklim koşulları	82	59.0
Nakilde hayvan refahına dikkat edilmesi hayvanların gittikleri yerde ortama adaptasyonlarının olumlu etkiler	Katılıyorum	102	73.4
	Kısmen katılıyorum	32	23.0
	Kararsızım	1	0.7
	Kısmen katılmıyorum	-	-
	Katılmıyorum	4	2.9
Nakilde hayvan refahına dikkat edilmesi hayvanların damızlık ve kasaplık verimlerini/kalitelerini artırmıştır.	Katılıyorum	65	46.8
	Kısmen katılıyorum	52	32.4
	Kararsızım	6	4.3
	Kısmen katılmıyorum	7	5.0
	Katılmıyorum	9	6.5

Nakilde stresin hayvanların araçlara yüklenmesiyle başladığı ve araçların boşaltılmasına kadar sürdüğü unutulmamalıdır. Ayrıca yükleme ve boşaltma elemanlarının tavır ve davranışları, kullanılan rampaların durumu, hayvanları strese sokan önemli diğer faktörlerdir (Güney & Toplu, 2017). Bu nedenle taşımada uygun yükleme ve boşaltma rampaları kullanılmalı, araç ve rampa zeminleri hayvanların kayma ve düşmesini önleyecek ve yeterli hareket alanı sağlayacak şekilde düzenlenmelidir. Gürültülü ortam ve kaygan zeminlerin hayvanları psikolojik ve fiziksel olarak strese sokabildiği, yaralanmalara hatta ölümlerine neden olabildiği dikkate alınmalıdır (Yıbar & Çetin, 2013). Ünal ve ark. (2008) kesimhaneye götürülen kasaplık sığır ve kuzuların yüklenme ve boşaltılmalarında kullanılan %9'luk açığa sahip rampaların standartlara uygun olduğunu bildirmişlerdir. Nitekim rampa açısının sığır ve koyunlarda 20-25 dereceden fazla olmaması (Kara & Koyuncu, 2011), açının artması durumunda rampa zemininin yürümeyi kolaylaştıracak materyallerle donatılması gerekir.

Diğer yandan hayvan başına ayrılan alan konusunda problem yaşamamak ve ek stres kaynağı oluşturmamak için hayvanların canlı ağırlıkları dikkate alınarak alan planlanmasına gidilmesi daha doğru olacaktır. Ayrıca farklı ağırlıkta olan hayvanların taşıtta farklı bölmelerde bulundurulması önerilmektedir (Kara & Koyuncu, 2011). Diğer yandan kavga gibi çeşitli anormal davranışların ortaya çıkmasına neden olabildiğinden nakil esnasında hayvanlar cinsiyetlerine göre de ayrı taşınmalı veya ayrı bölmelerde bulundurulmalıdır. Ünal ve ark. (2008) kesimhaneye götürülen 3719 kasaplık sığır ve 17896 kuzu ile yaptıkları ölçümlerde canlı ağırlığa göre araçta ayrılması gereken alanların beklenen değerlerden önemli düzeyde farklı olduğunu saptamıştır. Halbuki nakil sırasında araçtaki hayvan sayısı, hayvanların hareket durumu, araçtaki sarsıntılar, ortamın sıcaklık ve nemi, havalandırma durumu, su-yem kısıtlaması ile nakil süresi gibi faktörler stres oluşturarak refahı olumsuz yönde etkileyebilmektedir. Nitekim Yıldız & Saatçi (2009) Sarıkamış Tarım ilçe Müdürlüğü'nce üç yıllık sürede 3162 araçla yapılan hayvan sevklerini incelemiş, 54 araç sürücüsüne bir anket uygulamış, sevk sırasında araçlarda suluk ve yemlik bulunmadığını saptamıştır.

Hayvanların çiftlik şartlarında refahlarına gösterilen özenin nakil esnasında da gösterilmesi, hayvanların gittikleri ortama adaptasyonları açısından da önemlidir. Nitekim katılımcılar da bu durumu büyük oranda (%73.4+%23=%96.4) onaylamıştır (Çizelge 3). Bu nedenle nakil araçları güvenlik yanı sıra yeterli alan ve havalandırma sağlayacak şekilde tasarlanmalıdır. Eğer taşıtta alan yetersizliği varsa ve farklı sürülerin hayvanları beraber taşıyorsa artan nem ile hayvanlarda korku da oluşmaktadır. Diğer yandan yüksek sıcaklık,

refahın azalmasına ve ölüm oranının artmasına neden olmaktadır (Sağmanlıgil ve ark., 2015). Özellikle yüksek nem oranıyla birlikte artan sıcaklığın etkisi daha çarpıcı hissedilirken, doğrudan güneş ışığına maruz kalan ve hareketsiz duran nakil araçlarında ölüm riski daha fazla olmaktadır.

Nakilde refah, hayvanların verim performansı açısından da önemlidir. Bu yönde katılımcıların %79.2'si (%46.8+%32.4), nakilde hayvan refahı konularına dikkat edilmesinin hayvanların damızlık veya kasaplık performansı ile verim kalitesinde olumlu etkiler yaptığını, %11.5'i (%5+%6.5) ise bu görüşe kısmen veya tamamen katılmadığını ifade etmişlerdir (Çizelge 3). Nakil sırasındaki uygulamaların refah açısından değerlendirilmesinin bir diğer yolunun kesim sonrasında karkasın incelenmesi olduğu bilinmektedir. Çünkü karkasta ezik, yırtık ve lekelerin bulunması, nakil ve kesim sırasında refah şartlarının yeterli olmadığının önemli bir göstergesi olabilir. Bu süreçte hayvanlar üzerinde oluşan etkiler, fiziksel ve biyokimyasal değişikliklere neden olmakta, davranış problemlerine ve et kalitesinde bozulmalara yol açmaktadır (Erzurum & Yılmaz, 2016). Özellikle yüklemenin sıkıntılı olduğu durumlarda karkasta eziklere daha fazla rastlanmaktadır (Sağmanlıgil ve ark., 2015). Karkasta ezik bölgelerin artması etin kalitesinin düşmesine ve ekonomik kayıplara yol açmaktadır. Daha da ötesinde ölüm oranlarında artışa, hayvanlarda ağırlık kaybına ve karkasta deformasyon sonucu etin raf ömrünün kısılmasına neden olabilmektedir (Güngör & Ünal, 2020). Güngör & Ünal (2020), nakilde yaşanan olumsuzlukların etkisinin bakıcı, şoför, araç, mevsim, açlık, susuzluk, yolun durumu gibi birçok etmene bağlı olduğunu, bu etmenlere duyarlılığın ise türe, ırka, cinsiyete, yaşa, kondisyona ve yetiştirme şekline göre değiştiğini bildirmiştir.

