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From the Editorial Board

Why is Determining the Response of Plants to Multiple Stress Conditions Important for the Development of Breeding Materials?

Since about 30 years ago, when there were debates about whether or not global warming had started, we have now entered a period where we are facing the facts. In a discussion I had as a PhD student with Prof. John Herbert Beynon (Swansea-UK), one of the world's most renowned scientists, I argued that global warming could have devastating effects on agriculture and especially on plant breeding, apart from the popular discussion of melting glaciers and rising sea levels. I had stated that global warming should not have been associated only with an increase in temperature. I clearly remember saying that we might face an increase in other abiotic stressors such as salinity and drought, and other abiotic stressors. When I said that we might even encounter more aggressive races of pathogens as they adapt to these adverse conditions, that concept was a part of my PhD work at that time. He had wisely shown an interest with a mature attitude, and he had said that this could only be fiction, and that the scientific world was approaching it with an exaggeration. From that time, considerably a very short time has passed, and our memory is still fresh to remember those days. Global warming not only results in increases in air, soil, and water pollution but also contributes to ozone depletion through increased CO₂ emissions, allowing UV rays, especially UVB and UVC, to easily reach the earth's surface and cause serious DNA damage to organisms. While high temperatures and drought stress cause crop loss in plants during the period of their presence, heavy metals and UV rays cause fruit and flower deformations both during the period of their presence and in the following years by transferring damaged DNA molecules to the next generation and seriously threaten our future. Under the above conditions, the interaction and combination of almost any abiotic stress factors with each other is possible, as is the interaction and combination of abiotic and biotic stress factors with each other. In recent years, studies on pathogens exposed to abiotic stress have shown that pathogens have become even more aggressive in abiotic stress conditions and even mild pathogens act as serious pathogens under abiotic stress conditions.

In general, when a plant is exposed to biotic stress, it activates its defense mechanism, and when it is exposed to abiotic stress, it either tries to tolerate the stressors by storing them in its vacuoles or other cell cavities, or it tries to escape from the abiotic stressors. To do this, it either closes its stomata or, depending on the situation, increases

its respiratory capacity and tries to escape stress by rapidly producing energy. Even if plants are genetically resistant to such conditions, structural and biochemical disturbances in defense or tolerance mechanisms can occur when stress becomes chronic.

In general, plants exposed to dual or multiple stresses may show the additive effect of both stress factors, as well as triggering the defense mechanism of the pathogen, making it more aggressive; studies in this area have accumulated ample amount of data in recent years. In many cases, the plant defense system collapses rapidly due to the destructive effect of combined stress in the short term. In the long run, on the other hand, yield and quality losses are inevitable.

Dual stress can occur between abiotic + abiotic factors as well as abiotic + biotic factors. Stress factors possessing different characteristics can occur simultaneously or sequentially. In this case, the plant's defense mechanism deteriorates unpredictably. Because abiotic and biotic stress factors activate different signaling molecules and enzymatic activities in the plant. Signaling molecules can negatively interact with each other and disrupt the plant's defense system at an early stage. In the mechanism of plant breeding, only plants that can produce the mechanism to resist both stressors will survive. In other words, no matter how resistant a plant can become to a pathogen, the destruction of the defense mechanism will be inevitable if it is exposed to an abiotic agent. Under these conditions, plant breeding will be very complex and take a long time. Acclimation or adaptation of pathogens to constantly changing abiotic stress conditions might also prolong and make plant breeding difficult since the ability of bred plants may be lost during that time.

Mechanisms of induced systemic resistance (ISR) and systemic acquired resistance (SAR) activating the plant immune system become controversial under multiple stress conditions due to conflicting views; therefore, we need comprehensive and detailed studies to clarify this situation. Under these circumstances, we cannot complete highly complex studies with classical experimental methods as in the past and the results of different experimental groups cannot be explained by classical statistical methods. The relationship between each metabolite or molecule in complex stress

conditions should be evaluated in a network, and the significant molecular and biochemical pathways which stand out under different stress conditions and combinations should be analyzed. We could then perform breeding and other immunity studies after revealing the pathways. Otherwise, our work, efforts, financial budgets, energy, and most of all our hopes would be wasted.

These studies can even be modelled with Artificial Neural Network (ANN) studies, and even complex structures with no linear relationship between them can be analyzed with ANN. With artificial intelligence methods, even difficult experimental materials or inadequate sampling can be measured with these new techniques. Metabolites and all intracellular and extracellular parameters can be analyzed by the Correlation Network Analysis (CNA) method and the detailed pathway studies can be revealed.

In many parts of the world, especially in the European Union and the United States of America, the process of transition from descriptive biology to modelling biology has already started and it is known that this process will be completed in 2035, and modelling biology studies will be accelerated. For this purpose, the biological pathway mechanisms of each plant are being developed, and the pathway diagnosis of about 10 plants or tree species has already been made. For animal pathways, studies have been conducted on a much larger number of animal species, and almost all human metabolic pathways have been identified. The only remaining areas of study are how these mechanisms change under stress or how they can be maintained and improved. Fewer pathway mechanisms have been studied in plants compared to other species. Pathway studies in other plants are ongoing by researchers at many of the world's major universities and research institutes. The pathways to be uncovered in plants are attempted to be explained with the pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, but as these pathways are identified in more plants, the specific pathways of each plant will be revealed and the pathway mechanism under stress will be better understood. This will add a new dimension to breeding studies and provide a more solid foundation for plant resistance, tolerance and genetic resistance mechanisms, and it will be possible shortly to breed plants with the desired quality and traits using modelling biology. There may also be a ray of hope for the problem of famine caused by rapid population growth, global warming, and environmental and biological stressors. For example, the

FAO estimates that about half of the world's irrigated land is under stress, and the area of these lands continues to increase due to unintentional irrigation, desertification, and high temperatures. Therefore, it is imperative to act quickly in this field and to put the studies into practice as soon as possible.

To maintain healthy cultivation of all crops, all stress conditions to which they are or may be exposed should be studied within a matrix and a laboratory with a state-of-the-art approach should be established on a national basis for this purpose. This laboratory should have the equipment and knowledge to study the stress factors to which many plant species such as cool climate crops (wheat, barley, oats, etc.), industrial crops (cotton, corn, sesame, potatoes, etc.), vegetable crops (tomatoes, peppers, eggplants, etc.), edible legumes (lentils, chickpeas, etc.), ornamental plants, fruit trees, etc. are exposed. Our country has trained researchers in this field and these studies should take their rightful place as soon as possible within the framework of international cooperation with young and dynamic staff.

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Effect of hot air drying process on color and antioxidant attributes of the flavedo of bitter orange (*Citrus aurantium* L.)