Hayvan refahı yönetmeliğinin yürürlüğe girmesiyle birlikte yetiştiricilerin hayvan refahı konusunda daha duyarlı olmaya başladığını ifade eden katılımcıların (%31.7+32.4=%64.1) yanı sıra, bunun tam olarak doğru olmadığını belirten veteriner hekimler de (%8.6+15.8=%24.4) vardır (Çizelge 4). Hayvan refahı konusunda duyarlılığın arttığını söyleyenlerin oranı, katılımcı yaş grubunun artması ile artış göstermiştir ($X^2=24.381$; $sd=12$; $P=0.018$). Söz konusu duyarlılığın oluştuğunu reddedenlerin daha çok gençler olduğu saptanmıştır. Bu durum genç veterinerlerin 2011 yılında yürürlüğe giren 'Hayvan Nakilleri Sırasında Refahı ve Korunması Yönetmeliği'nin yayınlanması öncesindeki uygulamaları yeterince bilmemelerinin bir sonucu olarak şekillenmiş olabilir.

Hayvan refahı yönetmeliğinin yürürlüğe girmesiyle nakil işlemlerinde hayvanların psikolojik ve fizyolojik olarak strese maruz kaldığı durumların azaldığını ifade edenlerin oranı %81.3 (%41+%40.3) iken, uygulamada bir değişiklik olmadığını belirtenlerin oranı sadece %15.8'dir (Çizelge 4). Bu soruya verilen cevaplar veteriner hekimlerin mesleki tecrübe gruplarına (0-9; 10-19; 20-29; 30 yıl ve üzeri) göre önemli ($X^2=21.654$; $sd=12$; $P=0.042$) değişimler göstermiştir. Fakat alt gruptaki gözlem sayılarının yetersizliği bu ilişkiyi net olarak ortaya koyamamıştır (Fisher's Exact Test $P=0.071$). Fakat eğilim olarak mevcut duruma bakıldığında yönetmeliğin uygulanması ile stres durumlarında bir azalma olduğunu kabul edenlerin oranı, katılımcıların mesleki tecrübelerindeki artışa bağlı olarak %72'lerden %100'lere çıkmıştır. Konu hakkında kararsızların hepsinin 10-19 yıl tecrübe grubunda toplandığı saptanmıştır. Genel değerlendirildiğinde yeni düzenlemeler ile nakilde stres faktörleri tamamen yok olmasa da belirli ölçülerde azaldığı anlaşılmaktadır. Nitekim katılımcılara göre yönetmeliğin uygulanması, hayvanların korunması ve refahlarının güvence altına alınmasını önemli ölçüde (%28.8+%40.3=%69.1) sağlanmıştır (Çizelge 4). Bu oran içinde durumu kısmen olumlu karşılayanların payı yüksek (%40.3) olsa da yönetmeliğin yürürlüğe girmesi ile hayvanların korunması ve refahında olumlu değişimler başladığı anlaşılmaktadır. Diğer yandan katılımcıların %22.3'ü (%9.4+%12.9) yönetmeliğin hayvanların korunması ve refahını güvence altına aldığı görüşüne katılmadıklarını belirtmiştir (Çizelge 4).

Katılımcıların %94.2'si (%82+%12.2) hayvan nakillerinin, hayvan refahına uygun koşullarda gerçekleştirilmesi için yapılan yasal düzenlemelerin gerekli olduğunu, %2.9'u ise gerekli olmadığını ifade etmiştir. Yasal düzenlemelerin gerekliliği konusundaki tepkiler cinsiyete göre değişkenlik göstermiştir ($X^2=15.551$; $sd=3$; $P=0.013$; Fisher's Exact Test $P=0.017$). Erkeklerin %84'ü, kadınların %71'i yasal düzenlemelerin gerekli olduğu görüşüne katılmış, kadınların %17.6'sı erkeklerin %1'i bu konuda kararsız kalmıştır. Kısmen katılmayanlar her iki cinsiyette de bulunmazken, kısmen katılanların oranı ikisinde de %12'dir (Çizelgede verilmemiştir). Bu sorudaki cinsiyete göre oluşan görüş farklılıkları önemli değişim gösterse de ankete katılan kadın sayısının azlığı (17 kişi) bu ilişkiye ait sonucun geçerliliğini önemli ölçüde zayıflatmaktadır.

Diğer bir soruda ele alındığı gibi katılımcıların çoğu (%26.6+%45.3=%71.9) yönetmeliğin uygulanması ile hayvanların nakillerinde yaşanan olumsuzlukların azaldığını, %19.4'ü (%7.2+%12.2) ise azalmadığını söylemişlerdir (Çizelge 4).

Veteriner hekimlerin %83.4'ü (%48.9+%34.5) nakil raporu düzenlerken hayvan sağlığını korumanın yanında hayvanların refahını da düşünmenin mesleki sorumluluklarını artırıp, endişelerini azalttığını, %6.5'i (%0.7+%5.8) ise bu görüşe katılmadıklarını ifade etmiştir (Çizelge 4).

Nakil araçlarının yapısı ve kullanım şekli hayvan refahını olumlu ya da olumsuz etkilemektedir (Altınçekiç & Koyuncu, 2010). Ankete katılanların %54.7'si (%7.2+%47.5) hayvan nakillerinde kullanılan vasıtaların donanımlarının hayvan refahı için tamamen veya kısmen yeterli olduğunu ifade ederken, %36.7'si (%17.3+%19.4)

araçları yetersiz bulduklarını belirtmiştir (Çizelge 5). Bu nedenle nakil öncesi hayvanların ve araçların nakle uygunlukları mutlaka hayvan refahı açısından incelenmelidir. Nitekim katılımcıların %85.6'sı (64.7+%20.9) hayvan nakillerinin profesyonel nakliye şirketleri tarafından yapılması gerektiğini belirtirken, %10.8'i (%3.6+%7.2) ise bu görüşe katılmadığını ifade etmiştir (Çizelge 5).