Sıcak hava kurutma prosesinin turunç (Citrus aurantium L.) flavedosunun renk ve antioksidan özellikleri üzerine etkisi

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ABSTRACT

This research investigated the effects of different drying temperatures (40, 50, and 60°C) on changes in color, total phenolic, total flavonoid, ascorbic acid, and antioxidant activity of bitter orange flavedo. In comparison to the fresh sample, the L* and a* values increased, while b* and h° values decreased at all air drying temperatures. The lowest total color change (TCC) values were achieved at drying temperatures of 40 and 60°C. Drying temperatures induced an increment in total phenolic and antioxidant activity, the highest total phenolic content (16.35±0.54 mg GAE g⁻¹) and DPPH radical scavenging activity (IC₅₀: 14.61±0.53 mg mg⁻¹) were determined in flavedo dried at 40°C, and the highest FRAP value (52.19±1.49 mmol TE g⁻¹) was achieved in flavedo dried at 50°C. However, the applied drying temperatures resulted in a decline in the flavedo's total flavonoid content (52.79–64.75%) and ascorbic acid content (36.63–45.54%). Based on the results obtained from this work, bitter orange flavedos can be dried at lower temperatures (40-50°C) to minimize color changes and enhance phenolic and antioxidative activity.

Key Words: *Citrus aurantium* L., drying temperature, total phenolic, IC₅₀, FRAP

ÖZ

Bu araştırmada, farklı kurutma sıcaklıklarının (40, 50 ve 60 °C) acı portakal flavedosunun renk, toplam fenolik madde, toplam flavonoid, askorbik asit ve antioksidan aktivitesindeki değişimlere olan etkileri incelenmiştir. Taze numuneyle karşılaştırıldığında, tüm hava kurutma sıcaklıklarında L* ve a* değerleri artarken, b* ve h° değerleri azalmıştır. En düşük toplam renk değişikliği değerleri 40 ve 60 °C kurutma sıcaklıklarında elde edilmiştir. Kurutma sıcaklıkları toplam fenolik madde ve antioksidan aktivitede artışa neden olmuş, en yüksek toplam fenolik madde içeriği (16.35±0.54 mg GAE g⁻¹) ve DPPH radikal süpürücü aktivitesi (IC₅₀: 14.61±0.53 mg mg⁻¹) 40 °C'de kurutulan flavedoda belirlenmiş ve en yüksek FRAP değeri (52.19±1.49 mmol TE g⁻¹) 50 °C'de kurutulan flavedoda elde edilmiştir. Ancak uygulanan kurutma sıcaklıkları flavedonun toplam flavonoid içeriğinde (%52.79-64.75) ve askorbik asit içeriğinde (%36.63-45.54) düşüşe neden olmuştur. Bu çalışmadan elde edilen sonuçlara göre, acı portakal flavedoları, renk değişimlerini en aza indirmek ve fenolik ve antioksidan aktiviteyi artırmak için daha düşük sıcaklıklarda (40-50°C) kurutulabilir.

Anahtar Kelimeler: *Citrus aurantium* L., kurutma sıcaklığı, toplam fenolik, IC₅₀, FRAP

Introduction

Citrus fruits, belonging to the Rutaceae family, are the most widely cultivated horticultural products worldwide, with a total production of 161.8 million tons. Türkiye ranks 8th in the world, with a cultivation area of 180 thousand hectares and a total quantity of 7.87 million tons (FAO, 2022; TÜİK, 2023). The coastlines of the Southern Aegean and Mediterranean regions of Turkey have favorable ecological conditions (climate, soil characteristics, etc.) for citrus cultivation. The main citrus species planted in Turkey are orange, mandarin, lemon, and grapefruit. Fewer amounts of other citrus fruits such as kumquat, bitter orange, lime, and bergamot are also cultivated.

Citrus fruits are mainly processed into juice in the food industry. They also make jams, jellies, soft drinks, essential oils, flavoring agents and bioactive extracts. The citrus processing industry generates considerable amounts of by-products such as peel, pulp, and seeds. These by-products are an important source of vitamins, minerals, essential oils, dietary fibers, and bioactive compounds, especially phenolic acids and flavonoids, which may possess anti-inflammatory, antioxidant, anti-infective, neuroprotective effects, and anticancer properties (Gómez-Mejía et al., 2019; Sharma et al. 2022). Therefore, citrus processing wastes have the potential to be employed as a source of ingredients, natural additives, and raw materials in cosmetics, dietary supplements, and food products. Furthermore, the conversion of citrus wastes into value-added products can provide an effective and environmentally friendly platform (Rafiq et al., 2018).

Bitter orange (*Citrus aurantium* L.) is a species of citrus fruit that originated in Southeast Asia. It is known by several names worldwide, including sour orange and Seville orange (Pawar et al., 2020). Turkey has witnessed a 65% increase in bitter orange production over the past decade, with a total yield of 3,581 tons in 2023. Approximately 77% of this production is accounted for by the Mediterranean region.

(TÜİK, 2023).

Bitter orange is typically employed as a rootstock for citrus cultivation. It exhibits morphological similarities to the orange but possesses a sour and bitter taste. Therefore; it is not suitable for consumption in its fresh form. In some regions, the juice of the bitter orange is utilized as a substitute for lemon juice in salads, imparting a sour taste. The flowers, leaves, and peels of the fruit have been used for a long time as a sedative, stomach stimulant, weight loss aid, eyelid inflammation, central nervous system disorders, muscle pain, and skin bruising. During the jam processing, the flavedo parts of fruits are removed to the debittering of peels. Several researchers have revealed that the flavedo parts of citrus fruits contain phytochemicals with health-related benefits and display mighty antioxidant activity. (Escobedo-Avellaneda et al., 2014; Muzykiewicz et al., 2019; Ramful et al., 2010; Badalamenti et al., 2022). Owing to its inherent moisture content, citrus flavedo is unsuitable for storage over extended periods. The drying process represents an efficacious preservation method to extend the shelf stability of flavedo (Farahmandfar et al., 2020). The principal objective of the drying operation is to diminish the moisture content of the material to a level that will inhibit microbial growth during storage. Furthermore, the drying process inactivates chemical reactions that cause deterioration (Suri et al., 2022).

Hot air drying is one of the most prevalent methods due to its simplicity, cost efficiency, operational suitability, and capability to provide hygienic products. Drying temperature and duration are the main factors affecting final product quality (Önal et al., 2019; Senadeera et al., 2020).

Dried flavedo can be offered for the formulation of various food products, including beverages, dairy, bakery, and confectionery, as well as for the extraction of valuable bioactive substances in cosmetic and pharmaceutical applications. The impacts of drying on the antioxidant attributes of citrus processing wastes

have been documented by numerous researchers, confirming that drying affects antioxidant activity and bioactive components. However, there is limited research (Farahmandfar et al., 2020) on the influence of drying on the antioxidant features of bitter orange flavedo.

This study aimed to effects of drying temperatures on color parameters, total phenolic, total flavonoid, ascorbic acid content, and antioxidant activity of the flavedo part of bitter orange fruit.

Material ve Method

Bitter oranges (Yerli F1 cultivar) were collected from citrus orchards in the Western Mediterranean Agricultural Research Institute, Antalya province, in winter season (January-2023). Then, the fruits were washed with water procured from a tap and wiped through a cloth. A kitchen grater was used to remove the flavedo layer from the fruit (20 pieces).

Hot air drying experiments

The flavedo parts were spread on glass petri dishes and dried in a pilot-scale hot-air oven (Eksis, Isparta, Türkiye) at temperatures of 40, 50 and 60 °C, with a stationary air speed of 1.3 m s⁻¹. Approximately 100 g of samples were used for each drying experiment. Drying experiments were continued until a moisture content of 10% was reached and performed in triplicate. The drying treatments took about 185, 165, and 130 minutes for 40, 50, and 60 °C, respectively. The moisture content was assessed using an oven at 105 °C until the sample weight was constant. The dried samples were kept in plastic bags at -18 °C until analyses.

Analyses

Color measurements

L*, a*, b*, and hue angle parameters of flavedos were determined using a Minolta CR 400 (Osaka, Japan) color measurement device using a D65 light source from three different points. Total color changes were reckoned using the following equation 1 (Dağ et al., 2017).

$$TCC = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Extraction Procedure

The extraction procedure of dried samples was conducted according to the method used by Ramful et al. (2010) with certain modifications. Approximately 5 and 2 g of fresh and dried flavedo samples were added to falcon tubes containing 25 mL of methanol solution (80%; v/v) and vortexed for 1 minute. The mixtures were subjected to extraction on an orbital shaker at 180 rpm and room temperature for 24 hours and subsequently, the sample was subjected to centrifugation at 7000 rpm for 5 minutes. After centrifuging, the upper phases were collected and the volumes were completed to 25 mL with extraction solvent. The extracts were conserved at a temperature of -18 °C until the subsequent phytochemical analysis.

Total phenolic content

The total phenolic content of flavedo extracts was ascertained by the Folin–Ciocalteu colorimetric method suggested by Phuyal et al. (2020). Briefly, 950 µL of distilled water, 5 mL of Folin–Ciocalteu solution (10%; v/v), and 4 mL of Na₂CO₃ solution (7.5%; w/v) were added to 50 µL of extract, respectively. The mixture was vortexed and allowed to incubate for 90 minutes at room temperature in the absence of light. After incubation, the absorbance of the mixture was read to blank at 765 nm utilizing a spectrophotometer (Shimadzu UV-Vis 160A, Japan). Results were assessed as mg GAE (Gallic acid equivalent) g⁻¹ dm (dry matter) from the gallic acid calibration curve.

Total Flavonoid content

The total flavonoid content of the samples was analyzed using the aluminum chloride colorimetric method referred to as the Zhishen method (Zhishen et al., 1999). According to this method, 500 µL of the extract was transferred to a test tube, followed by the addition of 4.5 mL of distilled water and 300 µL of 5% (w/v) NaNO₂ solution. 600 µL of 10% (w/v) AlCl₃ was added

after five minutes. Then, at the 6th minute, 2 mL of 1M NaOH was added to the mixture and the total volume was achieved by the addition of 2.1 mL of distilled water. Following the thorough mixing of the solution, the absorbances of the samples were measured at a wavelength of 510 nm against the blank containing no extract. The total flavonoid values of the samples were presented as milligrams of catechin equivalent (CE) per gram of dry matter.

Ascorbic acid determination by HPLC

The analysis of ascorbic acid was conducted using a high-performance liquid chromatography (HPLC) device, as described by Sdiri et al. (2012), with alterations. For this purpose, flavedo samples were extracted with a 3% (w/v) metaphosphoric acid (HPO₃) solution. After centrifugation, the extracts were filtered through a 0.45 µm membrane filter and given to the HPLC (Shimadzu 2030 C 3 d Prominence-i, Japan) equipped with PDA (Photo-diode Array) detector. The separation was run on an Inertsil ODS3 C-18 column (5µm, 250x4.6 i.d.) (GL Sciences, Japan) at 25°C. The 25 mM potassium dihydrogen phosphate (KH₂PO₄; pH:2.3) was used as mobile phase with the flow rate of 0.6 mL min⁻¹, and the

detection wavelength was at 250 nm. The injection volume was set to 10 µL. Ascorbic acid was quantified as mg g⁻¹ dm according to the standard curve of ascorbic acid in the concentration range 1-200 mg L⁻¹ and the formula defining this curve.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The procedure for the DPPH method was as follows; 5 different volumes (25, 50, 75, 100, and 150 µL) of extracts were added to 600 microliters of 0.1 M DPPH solution in test tubes. Subsequently, the volume was brought to 6 mL with methanol and vortexed. The mixtures were left in the dark at room temperature for 15 minutes. The absorbance of the samples at 515 nm was read against methanol in a spectrophotometer. Methanol was used instead of extract as a control. With the help of the equation 2 below, the % inhibition data for different concentrations were calculated and the IC₅₀ value (the concentration required to reduce 50% of the initial DPPH concentration) was determined as mg mg⁻¹ from the curve prepared with these data (Cemeroğlu, 2010).

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{DPPH}} - \text{Absorbance}_{\text{extract}}) / \text{Absorbance}_{\text{DPPH}}] \times 100 \quad (2)$$

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP solution was generated by combining 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ), and 20 mM ferric chloride (FeCl₃ 6H₂O) at a ratio of 10:1:1 (v/v/v). 150 µL of extract and 2750 µL of FRAP reagent were blended and incubated in ambient conditions away from light for 30 minutes. Then, The absorbance was quantified by measuring the mixture against the blank in a spectrophotometer at 593 nm. The results were calculated from the calibration graph of standard Trolox and expressed as mmol Trolox equivalent (TE) g⁻¹ dm (Thaipong et al., 2006).

Statistical assessment

Drying studies were carried out in 3 repetitions and all values are given as “mean ± standard deviation”. Data were evaluated using one-way analysis of variance (ANOVA) using the SAS statistical program (Version 6.12). A comparison of the means were appraised by Duncan’s Multiple Range Test at P < 0.05 confidential interval.

Results and Discussion

As can be seen from Table 1, drying temperatures significantly affected the L*, a*, b*, and hue angle values of bitter orange flavedo (P ≤0.05). After drying, the L* value, indicating

lightness, and the a^* value, pointing to redness, increased compared to fresh flavedo. The closest L^* value to fresh flavedo was measured at drying temperatures of 50 and 60°C. However, the highest lightness was observed in flavedo dried at 40°C ($P<0.05$). The increment of a^* is related to browning reactions and carotenoid degradation (Ghanem et al., 2012; Özcan-sinir et al., 2018) and the highest increase occurred at 50°C drying temperature. Ghanem Romdhane et al. (2015) found an increase in the A^* value of lemon peels dried at 40 °C, 50 °C and 60 °C. The b^* value, an indicator of yellowness, increased after drying at 40°C and decreased at other drying temperatures. The decomposition of carotenoid pigments in the

samples may be responsible for the decrease in b^* (Ghanem Romdhane et al., 2015; Rafik et al., 2019). The hue angle closer to 90° indicates a more yellowish tone, and closer to 0° expresses a reddish tone (Wang et al., 2023). The h° value of fresh flavedo decreased with drying temperature and the lowest yellowness was recorded at 50 and 60°C drying. Hot air drying caused changes in the color of fresh flavedo and the lowest TCC occurred at 40 and 60°C. It is assumed that color alterations during the drying of plant materials arise due to various factors, including degradation of color pigments, oxidation and browning reactions (Ghanem Romdhane et al., 2015).