Çizelge 4. Yasal düzenlemelerin nakilde hayvan refahına etkisi

Table 4. Effect of legal regulations on animal welfare during transport

Anket sorusu	Seçenekler	Adet	%
Nakilde hayvan refahı yönetmeliği uygulaması, yetiştiricilerin hayvan refah konusunda daha duyarlı olmaları yönünde bir algı oluşturmıştır	Katılıyorum	44	31.7
	Kısmen katılıyorum	45	32.4
	Kararsızım	16	11.5
	Kısmen katılmıyorum	12	8.6
	Katılmıyorum	22	15.8
Nakilde hayvan refahı yönetmeliğinin uygulanması, hayvanların psikolojik ve fizyolojik olarak strese maruz kaldığı durumları azaltmıştır	Katılıyorum	57	41.0
	Kısmen katılıyorum	56	40.3
	Kararsızım	4	2.9
	Kısmen katılmıyorum	7	5.0
	Katılmıyorum	15	10.8
Nakilde hayvan refahı yönetmeliğinin uygulanması, hayvanların korunması ve refahını güvence altına almıştır	Katılıyorum	40	28.8
	Kısmen katılıyorum	56	40.3
	Kararsızım	12	8.6
	Kısmen katılmıyorum	13	9.4
	Katılmıyorum	18	12.9
Hayvan nakillerinin, hayvan refahına uygun koşullarda gerçekleştirilmesi için yasal düzenlemeler gerekliydi	Katılıyorum	114	82.0
	Kısmen katılıyorum	17	12.2
	Kararsızım	4	2.9
	Kısmen katılmıyorum	-	-
	Katılmıyorum	4	2.9
Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliği'nin uygulanması ile nakliye sonrası duyulan olumsuzluklar azalmıştır	Katılıyorum	37	26.6
	Kısmen katılıyorum	63	45.3
	Kararsızım	12	8.6
	Kısmen katılmıyorum	10	7.2
	Katılmıyorum	17	12.2
Nakilde hayvan sağlığını korumanın yanında hayvan refahını da gözeterek olmam mesleki anlamda sorumluluklarımı artırmış, endişelerimi azaltmıştır	Katılıyorum	68	48.9
	Kısmen katılıyorum	48	34.5
	Kararsızım	14	10.1
	Kısmen katılmıyorum	1	0.7
	Katılmıyorum	8	5.8

Çizelge 5. Hayvan taşımacılığının ve nakillerde kullanılan araçların durumu

Table 5. Status of animal transportation and vehicles used in transportation

Anket sorusu	Seçenekler	Adet	%
Kullanılan nakil vasıtalarının taşıma standartları (araç donanımı vs.) hayvan refahı için yeterlidir	Katılıyorum	10	7.2
	Kısmen katılıyorum	66	47.5
	Kararsızım	12	8.6
	Kısmen katılmıyorum	24	17.3
	Katılmıyorum	27	19.4
Hayvanların nakilleri profesyonel nakliye şirketleri tarafından yapılmalıdır	Katılıyorum	90	64.7
	Kısmen katılıyorum	29	20.9
	Kararsızım	5	3.6
	Kısmen katılmıyorum	5	3.6
	Katılmıyorum	10	7.2

Ankete katılan veterinerlerin beyanlarına göre Aydın ilinden bir yıl içerisinde gerçekleştirilen hayvan nakillerine yönelik düzenlenen sevk belgelerinin çoğunluğunun sığır nakillerine ait olduğu, daha sonra sırasıyla küçükbaş ve kanatlı nakillerinin geldiği saptanmıştır (Çizelge 6). Nitekim Çizelge 6-Çizelge 11'de sunulan kriterlerde sütun karşılaştırmaları, 'En çok' ve 'Çok' satır toplamalarının söz konusu genel satır toplamı içindeki payı üzerinden

yapılmıştır. Buna göre Aydın ilinde gerçekleşen sığır, küçükbaş ve kanatlı nakillerinin toplam nakiller içindeki payı sırasıyla 0.56, 0.25 ve 0.14'dür (Çizelge 6).

Çizelge 6. Aydın'daki hayvan nakillerinde daha çok hangi tür için nakil raporu düzenlenmektedir?

Table 6. For which species are transport reports prepared most often in animal transports in Aydın?

Düzyey	Sığır		Küçükbaş		Kanatlı		Kedi-Köpek		Tek Tırnaklı		Satır Toplam
	Kişi	%	Kişi	%	Kişi	%	Kişi	%	Kişi	%	
En çok	114	82.0	14	10.1	14	10.1	5	3.6	1	0.7	
Çok	15	10.8	44	31.7	18	12.9	3	2.2	1	0.7	
Ara Toplam*	129	0.56	58	0.25	32	0.14	8	0.04	2	0.01	229
Orta	7	5.0	54	38.8	34	24.5	9	6.5	4	2.9	
Az	-	-	18	12.9	36	25.9	34	24.5	43	30.9	
En az	1	0.7	4	2.9	10	7.2	16	11.5	17	12.2	
Düzenlemedim	2	1.4	5	3.6	27	19.4	72	51.8	73	52.5	
Toplam	139	100	139	100	139	100	139	100	139	100	

*En fazla ve fazla düzeylerin toplamaları ve bu toplamın satır toplamı içindeki oranı

Büyükbaş hayvan nakillerinde sevk işlemlerinin ağırlıklı olarak sırasıyla damızlık satış (%35), kesime gönderme (%29) ve besi amaçlı (%28) olduğu bildirilmiştir (Çizelge 7). Küçükbaş nakillerindeki durum da büyükbaşta ki benzerdir (Çizelge 8). Fakat Aydın ilinde göçer hayvancılık nakilleri küçükbaşlarda daha fazladır (%15).

Çizelge 7. Aydın'daki büyükbaş hayvan nakillerinin amacı

Table 7. Purpose of cattle transport in Aydın

Düzyey	Damızlık		Besilik		Kesim için		Göçer		Diğer		Satır Toplam
	Kişi	%	Kişi	%	Kişi	%	Kişi	%	Kişi	%	
En çok	61	43.9	30	21.6	46	33.1	3	2.2	3	2.2	
Çok	36	25.9	46	33.1	35	25.2	10	7.2	6	4.3	
Ara toplam*	97	0.35	76	0.28	81	0.29	13	0.05	9	0.03	276
Orta	26	18.7	46	33.1	41	29.5	37	26.6	19	13.7	
Az	7	5.0	9	6.5	10	7.2	34	24.5	23	16.5	
En az	3	2.2	2	1.4	5	3.6	24	17.3	26	18.7	
Düzenlemedim	6	4.3	6	4.3	2	1.4	31	22.3	62	44.6	
Toplam	139	100	139	100	139	100	139	100	139	100	

*En fazla ve fazla düzeylerin toplamaları ve bu toplamın satır toplamı içindeki oranı

Çizelge 8. Aydın'daki koyun ve keçi nakillerinde amaç

Table 8. Purpose of sheep and goat transport in Aydın

Düzyey	Damızlık		Besilik		Kesim için		Göçer		Diğer		Satır Toplam
	Kişi	%	Kişi	%	Kişi	%	Kişi	%	Kişi	%	
En çok	46	33.1	14	10.1	35	25.2	22	15.8	3	2.2	
Çok	32	23.0	35	25.2	27	19.4	12	8.6	5	3.6	
Ara toplam*	78	0.34	49	0.21	62	0.27	34	0.15	8	0.03	231
Orta	42	30.2	50	36.0	39	28.1	33	23.7	22	15.8	
Az	12	8.6	22	15.8	20	14.4	37	26.6	24	17.3	
En az	1	.7	9	6.5	7	5.0	15	10.8	26	18.7	
Düzenlemedim	6	4.3	9	6.5	11	7.9	20	14.4	59	42.4	
Toplam	139	100	139	100	139	100	139	100	139	100	

*En fazla ve fazla düzeylerin toplamaları ve bu toplamın satır toplamı içindeki oranı

Nakillerin hangi yollarla gerçekleştirildiği sorulduğunda, veteriner hekimler saha tecrübelerine dayanarak nakillerin neredeyse tamamının (%94) karayolu ile yapıldığını ortaya koymuştur (Çizelge 9). Bu durumda nakilde araç sürüş kalitesi ön plana çıkmaktadır. Çünkü araçların yol ve yük durumuna uygun olmayan kötü sürüş teknikleriyle kullanılması, hayvanların refahını olumsuz etkilemektedir. Ayrıca araçların fazla kattan oluşması, süspansiyon ve yalıtım gibi araç özellikleri, araç içi iklimlendirme koşulları, naklin kalitesine doğrudan etki eden etmenler olduğu unutulmamalıdır. Diğer yandan araçlarındaki bölmelerin yüksekliği, hayvanların doğal pozisyonlarına uygun olmalıdır. Nakil araçlarının çevresi özellikle uzun nakillerde aşırı iklim şartlarının etkisini azaltacak şekilde yalıtımlı olmalı, yeterli havalandırma açıklıkları veya havalandırma sistemi araçta

bulunmalıdır. Araçlar temiz ve dezenfekte edilmiş olmalı, zemin yüzeyi kaygan olmamalı, aydınlatma sistemi bulunmalıdır (Kara & Koyuncu, 2011; Sağmanlıgil ve ark., 2015).

Çizelge 9. Aydın'da gerçekleşen hayvan nakillerinde kullanılan araçlar

Table 9. Vehicles used in animal transport in Aydın

Düzyey	Karayolu		Demiryolu		Denizyolu		Havayolu		Satır Toplam
	Kişi	%	Kişi	%	Kişi	%	Kişi	%	
En çok	128	92.1	-	-	-	-	-	-	
Çok	9	6.5	-	-	4	2.9	4	2.9	
Ara toplam*	137	0.94	-	-	4	0.03	4	0.03	146
Az	-	-	12	8.6	16	14.4	21	15.1	
Olmadı	2	1.4	127	91.4	119	7.9	114	82.0	
Toplam	139	100	139	100	139	100	139	100	

*En çok ve çok düzeylerin toplamı ve bu toplamın satır toplamı içindeki oranı

Nakilde refahı etkileyen önemli faktörlerden biri de nakil mesafesi ve buna bağlı oluşan nakil süresidir. Son bir yıl içinde Aydın ilinde gerçekleşen hayvan nakillerindeki taşıma mesafeleri dört grup (0-50 km, 51-100 km; 101-200 km ve 200 km'den daha uzak) altında sorgulanmıştır. İl içi nakil işlemlerinin takibinin görevli veteriner hekimler tarafından gerçekleştirildiğinden ve saha çalışmalarında ilçelerin demografik yapısı, besi ve damızlık süt işletmelerinin hangi ilçelerde yoğun olduğu, besilik ve damızlık hayvan hareketlerinin hangi ilçe güzergahları yönünde olduğu, hayvancılık işletmelerindeki gözlem ve değerlendirmeleri veterinerlere il içi hayvan hareketlerinin mesafeleri hakkında önemli fikirler vermektedir. Bu tecrübelerle dayanarak veteriner hekimlerin yaptıkları değerlendirmelere göre nakillerin taşıma mesafesi gruplarına dağılım oranlarının (%22-%29) birbirine yakın olduğu, %29 ile en fazla taşımanın 51-100 km mesafe grubunda gerçekleştiği belirlenmiştir (Çizelge 10). Ünal ve ark. (2008) ortalama nakil süresi ve mesafesini kesimhaneye götürülen kasaplık sığırlarda sırasıyla 1.85 saat ve 92.33 km, kuzularda ise 1.91 saat ve 74.05 km olarak saptamışlardır. Bu düzeyler, çalışmamızdaki en yaygın 51-100 km ile uyumludur. Bununla birlikte Sarıkamış Tarım İlçe Müdürlüğü'nce üç yıllık sürede 3162 araçla yapılan hayvan sevklerinin %52.8'inin 8 saatten uzun sürdüğü bildirilmiştir (Yıldız & Saatçi, 2009).

Çizelge 10. Aydın'da gerçekleşen hayvan nakillerinde taşıma mesafesi

Table 10. Transport distance in animal transport in Aydın

Düzyey	0-50 Km		51-100 Km		101-200 Km		200 km'den fazla		Satır Toplam
	Kişi	%	Kişi	%	Kişi	%	Kişi	%	
En çok	40	28.8	27	19.4	31	22.3	29	20.9	
Çok	19	13.7	45	32.4	30	21.6	24	17.3	
Ara Toplam*	59	0.24	72	0.29	61	0.25	53	0.22	245
Az	50	36.0	-	-	-	-	-	-	
Olmadı	30	21.5	67	48.2	78	56.1	86	61.9	
Toplam	139	100	139	100	139	100	139	100	

*En çok ve çok düzeylerin toplamı ve bu toplamın satır toplamı içindeki oranı

Taşınmada alışık olmadıkları durumlarla karşılaşan hayvanlar, naklin ilk saatlerinde yoğun stres altında kalırken zamanla taşınmaya belirli bir düzeyde uyum sağlamaktadır. Bu uyumun düzeyi türe ve taşıma koşullarına göre değişim gösterebilir. Diğer yandan taşıma süresi uzadıkça stresin boyutu artmakta ve refaktan uzaklaşmaktadır (Yıbar & Çetin, 2013). Bu nedenlerle nakil süresi olabildiğince kısa tutulmalıdır. Bu çalışmada nakilde geçen süreler, 0-4, 5-8 ve 8 saatten fazla şeklinde üç grupta sorgulanmıştır. Katılımcıların beyanına göre nakillerin %59'unun 0-4 saat sürdüğü, %31'inin 5-8 saat arasında gerçekleştiği, 8 saatten fazla süren nakillerin oranının ise sadece %10 olduğu saptanmıştır (Çizelge 11). Bu durumda nakillerin büyük çoğunluğunun 5 saatin altında gerçekleştiği anlaşılmaktadır. Ünal ve ark. (2008) Ankara Ticaret Borsası Kesimhanesinde yürüttükleri çalışmalarında 8 saati aşan nakillerin yüzdesinin kasaplık sığırlarda %1.69 olduğunu, kuzularda ise 8 saati aşan nakil bulunmadığını bildirmişlerdir.

Canlı hayvan nakilleri sırasında oluşan ağırlık kaybı, naklin başlarında fazlayken zamanla azalmaktadır. Bu azalmanın nedeninin canlılarda zaman içinde oluşan adaptasyonun bir sonucu olduğu düşünülmektedir (Erzurum & Yılmaz, 2016). Nakil süresi veya mesafesine bağlı olarak hayvanlar aç ve susuz kalmakta, dehidrasyon, enerji tüketimi ve protein katabolizması sonucu et kalitesi önemli düzeyde olumsuz etkilenmektedir (Güney & Toplu, 2017).