Table 1. Color values of fresh and dried flavedos*

Flavedo samples	L^*	a^*	b^*	h°	TCC
Fresh	68,97±0,85b	18,11±0,68d	50,5±3,19a	70,22±1,83a	-
40°C	74,18±1,16a	26,58±0,53b	52,01±1,45a	62,92±0,94b	15.53±0.45b
50°C	69,94±1,12b	28,56±0,31a	46,28±1,54b	58,31±0,87c	16.37±0.37a
60°C	69,85±0,39b	24,28±0,33c	42,14±0,41c	60,04±0,16c	15.70±0.12b

* Different exponential letters in the same line show that the means are significantly different ($P<0.05$)

The total phenolic and flavonoid contents of fresh and dried bitter orange flavedo are illustrated in Figure 1. The total phenolic content of fresh flavedo (11.84 ± 2.05 mg GAE g^{-1}) increased by about 38.1% and 26.69% drying at 40 (16.35 ± 0.54 mg GAE g^{-1}) and 50°C (15.00 ± 1.19 mg GAE g^{-1}), respectively. The increase in total phenolic content can be attributed to the temperature-induced release of some phenolic acids and flavonoids, which are mostly present in bound form in the plant matrix, and the decrease in the activity of polyphenoloxidase, an enzyme known to facilitate the oxidation of polyphenols (Papoutsis et al., 2017). Sultana et al. (2012)

observed an increase in total phenolics in oven-dried apricots and explained this with the release of high molecular weight phenolics due to heat treatment. Güçlü et al. (2022) reported that different drying methods increased the amount of phenolic substances in citrus peels. On the other hand, no significant difference was observed between fresh and dried flavedo at 60°C (11.99 ± 1.00 mg GAE g^{-1}) ($P\geq0.05$). A similar finding has been reported by Abd Rahman et al. (2018) for the pomelo flavedo.

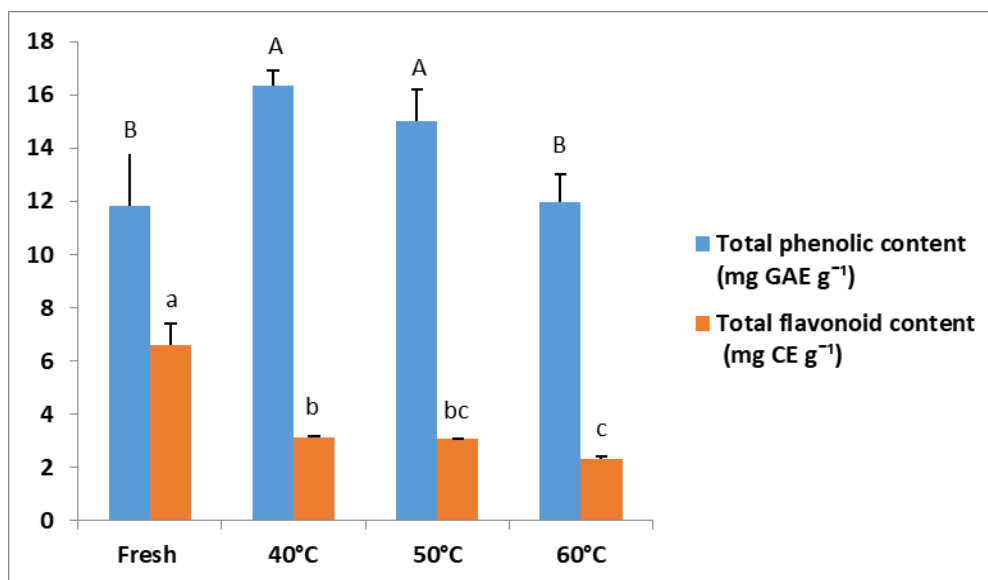


Figure 1. Total phenolic and flavonoid contents of fresh and dried flavedo samples*

*Different capital letters (A-B) on the bars show significant differences between total phenolic values and different lowercase letters (a-c) on the bars show significant differences between total flavonoid values ($P < 0.05$).

The contents of total flavonoids in fresh and dried flavedo varied between 2.33 ± 0.11 – 6.61 ± 0.78 mg CE g⁻¹ (Figure 1). In contrast to the total phenolic content, the total flavonoid content of fresh flavedo decreased by 52.79–64.75% with drying temperatures. This phenomenon is likely attributable to the chemical, enzymatic, or thermal deterioration of certain flavonoids (Rafiq et al., 2019). The highest total flavonoid content was found in fresh flavedo, followed by flavedo dried at 40°C (3.12 ± 0.05 mg CE g⁻¹), 50°C (3.07 ± 0.02 mg CE g⁻¹) and 60°C (2.33 ± 0.11 mg CE g⁻¹), respectively. Similarly, Lai et al (2022) found that total flavonoids reduced with increasing temperature during oven drying of orange peels, which could be attributed to the decomposition of some heat-sensitive flavonoids.

The ascorbic acid content of the flavedo samples exhibited a concentration range of 0.55

to 1.01 mg g⁻¹. The ascorbic acid content was highest in fresh flavedo (Figure 2). Drying temperatures caused losses of the ascorbic acid content of fresh flavedo by 36.63–45.54%. The lowest ascorbic acid losses were observed during drying at 50°C (0.64 ± 0.02 mg g⁻¹). Rafik et al. (2019) reported the reduction of ascorbic acid in kinnow peel after different drying techniques. The degradation of ascorbic acid during food processing can be attributed to several factors including pH, temperature, light, enzymes, oxygen, and metal ion catalyzers. The oxidation of ascorbic acid due to high drying temperatures or long drying times, as well as the depletion of this acid to prevent polyphenol oxidation, may be the cause of vitamin C losses during drying (Kamiloğlu et al. 2016; Yeasmin et al., 2021).

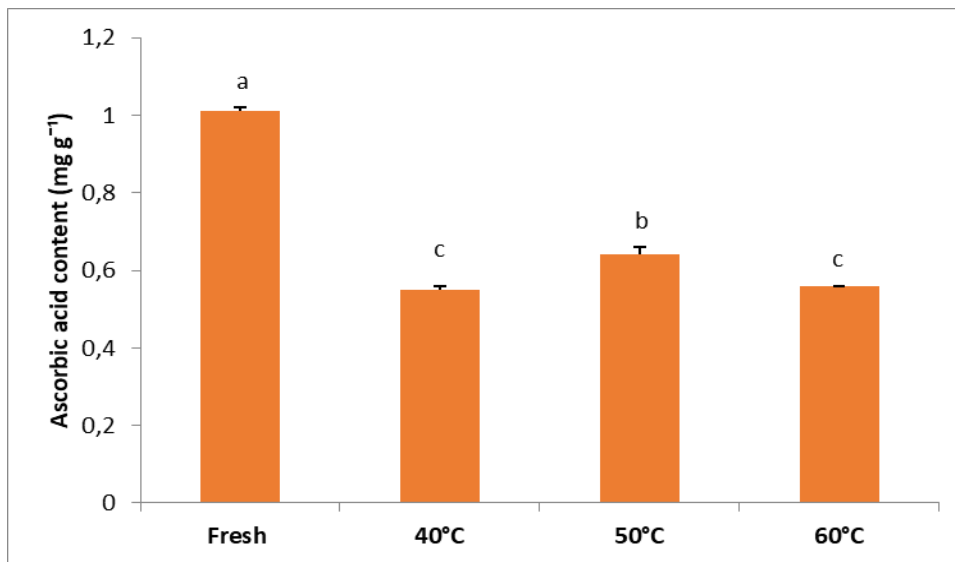


Figure 2. Ascorbic acid contents of fresh and dried flavedo samples*

*Different lowercase letters (a-) on the bars show significant differences between ascorbic acid values ($P < 0.05$).

Drying temperatures significantly decreased the IC_{50} value ($92.25 \pm 1.92 \text{ mg mg}^{-1}$) of fresh flavedo. In other words, DPPH radical scavenging activity increased. While the differences in IC_{50} values of the samples were inconsequential concerning drying temperatures ($P \geq 0.05$), the highest IC_{50} value was found in the samples dried at 60°C ($16.61 \pm 0.6 \text{ mg mg}^{-1}$), while the lowest IC_{50} value was achieved in the sample dried at 40°C ($14.61 \pm 0.53 \text{ mg mg}^{-1}$). The FRAP value of fresh flavedo ($40.28 \pm 1.22 \text{ mmol TE g}^{-1}$) increased with drying temperature. The FRAP values of the dried samples varied between 41.47 ± 2.21 and $52.19 \pm 1.49 \text{ mmol TE g}^{-1}$. The highest and lowest FRAP values were obtained at 50°C and 40°C ,

respectively (Figure 3). This trend is consistent with reports from previous studies conducted on various citrus cultivars. Abd Rahman et al. (2018) reported that the DPPH radical scavenging activity of pomelo flavedo was raised by oven drying at 50°C and 60°C . DPPH and FRAP values were significantly increased in dried grapefruit peel samples compared to fresh peels in the study by Castro-Vazquez et al. (2016). Chen et al., (2011) found that IC_{50} values of orange peels dried at 50°C , 60°C , and 70°C were higher than those of fresh ones. Özcan-Sinir et al. (2018) determined an increment in DPPH free radical scavenging activity of kumquats dried at 70°C and 80°C air temperatures.

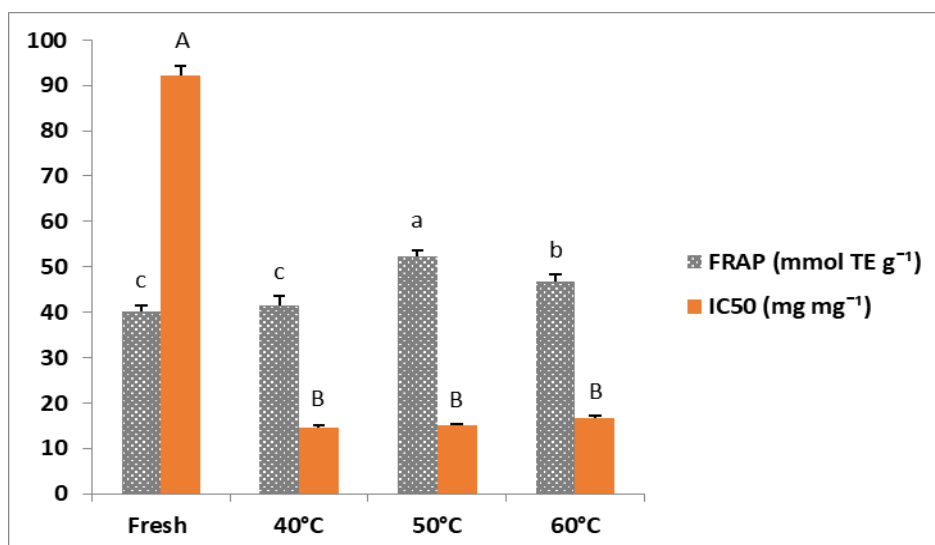


Figure 3. IC_{50} and FRAP values of fresh and dried flavedo samples*

*Different capital letters (A-B) on the bars show significant differences between IC_{50} values and different lowercase letters (a-c) on the bars show significant differences between FRAP values ($P < 0.05$).

It is thought that the antioxidant activities of dried flavedos are higher than the fresh sample, which may be due to the inactivation of the polyphenoloxidase enzyme during the drying process, as well as the decline in moisture content and the increment in dry matter content (Ghafoor et al. 2020). Moreover, high drying temperatures can stimulate Maillard reactions, resulting in the formation of Maillard products that possess antioxidant properties (Saikia et al., 2015). Previous studies have revealed that phenolic compounds and ascorbic acid are the primary contributors to the antioxidant activity observed in citrus fruits (Zou et al., 2016). However, our results showed that hot air drying increased the total phenolic and decreased the total flavonoid and ascorbic acid. Changes in antioxidant activity depend on individual phenolic and other antioxidant compounds, as well as their susceptibility to thermal and conformational modifications (Saikia et al. 2015).

Conclusion

The effect of the drying temperatures on the color, total phenolics, total flavonoids, ascorbic acid contents, DPPH radical scavenging activity and Ferric Reducing Antioxidant Power Assay of the flavedo parts of bitter orange was investigated in this work. Drying temperatures affected the color parameters measured. The drying temperatures were observed to reduce the brightness and redness values while simultaneously decreasing the h value, compared to those of the fresh flavedo. The flavedo subjected to drying at 40°C exhibited the highest b* value; however, the b* values decreased at the other drying temperatures. The total phenolic content was significantly higher at both 40°C and 50°C compared to the initial fresh sample. Nevertheless, the total flavonoid content of the bitter orange flavedo exhibited a decline as the drying temperature increased. As expected, drying temperatures caused the loss of ascorbic acid and a maximum loss of 45.54% was observed in samples dried at 40 °C. Hot air drying

significantly enhanced the antioxidant activity evaluated through the DPPH and FRAP assays. This study demonstrates that the drying temperatures applied have a significant influence on both the color and the antioxidant properties of bitter orange flavedos. It is recommended that bitter orange flavedos be dried at lower temperatures (40-50°C) to minimize color changes and enhance phenolic and antioxidative activity.