Çizelge 11. Aydın'da hayvan nakillerinde ortalama taşıma süresi (saat)

Table 11. Average transportation time for animals in Aydın (hours)

Düzye	0- 4 saat	arası	5- 8 saat	arası	8 saatten	fazla	Satır
	Kişi	%	Kişi	%	Kişi	%	Toplam
En çok	86	61.9	11	7.9	8	5.8	
Çok	28	20.1	48	34.5	12	8.6	
Ara Toplam*	114	0.59	59	0.31	20	0.10	193
Az	4	2.9	63	45.4	63	45.4	
Olmadı	21	15.1	17	12.2	56	40.2	
Toplam	139	100	139	100	139	100	

*En çok ve çok düzeylerin toplamı ve bu toplamın satır toplamı içindeki oranı

Türkiye'de hayvan hareketleri özellikle Kurban Bayramı sezonunda artış göstermektedir. Bu nakiller genellikle karayolları ile gerçekleşmektedir. Türkiye'de hayvan taşımacılığın en yoğun yaşandığı rotalar, Erzurum'dan başlayan ve İzmir, İstanbul, Ankara, Konya ve Samsun illerine giden sırasıyla 1457, 1225, 877, 955 ve 571 km'lik karayollarıdır. Örneğin Erzurum-İstanbul rotası 20-30 saat sürmektedir. Bunun gibi uzun yol şartları nedeniyle hayvanlarda yorulma, ezilme ve hatta boğulma durumlarıyla karşılaşılabilir (Antalyalı, 2007). Çünkü uzun süreli nakillerde yorgunluk, enerji yetersizliği ve hastalıklara karşı duyarlılık refahın kötüleşmesine neden olmaktadır. Bu nedenle karayoluyla nakillerde taşıma süresinin 8 saati aşmaması, bu süreyi aşması durumunda ise ilave tedbirlerin alınması zorunludur (Altınçekiç & Koyuncu, 2010; Kara & Koyuncu, 2011). Uzun nakillerde altlık kullanılmalı, nakil araçlarında havalandırma bulunmalı, hayvanlara yiyecek-su sağlanmalı, hayvanların türü, yaşı, fizyolojik durumuna göre seyahat süre ve şekilleri için yönetmelik (Anonim, 2011b, Anonim, 2015b) kuralları takip edilmeli, uzun sürecek nakillerde kontrol ve dinlenme alanlarında hayvanlar dinlendirilmelidir.

Hayvan nakillerinde gerek sevk raporu düzenlenirken gerekse yol kontrollerinde resmi makamlarca çeşitli denetimler yapılmaktadır. Nitekim yapılan denetimlerin çoğunun taşıma öncesinde gerçekleştirildiği (%77.7), taşıma sırasında, çıkış noktalarında, sınır kontrol noktalarında, varış yerinde ve taşıma sonrasında da bazı denetimler yapıldığı görülmektedir (Çizelge 12).

Çizelge 12. Hayvan taşımacılığında yapılan denetimler (çoklu seçenek mümkün)

Table 12. Inspections carried out in animal transport (multiple options possible)

Yapılan denetimlerin zaman veya yeri	Adet	%*
Taşıma öncesinde	108	77.7
Taşıma sırasında	84	60.4
Çıkış noktasında	74	53.2
Sınır kontrol noktalarında	32	23.0
Varış yerinde	25	18.0
Taşıma sonrasında	16	11.5

*139 katılımcıdan olguyu onaylayanların oranı

Hayvanların nakil araçlarına bindirilmesi ve indirilmesi işlemlerinin büyük oranda (%60) denetlenmediği, yapılan denetimlerin hayvan satış yerleri veya az da olsa hayvancılık işletmelerinde olduğu saptanmıştır (Çizelge 13).

Ankete katılan veteriner hekimler nakil esnasında bakıcı ve sürücülere verilen yeterlilik belgelerinin, nakil araçlarının uygunluğunun ve onay belgelerinin, taşınacak hayvanların sağlık durumlarının, hayvanlara ayrılan alanların asgari düzeylerinin sağlanıp sağlanmadığının büyük oranlarda kontrol edildiğini ifade etmişlerdir (Çizelge 14). Çizelge 14'teki oranların %100 olmaması, denetimlerin sadece sevk raporu düzenlenirken değil, denetimlerin bir kısmının nakil esnasında kolluk kuvvetleri eşliğinde yol kontrol noktalarında yapılan denetimler olmasından kaynaklanmaktadır. Çünkü yol denetimlerinde hareket yeri ile varış noktası arasında 65 km'den az mesafe bulunan nakillerde mevzuatlar gereği soru sıklarında yer alan bazı belgelerin bulundurulması zorunlu değildir. Çoklu seçim yapılabilen bu soruda gerçekleşen kontrol sonuçlarına ilişkin genel eğilim ortaya konmuştur.

Çizelge 13. Hayvanların nakil araçlarına bindirme ve indirme işlemlerinin denetlenmesi (çoklu seçenek mümkün)

Table 13. Inspection of loading and unloading of animals from/to transport vehicles (multiple options possible)

Denetim yeri	Adet	%
Hayvan pazarında	54	38.8
Geçici kurban satış yerinde	31	22.3
Hayvancılık işletmesinde	25	18.0
Denetlenmiyor	83	59.7

Çizelge 14. Nakil esnasında kontrol edilen noktalar (çoklu seçenek mümkün)

Table 14. Checked points during transport (multiple options possible)

Yapılan kontrollerde ele alınan noktalar	Adet	%*
Hayvanlara refakat eden bakıcıların yeterlilik belgesi	75	54.0
Nakil araçlarının sürücülerine verilen yeterlilik belgeleri	118	84.9
Hayvan naklinde kullanılan araçların onay belgeleri	117	84.2
Nakil araçları, konteyner ve bunların donanımlarının hayvan taşımaya uygunluğu	106	76.3
Taşınamak hayvanların sağlık şartlarının yolculuk yapmaya uygun olup olmadığı	118	84.9
Nakil sırasında hayvanlara ayrılan alanların asgari düzeylerinin sağlanıp sağlanmadığı	101	72.7

*139 katılımcıdan olguyu onaylayanların oranı

'Hayvanların nakiller sırasında hayvan refahına uygun olarak korunduğunu düşünüyor musunuz?' sorusuna katılımcıların %69.1'i kısmen, %25.2'si ise hayır cevabını vermiştir (Çizelge 15). Katılımcıların sadece %5.8'i nakillerde hayvan refahı kurallarına uygun davranıldığını ifade etmişlerdir. Bu durumda nakilde görev alan tüm paydaşların (bakıcı, şoför vd.) nakilde hayvan refahı konusunda bilinçlendirilmeleri gerekliliği ortaya çıkmaktadır. Çünkü taşımada refah kayıplarının bir bölümünün bakıcıların nakil sürecinde hayvanlara uyguladıkları kötü davranışların bir sonucu olduğu unutulmamalıdır. Bu nedenle bakıcıların uygun kabiliyet, bilgi ve mesleki yeterliliğe sahip olması gerekir.