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Conflict of interest

The authors declare that they have no conflict of interest

Author contributions

DYT: Conducting experimental studies, data collection, analysis, writing original draft, review, and editing; MG: Project administration; conceptualization; supervision, analysis, review and editing; BB: Supervision, analysis, review, and editing; HT: Analysis, review and editing; OÇ: Analysis, review and editing; ET: Provision of material; review and editing.

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The effect of post-sowing potassium fertilizer application on the yield and some fiber technological properties of cotton (*Gossypium hirsutum* L.)

Ekimden sonra uygulanan potasyum gübresinin pamuğun (*Gossypium hirsutum* L.) verim ve bazı lif teknolojik özelliklerine etkisi

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ABSTRACT

This study was conducted to determine the effects of different post-sowing potassium fertilizer doses on cotton (*Gossypium hirsutum* L.) yield and fiber properties in Haliliye district of Şanlıurfa province in 2020-2021. The experiments were arranged in a randomized split-plot design with three replications. The Fiona cotton variety was used as plant material, and six-row plots were sown (row spacing: 75 cm, plant spacing: 5-6 cm). Main plots were defined by post-sowing days (30, 40, 50 days), while subplots consisted of potassium doses (control, 10, 20, 30 kg da⁻¹). Results showed that seed cotton yield ranged between 396 and 520 kg da⁻¹, with the highest yield achieved with 50 days post-sowing and 30 kg da⁻¹ KCl application (520 kg da⁻¹). Plant height varied from 77.95 to 85.41 cm in 2020 and from 81.13 to 87.63 cm in 2021. The highest boll weight (6.97 g) was recorded in 2021 with 50 days post-sowing and 30 kg da⁻¹ KCl application. The number of fruiting branches and bolls per plant varied across years, with the highest boll count observed with 40 days post-sowing and 20 kg da⁻¹ KCl application. Fiber uniformity index ranged between 82.23% and 85.40%, while short fiber content varied from 6.70% to 11.70%. As a result, potassium fertilizer doses were found to be effective in increasing cotton yield and fiber quality; in particular, the application of 30 kg da⁻¹ KCl on the 50th day gave the best results.

Keywords: Cotton, Fertilization, Fiber properties, Potassium, Yield

Öz

Bu çalışma, ekim sonrası farklı potasyum gübresi dozlarının pamuk (*Gossypium hirsutum* L.) verimi ve lif özelliklerine etkisini belirlemek amacıyla 2020-2021 yıllarında Şanlıurfa İli Haliliye İlçesi'nde yürütülmüştür. Denemeler, tesadüf bloklarında bölünmüş parseller deneme desenine göre üç tekerrürlü olarak gerçekleştirilmiştir. Fiona pamuk çeşidi kullanılarak 6 sıralı parsellerde (sıra arası 75 cm, sıra üzeri 5-6 cm) ekim yapılmıştır. Ana parsellerde ekim sonrası gün sayısı (30, 40, 50 gün), alt parsellerde ise potasyum dozları (kontrol, 10, 20, 30 kg da⁻¹) değerlendirilmiştir. Sonuçlar, kütlü pamuk veriminin 396-520 kg da⁻¹ arasında değiştiğini ve en yüksek verimin 50. gün 30 kg da⁻¹ KCl uygulamasıyla elde edildiğini göstermiştir (520 kg da⁻¹). Bitki boyu 2020'de 77.95-85.41 cm, 2021'de 81.13-87.63 cm arasında değişmiştir. En yüksek koza ağırlığı (6.97 g), 50. gün 30 kg da⁻¹ KCl uygulamasında 2021 yılında kaydedilmiştir. Meyve dalı ve koza sayıları yıllara göre değişim göstermiş, en yüksek koza sayısı 40. gün 20 kg da⁻¹ uygulamasında belirlenmiştir. Lif özellikleri açısından üniformite indeksi %82.23-%85.40 arasında, kısa lif oranı ise %6.70-%11.70 arasında değişmiştir. Sonuç olarak, potasyum gübresi dozları pamuk verimi ve lif kalitesini artırmakta etkili bulunmuş; özellikle 50. gün 30 kg da⁻¹ KCl uygulaması en iyi sonuçları vermiştir.

Anahtar Kelimeler: Pamuk, Gübreleme, Lif özellikleri, Potasyum, Verim

Introduction

Clothing, an indispensable aspect of life, continues to underscore the growing importance of cotton fibers in meeting protection and covering needs. Cotton stands as the most significant fiber crop in the world. As the most valuable raw material in the global textile industry, cotton can only be cultivated in a limited number of countries due to its specific climatic requirements. Supplying raw materials to approximately fifty industrial sectors, cotton is not only an industrial crop but also a strategic product of great significance. Factors such as its natural composition, high moisture absorption capacity, ability to retain its properties even after multiple washes, greater durability compared to other fibers, low static electricity conductivity, excellent air permeability due to its high porosity, hygienic characteristics, and the inability of synthetic fibers to replicate its natural qualities all contribute to the increasing importance of cotton (Usta, 2003).

According to the data from the International Cotton Advisory Committee (ICAC) for the years 2016-2021, cotton cultivation worldwide during this six-year period covered an average area of 33.128 million hectares, with an annual average production of 25.57 million tons of cotton fiber. The United States ranked as the world's largest exporter, with an average annual export volume of 3.35 million tons, while China was the largest importer, with an annual average import volume of 1.87 million tons. Turkey, on the other hand, imported an annual average of 0.99 million tons of cotton during this period. In the 2020-2021 production season, the global cotton cultivation area was recorded at 32.1 million hectares. India had the largest cotton cultivation area, followed by the United States and China. Although Turkey ranked ninth globally in terms of cotton cultivation area, it held third place in productivity, following Australia and China. In Turkey, 814,000 tons of cotton fiber were produced on 477.8 thousand hectares during the 2019/2020 production season, but this figure declined to

656,000 tons on 359.2 thousand hectares in the 2020/2021 season (ICAC, 2022).

According to the Turkish Statistical Institute (TÜİK), in 2019, 55% of the cotton produced in Turkey was grown in the Southeastern Anatolia Region, 22% in the Aegean Region, 22% in the Çukurova region, and 1% in the Antalya region. Cotton is not only an agriculturally significant crop at both national and international levels due to its economic and strategic importance but also an indispensable raw material for the sustainability of the textile industry. Ensuring the continued success of Turkey's clothing sector, which plays a crucial role in the nation's economy and development, depends on increasing the production of cotton fiber, often referred to as "white gold." In the 2020 cotton production season, cotton was cultivated on 224,000 hectares in Şanlıurfa, compared to 232,000 hectares in 2019. These figures indicate that Şanlıurfa accounts for approximately 45% of Turkey's cotton production and 75% of the Southeastern Anatolia Region's output (TÜİK, 2020). To increase yield per unit area, it is essential to implement practices such as selecting high-quality seeds suitable for the region, optimal irrigation, proper fertilization, and pesticide application methods that do not disrupt the natural balance or harm predators, all executed at the right time and with maximum efficiency. Among these practices, fertilization holds a particularly critical role as it enhances the effectiveness of other agricultural inputs and is one of the most significant factors in improving both the yield and quality of agricultural production.