Ankete katılanların %40.3'ü nakil esnasında hayvanlara tür ve yaşlarına uygun biçimde su, yem ve dinlenme imkânının sağlanmadığını, %54'ü ise kısmen bu imkanların sağlandığını bildirmişlerdir. Katılımcıların sadece %5.8'i nakillerde hayvanlara uygun su, yem ve dinlenme imkânı verildiğini ifade etmiştir (Çizelge 15).

Hayvanların gereksiz acı çekmelerine neden olabilecek şartlarda taşındığı veya muamele edildiği durumlarla denetimlerde karşılaşmış katılımcıların karşılaştıkları sorulduğunda veteriner hekimlerin %30.2'si karşılaştıklarını, %69.8'i ise bu gibi durumlarla karşılaşmadıklarını ifade etmişlerdir (Çizelge 15).

Çizelge 15. Hayvanların taşınma şartları

Table 15. Conditions for transport of animals

Anket sorusu	Seçenekler	Adet	%
Sizce hayvanların nakilleri sırasında hayvan refahına uygun olarak korunduğunu düşünüyor musunuz?	Evet	8	5.8
	Hayır	35	25.2
	Kısmen	96	69.1
Nakliye sırasında, hayvanlara, uygun aralıklarla tür ve yaşlarına uygun biçimde su, yem ve dinlenme imkânı sağlandığını düşünüyor musunuz?	Evet	8	5.8
	Hayır	56	40.3
	Kısmen	75	54.0
Hayvanların gereksiz yere acı çekmelerine neden olacak şartlarda taşındığı durumlarla karşılaştınız mı?	Evet	43	30.9
	Hayır	96	69.1
Taşımada hayvanlara gereksiz yere acı verecek biçimde muamele edildiği durumlarla karşılaştınız mı?	Evet	42	30.2
	Hayır	97	69.8

Diğer yandan katılımcıların %12.2'si son bir yıl içinde yapılan denetimlerde yetki belgesi askıya alınmış ya da iptal edilmiş olmasına rağmen hala bu işi yapan nakliyeciler olduğunu, ayrıca denetimlerde yetki belgesi olmayan nakliyecilerin de bulunduğunu (%25.9) ifade etmişlerdir (Çizelge 16).

Katılımcıların %29.5'i denetimlerde onay belgesi olmayan araçlar saptandığını, asgari şartları sağlamayan nakil araçlarının bulunabildiğini (%25.9), onay belgesi askıya alınan yada iptal edilen nakil araçlarının saptandığını (%11.5), yeterlilik belgesi olmayan (%31.7) veya bu belgesi geri alınan sürücüler bulunduğunu (%2.2), yeterlilik belgesi geri alınan bakıcılar saptamadıklarını bildirmişlerdir (Çizelge 16). Genel olarak katılımcıların %17.3'ü nakliyenin öngörülen şartlarda yapılmadığını, fakat %82.7'sinin ise gerekli uygunluğun bulunduğunu ifade etmişlerdir. Bu olgular bir arada değerlendirildiğinde büyük oranda kurallara uyulduğunu fakat hala dikkat edilmesi gereken bazı eksikliklerin bulunduğunu, denetimlerin daha sıkı ve düzenli yapılmasının bu eksikliklerin giderilmesini sağlayabileceğini göstermektedir. Nakilde kullanılan araç ve donanımların her zaman yeterli olmadığı, bu nedenle nakillerin daha profesyonel firma ve kişiler tarafından yapılmasının daha uygun olacağı fikrini gündeme getirmiştir (Erzurum & Yılmaz, 2016). Yönetmeliklerin uygulanmasında yaşanan güçlükler, denetimlerin yetersizliği, alt yapı yetersizlikleri hayvan refahı konusunda sahada istenen düzeyde başarıya ulaşılmasını engelleyecek noktalardır (Sert & Uzmay, 2017).

Çizelge 16. Denetlenen kişi ve araçların yetkinlik durumu

Table 16. Competence status of inspected persons and vehicles

Anket sorusu	Seçenek	Adet	%
Son bir yıl içinde yetki belgesi askıya alınan ya da iptal edilen nakliyecisi oldu mu?	Evet	17	12.2
	Hayır	122	87.8
Son bir yıl içinde hayvan taşımada yetki belgesi olmayan bir nakliyecisi saptandı mı?	Evet	36	25.9
	Hayır	103	74.1
Son bir yıl içinde hayvan taşımada kullanılan araçlardan onay belgeleri olmayan bir araç saptandı mı?	Evet	41	29.5
	Hayır	98	70.5
Son bir yıl içinde teknik ve sağlığa uygunluk bakımından asgari şartları sağlamayan nakil araçları saptandı mı?	Evet	36	25.9
	Hayır	103	74.1
Son bir yıl içinde onay belgesi askıya alınan ya da iptal edilen nakil aracı oldu mu?	Evet	16	11.5
	Hayır	123	88.5
Son bir yıl içinde nakil sırasında yeterlilik belgesi olmayan sürücü saptandı mı?	Evet	44	31.7
	Hayır	95	68.3
Son bir yıl içinde taşıma yönetmeliğine göre yeterli bilgi ya da bilince sahip olmaması nedeniyle yeterlilik belgesi geri alınan sürücü oldu mu?	Evet	3	2.2
	Hayır	136	97.8
Son bir yıl içinde taşıma yönetmeliğine göre yeterli bilgi ya da bilince sahip olmaması nedeniyle yeterlilik belgesi geri alınan bakıcı oldu mu?	Evet	-	-
	Hayır	139	100
Son bir yıl içinde nakliyenin öngörülen şartlarda yapılamadığı bir durum gerçekleşti mi?	Evet	24	17.3
	Hayır	115	82.7

SONUÇ ve ÖNERİLER

Elde edilen sonuçlar, nakliyenin hayvanların hayatları boyunca yaşadıkları en stresli dönem olduğunu, bu nedenle refah konusunda çiftlikte gösterilen özenin hayvanların nakli sırasında da gösterilmesi gerektiğini ortaya koymuştur. Taşınacak hayvanların tür, ırk, cinsiyet, yaş, kullanım amacı (kasaplık, damızlık) doğrultusunda refah ihtiyaçları belirlenmeli, ilgili mevzuatlar kapsamında alınacak önlemlerle nakliye sırasında olabilecek olumsuzluklar minimize edilmelidir.

Aydın ilinden diğer illere ve il içinden yapılan nakillere ait sağlıklı rakamlara ulaşılamasa da ülke çapındaki rakamlar konunun düzeyi ve önemini ortaya koymuştur.