In large-scale field agriculture conducted in Turkey, fertilization typically focuses on the essential nutrients nitrogen and phosphorus, while applications of other nutrients, particularly potassium, remain quite limited. However, according to the "Law of the Minimum," also known as "Liebig's Law," which is critical for plant productivity, the deficient nutrient acts as a limiting factor for growth, and its uptake by the plant is of vital importance. Cotton is known as a

crop that consumes large amounts of potassium, making potassium one of the most crucial plant nutrients for its cultivation. Potassium plays a critical role in both increasing yield and improving fiber quality. Addressing potassium deficiencies has emerged as a strategic necessity for ensuring sustainability in cotton production and enhancing the sector's competitive edge.

In cotton plants, potassium is one of the most important nutrients influencing fruit quality. Its primary functions include its role in protein synthesis and photosynthesis, as well as facilitating the transport of sugars from leaves to fruits. Effective potassium application maintains leaf activity throughout fruit development, increases soluble matter content (more sugars) in the fruit at harvest, and reflects positively on yield. It has been observed that approximately 60-66% of the total potassium taken up by the plant is found in the fruit. Potassium also has beneficial effects on plants exposed to environmental stress factors and significantly reduces the adverse impacts of diseases and pests (Ramazanoglu, 2024). Various studies have reported that potassium applications substantially increase yield in cotton (Çağlayan and Demoglu, 2005).

Potassium deficiency can negatively impact numerous physiological activities in plants, such as reduced yield, poor growth, decreased enzyme activation, impaired water relations, and lower resistance to stress (Wang et al., 2012; Hu et al., 2016, 2017; Qi et al.). For instance, Adeli and Varco (2014) found that applying potassium doses of 0, 68, 136, and 204 kg/ha either as band applications alongside rows or broadcast over the field on non-acidic silty soils in Mississippi resulted in a linear increase in cotton yield. The same study reported fiber yields ranging between 1142 kg/ha and 1449 kg/ha, with the highest fiber yield achieved through the broadcast application of 13.6 kg da⁻¹ potassium. Similarly, a study conducted in China's Hebei region demonstrated that different potassium doses (0, 45, 90, 180 kg/ha) and six different application timings had significant effects on fiber yield.

In terms of fiber yield, potassium applications made particularly before planting and during the squaring stage resulted in a 5% to 8% increase compared to the control group (Yang et al., 2017). Another study conducted in the same region found no significant difference in yield between potassium sources (K₂SO₄ or KCl), but application timings led to variations in yield ranging from 23% to 103% (Yang et al., 2016). Additionally, polymer-coated potassium chloride was reported to enhance potassium release during the flowering and boll formation stages, significantly improving the number of bolls and seed cotton yield.

In large-scale agricultural production in Turkey, nitrogen and phosphorus, which are essential nutrients, are widely applied, whereas potassium fertilization is often neglected. However, potassium is as important as nitrogen for cotton. Soil analysis results indicate that the potassium levels in the experimental field, ranging from 98 to 108 kg da⁻¹, classify these soils as high in potassium content. Nevertheless, the ability of plants to utilize soil potassium depends on factors such as genetic makeup, soil characteristics, temperature, and humidity.

Various studies have found that potassium fertilization in cotton enhances seed cotton yield, the number and weight of bolls, fiber length, fiber fineness, and uniformity. Generally, yield and its components improve as potassium doses increase (Pervez et al., 2004; Khalifa et al., 2012; Zia-ul Hassan et al., 2014; Bumguardner, 2018). Additionally, it has been noted that potassium application as a foliar fertilizer boosts seed cotton yield and fiber quality, though soil applications are also necessary to meet the plant's potassium requirements.

This study was conducted in the Şanlıurfa region during 2020 and 2021 to determine the effects of timing and doses of potassium fertilization on yield, quality, and certain yield components in cotton.

Material and Method

Material

The study was conducted during the 2020 and 2021 cotton growing seasons in Haliliye District, Şanlıurfa Province. The plant material used in the study was the Fiona cotton variety (*Gossypium hirsutum* L.), one of the standard varieties of the region. Fiona is known for its high yield potential, medium-to-long growing season, short fruiting branches, and cluster structure, making it suitable for dense planting and mechanical harvesting.

It has a ginning outturn of 44–46%, medium-sized bolls, and strong boll opening characteristics. Approximately 12,000 seeds are found in 1 kilogram, and its high fiber quality makes it a preferred choice in the textile industry (agro.basf.com.tr/tr).

The study was conducted in the Harran Plain, characterized by red-brown soils with low organic matter content. The soils in this region are derived from alluvial parent material, have a flat to nearly flat slope, and exhibit a clayey structure with high lime content. Organic matter content decreases from surface to deeper layers, ranging from 0.9% to 0.3%. The cation exchange capacity increases in the lower layers due to the high clay content (Dinç et al., 1986; Öztürkmen et al., 2021). Soil samples taken from a depth of 0–20 cm in the experimental area were analyzed at the GAP Agricultural Research Institute (GAP TAEM) Soil Analysis Laboratory. According to these analyses, the experimental field was classified as having a clay-loam texture, with silt content ranging from 22% to 33% and sand content from 20.8% to 26.6%. The soil pH was measured as 7.76 in 2020 and 7.50 in 2021. Potassium content was found to range between 98.8 and 109.8 kg da⁻¹, classifying these soils as potassium.

The experimental area experiences a continental climate characterized by hot and dry summers and cold winters. Between April and November, the average temperatures ranged

from 11.4°C to 33.4°C in 2020 and from 12.6°C to 34.0°C in 2021, with the long-term average recorded as 12.8°C to 33.1°C. Total precipitation varied between 0 mm and 81.1 mm in 2020, and between 0 mm and 49.2 mm in 2021. The average relative humidity was recorded as 40.8%–62.9% in 2020 and 31.3%–66.5% in 2021. The average temperature across Turkey in 2020 and 2021 was 14.9°C, which is 1–1.4°C above the 1981–2010 average. In this context, 2020 was recorded as the third warmest year since 1971, while 2021 was the fourth warmest (MGM, 2022).