Tarım ve Orman Bakanlığı Aydın İl/İlçe Müdürlüklerinde görevli veteriner hekimler üzerinden gerçekleştirilen anket çalışması sonuçları Nakil Yönetmeliği'nin yayınlanarak yürürlüğe girmesi sonrasında yetiştiricilerin hayvan refahı konusunda algılarının arttığını, konuya daha duyarlı olduklarını, ancak olumsuz durumlarla hala karşılaştığını göstermektedir. Konu hakkında yasal düzenlemelerin yapılmasının hayvan refahına katkı sağladığı, fakat hayvan refahını tamamen güvence altına almadığı, nakilde kullanılan araç ve donanımların her zaman yeterli olmadığından nakillerin daha profesyonel firma ve kişilerce yapılması üzerinde de durulabilir.

Kontrol ve denetimlerin daha çok nakliye öncesi ve taşıma sırasında yapıldığı, kontroller sonucu olumsuz nakil koşullarında azalmalar olmasına rağmen son bir yıl içerisinde yetki belgesi olmayan nakliyecisi ve sürücüsü nedeniyle hayvanlarda stres oluşturan durumlarla karşılaştığı bildirilmiştir. Elde edilen sonuçlar, hayvan nakillerinin denetlenmesinin önemini net olarak ortaya koymuştur. Diğer yandan AB'nde yayınlanan yeni regülasyonlar nakillerle ilgili kuralları daha da detaylandırmış, nakil araç standartlarını yükseltmiş, uzun süreli nakillerle ilgili kurallar sertleştirilerek araçların uzaktan izlenmesi konuları ön plana alınmıştır.

Bu çalışmada hayvan nakillerin tamamına yakınının karayolu ile yapıldığı ve nakil süresinin genellikle 5 saatten kısa sürdüğü, sekiz saati aşan nakillerin sadece %6 civarında olduğu saptanmıştır. Bu durum, nakil süresinin 8 saati geçmemesi beklentisi ile uyumludur. Eğer nakil sekiz saati geçecekse mola verilerek hayvanlar dinlendirilmeli, yem ve su sağlama gibi ek tedbirler alınmalı, ayrıca araçlarda GPS ve havalandırma sistemi gibi ek donanımlar bulunmalıdır. Nakil yönetmeliğinde 31.12.2015 tarihinde uygulamaya geçileceği belirtilen GPS sistemi kullanım zorunluluğu yayınlanan ek yönetmelikle 31.12.2026 tarihine ertelenmiştir. Gerek uzun mesafe hayvan taşımada uygun araç sayısının yetersizliği gerekse hayvan dinlendirme istasyonlarının durumu 8 saati aşan yolculukların sıkıntılı olduğunu ortaya koymaktadır. Bu nedenlerle hayvanlar üzerinde strese neden olmaması için nakil süreci iyi planlanmalıdır. Kurban Bayramı gibi hayvan hareketlerinin yüksek düzeyde ve uzun mesafeli olduğu dönemlerde bu planlama daha da önemlidir.

Hem hayvan refahı hem de yüksek verim için optimum çevre koşullarının sağlanmasını hedefleyen mevzuatların hazırlanmasının önemi kadar, ülkede gerekli teknik alt yapının oluşturulması, eğitimlerle mevzuatın ilgililere öğretilmesi, tüketici ve üreticilerin hayvan refahı konusundaki duyarlılığı, mevzuatın etkin takibi ve denetimi oldukça önemli konulardır. Sonuç olarak, Türkiye'de hayvan nakilleri için gerekli mevzuat oluşturulmuş, yasal

düzenlemelerin takibinin yapılmaya başlanmıştır. Sistemin tüm unsurlarıyla çalışmaya başlaması ve alt yapı eksikliklerinin giderilmesi sonucu uygulamalarda karşılaşılan problemlerin giderek azalacağı açıktır. Diğer yandan uygulamaların ertelenmeden en kısa sürede hayata geçirilmesinin ülke hayvancılığına ve dolayısıyla ekonomisine olumlu katkılar yapacağı düşünülmektedir.

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.

KAYNAKLAR

- Altınçekiç, Ö.Ş., Koyuncu, M. (2010) Nakil koşullarının hayvan refahı üzerine etkileri. *Hayvansal Üretim* 51(1), 48-56.
- Anonim (2011a) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelik. Resmî Gazete, 17 Aralık 2011, Sayı 28145. <https://www.resmigazete.gov.tr/eskiler/2011/12/20111217-13.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2011b) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliği. Resmî Gazete, 24 Aralık 2011, Sayı 28152. <https://www.resmigazete.gov.tr/eskiler/2011/12/20111224-2.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2013) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelikte değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 14 Mayıs 2013, Sayı 28647. <https://www.resmigazete.gov.tr/eskiler/2013/05/20130514-16.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2014) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 5 Şubat 2014, Sayı 28904. <https://www.resmigazete.gov.tr/eskiler/2014/02/20140205-3.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2015a) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 29 Aralık 2015, Sayı 29577. <https://www.resmigazete.gov.tr/eskiler/2015/12/20151229-4.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2015b) Hayvan Nakillerinde Kontrol ve Dinlendirme İstasyonu Yönetmeliği. Resmî Gazete, 30 Mayıs 2015, Sayı: 29371. <https://www.resmigazete.gov.tr/eskiler/2015/05/20150530-13.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2015c) Hayvan Nakillerinde Kontrol ve Dinlendirme İstasyonu Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 29 Aralık 2015, Sayı: 29577. <https://www.resmigazete.gov.tr/eskiler/2015/12/20151229-3.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2016a) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 24 Aralık 2016, Sayı 29928. <https://www.resmigazete.gov.tr/eskiler/2016/12/20161224-7.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2016b) Hayvan Nakillerinde Kontrol ve Dinlendirme İstasyonu Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 24 Aralık 2016, Sayı: 29928. <https://www.resmigazete.gov.tr/eskiler/2016/12/20161224-6.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2017a) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 24 Aralık 2017, Sayı 30280. <https://www.resmigazete.gov.tr/eskiler/2017/12/20171224-12.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2017b) Hayvan Nakillerinde Kontrol ve Dinlendirme İstasyonu Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 24 Aralık 2017, Sayı: 30280. <https://www.resmigazete.gov.tr/eskiler/2017/12/20171224-11.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2018) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelikte değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 23 Şubat 2018, Sayı 30341. <https://www.resmigazete.gov.tr/eskiler/2018/02/20180223-5.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2019) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 31 Aralık 2019, Sayı 30995. <https://www.resmigazete.gov.tr/eskiler/2019/12/20191231-12.htm> (Alınma Tarihi: Şubat 2024).

- Anonim (2020a) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelikte değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 18 Şubat 2020, Sayı 31043. <https://www.resmigazete.gov.tr/eskiler/2020/02/20200218-13.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2020b) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelikte değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 28 Kasım 2020, Sayı 31318. <https://www.resmigazete.gov.tr/eskiler/2020/11/20201128-7.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2021a) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 10 Nisan 2021, Sayı 31450. <https://www.resmigazete.gov.tr/eskiler/2021/04/20210410-16.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2021b) Hayvanların Nakilleri Sırasında Refahı ve Korunması Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. Resmî Gazete, 30 Aralık 2021, Sayı 31705. <https://www.resmigazete.gov.tr/eskiler/2021/12/20211230M2-8.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2022) Yurt içinde canlı hayvan ve hayvansal ürünlerin nakilleri hakkında yönetmelikte değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 24 Mayıs 2022, Sayı 31845. <https://www.resmigazete.gov.tr/eskiler/2020/02/20200218-13.htm> (Alınma Tarihi: Şubat 2024).
- Anonim (2023) Hayvanların nakilleri sırasında refahı ve korunması yönetmeliğinde değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 23 Aralık 2023, Sayı: 32408. <https://www.resmigazete.gov.tr/eskiler/2023/12/20231223-10.htm> (Alınma Tarihi: Ocak.2024).
- Anonim (2024a) Hayvanların nakilleri sırasında refahı ve korunması yönetmeliğinde değişiklik yapılmasına dair yönetmelik. Resmî Gazete, 3 Eylül 2024, Sayı: 32651. <https://www.resmigazete.gov.tr/eskiler/2024/09/20240903-6.htm> (Alınma Tarihi: Eylül.2024).
- Anonim (2024b) Tarım ve Orman Bakanlığı. Gıda ve Kontrol Genel Müdürlüğü Sunumu. Mart 2024, <https://www.tarimorman.gov.tr/sgb/belgeler/sagmenuveriler/gkgm.pdf> (Alınma Tarihi: Kasım.2024).
- Antalyalı, A.A. (2007) *Avrupa Birliği ve Türkiye'de hayvan refahı uygulamaları*. TC. Tarım ve Köyşleri Bakanlığı. AB Uzmanlık Tezi. Ankara, 162s.
- Birli, İ., Emek, T., & Akbaş, Y. (2025). Aydın İlindeki Veteriner Hekimlerin Hayvan Refahı Algısı. *KSU Tarım ve Doğa Derg 28 (1)*, 274-284. DOI: 10.18016/ksutarimdog.vi.1538769.
- Demirel, A.F., Çak, B. (2016) Türkiye ve Avrupa Birliği'nde ilgili mevzuatlar açısından hayvan refahı uygulamalarının gıda güvenliğindeki önemi. *Van Veterinary Journal 27(2)*, 111-116.
- Erzurum, O., Yılmaz, A. (2016) Sığır nakillerinde refah ve davranış ilişkisi. *Kocatepe Veterinary Journal 9(4)*, 357-362. <https://doi.org/10.5578/kvj.34238>.
- Gökcam, Ö., Yelken, N., Çal, H., Akunal, T., Koşkan, Ö. (2012) Üniversite öğrencilerinin hayvan refahına bakış açısı: Isparta örneği. TMMOB Ziraat Mühendisleri Odası VIII. Öğrenci Kurultayı (1-2 Aralık 2012) Bildirileri, TMMOB Ziraat Mühendisleri Odası Yayınları, Ankara, 48-57.
- Güney, N., Toplu, H.D. (2017) Ruminantlarda nakil işlemlerinin refah ve et kalitesi üzerine etkileri. *Animal Health, Production and Hygiene 6(1)*, 487-492.
- Güngör, Ö.F., Ünal, N. (2020) Çiftlik hayvanlarında karayoluyla nakil koşullarının mortalite ve karkas kalitesine etkisi. *Türk Tarım – Gıda Bilim ve Teknoloji Dergisi 8(9)*, 1902-1909. DOI: <https://doi.org/10.24925/turjaf.v8i9.1902-1909.3518>.
- İkiz, F., Püskülcü, H., Eren, Ş. (2000). *İstatistiğe giriş*. Barış Yayınları. Fakülteler Kitabevi. İzmir, Türkiye.
- İzci, C., Çuhadar Erdal, F., Yıldız, M. (2021) Süt sığırlarında topallık: hayvan refahı ve davranışına etkisi üzerine bir değerlendirme. *Ege Üniversitesi Ziraat Fakültesi Dergisi 58(4)*, 629-639, <https://doi.org/10.20289/zfdergi.870888>.
- İzmirli, S. (2009) *Türkiye'de veteriner hekimler, veteriner hekimliği öğrencileri, hayvan sahipleri ve toplumun hayvan gönenci (refahı) tutumları üzerine anket çalışması*. Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, Konya, 131s.
- Kara, N.K., Koyuncu, M. (2011) Sığırlarda taşıma sırasında hayvan refahına etki eden faktörler. *Kafkas Univ Veteriner Fakültesi Dergisi 17(3)*, 511-516. <https://doi.org/10.9775/kvfd.2010.3703>.
- Kılıç, İ., Bozkurt, Z., Tekerli, M., Koçak, S., Çelikelioğlu, K. (2013) Afyonkarahisar ili koyunculuk işletmeleri çalışanlarının hayvan refahını etkileyen faktörlerle ilgili algıları. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 53(1)* 29-38.
- Özgür, A. (2010). Hayvanlarla yaşamı paylaşmak. *Veteriner Hekimler Derneği Dergisi 81(2)*, 9-13.
- Sağmanlıgil, V., Cengiz, F., Salırlı, Y., Atasoy, F., Ünal, N., Petek, M. (2015). *Hayvan davranışları ve refahı*. Anadolu Üniversitesi Açıköğretim Fakültesi, T.C. Anadolu Üniversitesi yayini no: 2332, Açıköğretim Fakültesi yayini no: 1329, Eskişehir, 225s.
- Sert, H., Uzmay, A. (2017) Dünya'da hayvan refahı uygulamalarının ekonomik ve sürdürülebilirlik açısından değerlendirilmesi. *Adnan Menderes Üniversitesi Sosyal Bilimler Enstitüsü Dergisi 4(4)*, 26,3-276.

- Turan, Ö. (2018) *Büyükbaş ve kanatlı hayvan yetiştiriciliğinde olası hayvan refahı uygulamalarına yönelik tüketici tercihlerinin belirlenmesi üzerine bir araştırma*. Bursa Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Tarım Ekonomisi Anabilim Dalı, Doktora Tezi, Bursa, 154s.
- Ünal, N., Teke, B., Özbeyaz, C. (2008) Ankara Ticaret Borsası Kesimhanesi'ne yapılan kasaplık hayvan nakillerinde bazı koşulların hayvan refahı bakımından incelenmesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 55, 51-56.
- Yıbar, A., Çetin, E. (2013) Hayvan refahının et kalitesi üzerine etkileri. *Uludag Üniversitesi Veteriner Fakültesi Dergisi* 32(2), 31-37.
- Yıldız, U., Saatci, M. (2009) An evaluation of the welfare in the large and small animal transportations made from Sarikamis. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 15(3), 363-368. <https://doi.org/10.9775/kvfd.2008.124-A>.