The experimental field was deeply plowed in the fall of 2019 and cultivated in April 2020. A trifluralin-based herbicide was applied to prepare the field for sowing. After harvesting, the field was plowed again in November 2020, and the same procedures were repeated for the 2021 growing season. Sowing was performed with a six-row pneumatic cotton seeder on April 25, 2020, and April 29, 2021. The study was designed with potassium application timings (30th, 40th, and 50th days after planting) assigned to main plots, and potassium doses (0 kg da⁻¹ as control, 10, 20, and 30 kg da⁻¹) assigned to subplots. Each plot consisted of six rows, with a row spacing of 75 cm and an intra-row spacing of 5.4 cm, and measured 12 meters in length. This arrangement resulted in a total area of 54 m² per plot.

Using these methods, the study aimed to determine the effects of potassium applications on cotton yield, the number and weight of bolls, ginning outturn, fiber quality, and other yield components. During the research period, maintenance, pest control, irrigation, and harvesting operations were carried out meticulously. In both experimental years, after achieving a uniform plant population in the plots, hand hoeing was performed twice for weed control once at the end of May and again in the first week of June. Subsequently, tractor hoeing was conducted twice in conjunction with top-dressing fertilization.

Method

Agricultural Pest Control

Table 1. Agricultural pest control measures applied in the experimental area

Pest	Application
Thrips (<i>Aphis gossypii</i>)	In both years, an insecticide containing Dimethoate (40%) was applied at a dose of 100 cc da ⁻¹ .
Red Spider Mite (<i>Tetranychus urticae</i> Koch)	In July, Nissoril (Hekythiozox) was applied at a dose of 100 cc da ⁻¹ .
Leafhopper (<i>Empoasca</i> spp.) and Whitefly (<i>Bemisia tabaci</i> Genn.)	In August, a mixture of Hekplan (Acetamiprid, 20 g da ⁻¹) and Sumigold (Esfenvalerate, 60 cc/da) was applied.
Helicoverpa (<i>Helicoverpa armigera</i> Hübner)	At the end of August, Hekplan (Acetamiprid, 20 g da ⁻¹) and Dursban-4 (Chlorpyrifos, 200 cc da ⁻¹) were applied.
Aphid (<i>Aphis gossypii</i>)	In 2020, Movento (Spirotetramat, 100 cc da ⁻¹) and Transform (Sulfoxaflor, 15 g da ⁻¹) were applied for control.

Irrigation

In both experimental years, flood irrigation was applied in April before sowing to ensure adequate moisture for germination. The irrigation process was carried out using the furrow method, with a total of ten irrigations performed. The first irrigation was applied 30 days after sowing, along with top-dressing fertilization. Subsequent irrigations were conducted on June 22, July 2, July 12, July 22, August 2, August 11, August 21, September 3, and September 15. This systematic irrigation schedule was meticulously followed to ensure optimal plant development.

Harvest

In both years, approximately 15 days after the final irrigation, 60 ml da⁻¹ of Bayer Finish Drop (ethephon + cyclanilide) defoliant and boll opener was applied. Depending on weather conditions, manual harvesting was performed 10–12 days later. To account for edge effects, 1 meter from the start and end of each plot was excluded, and the middle 10-meter-long 2 rows (15 m²) were harvested by hand.

Determination of Yield and Fiber Characteristics

The yield and fiber characteristics obtained in the study were evaluated according to the methods suggested by Şenel (1980). Yield components measured included seed cotton yield (kg da⁻¹), plant height (cm), the number of fruiting

branches (units/plant), the number of bolls (units/plant), boll weight (g), seed cotton weight per boll (g), 100-seed weight (g), and ginning outturn (%). Technological fiber characteristics such as fiber length, fiber fineness, fiber strength, uniformity, short fiber index, and brightness were analyzed using the HVI 900-A device at the Rubenis Textile Company Laboratory. These methods provided detailed results on cotton yield and fiber quality.

Data Analysis

The measurements, weights, and fiber analysis results obtained from the research were analyzed using the SPSS statistical package, following the randomized block design. Grouping was performed using Tukey's test. The homogeneity of the error variances across years was tested, and the years were combined for a unified evaluation of the results.

Results and Discussion

Seed Cotton Yield (kg da⁻¹)

The number of days after sowing (DAS), potassium doses, and the interaction between potassium application doses and DAS were statistically significant at the $p \leq 0.01$ level (Table 2). The seed cotton yield was determined as 416.36 kg da⁻¹ in 2020 and 401.41 kg da⁻¹ in 2021. It was found that potassium applications on the

30th, 40th, and 50th days after sowing had statistically significant effects on seed cotton yield, with average yield values ranging between 385.37 kg da⁻¹ and 437.41 kg da⁻¹. When the average yield values for 2020, 2021, and the two-year average were analyzed, the highest seed cotton yield was observed in the 30th-day application. Specifically, in 2020, the yields for the 30th, 40th, and 50th-day applications were 458.00, 407.73, and 383.35 kg da⁻¹, respectively. Similarly, in 2021, the yields were 416.82, 400.01, and 387.40 kg da⁻¹. The two-year average also followed a similar trend, with the highest yield recorded in the 30th-day application at 458.00 kg da⁻¹, followed by the 40th-day and 50th-day potassium applications.

Potassium doses also had a statistically significant effect on seed cotton yield. Yields varied between 376.25 kg da⁻¹ and 459.52 kg da⁻¹ depending on potassium dose. Based on the two-year average, the highest seed cotton yield was obtained with a 20 kg da⁻¹ potassium application, a trend that was also evident when the years were evaluated individually. The 20 kg da⁻¹ potassium dose was followed by the control, 10 kg da⁻¹, and 30 kg da⁻¹ potassium applications. These results highlight the critical role of an optimal potassium dose in improving seed cotton yield. When interactions between the number of days after sowing (DAS) and potassium doses

were analyzed, seed cotton yields ranged from 357.15 kg da⁻¹ to 496.97 kg da⁻¹. The highest yield, 496.97 kg da⁻¹, was obtained from the interaction between the 30th-day application and 20 kg da⁻¹ potassium dose (DAS30 × K20). Similarly, this interaction produced the highest seed cotton yield in both experimental years. However, a decrease in seed cotton yield was observed as the potassium application was delayed. These findings demonstrate that early potassium applications have a positive impact on yield, underscoring the importance of timely nutrient management.

The findings of this study are fully or partially consistent with the results of studies by Akhtar et al. (2003); Pervez et al. (2004); Çağlayan and Demoglu (2005); Khalifa et al. (2012); Adeli (2014); Ziaul Hassan et al. (2014); Shahzad et al. (2019); Hussain et al. (2021); Wang et al. (2012); Phipps et al. (2007); Mozaffari (2018); Yang et al. (2017); Kusi (2019); and Bumgardner et al. (2024). However, the finding by Bumgardner (2018), which reported that potassium fertilization had no effect on cotton yield, does not align with our study. This discrepancy may be attributed to differences in the ecological conditions under which the experiments were conducted, the genotypic characteristics of the cotton seeds used, or variations in cultural practices applied during the trials.

Table 2. Variance Analysis Results, Mean Values, Tukey-HSD Test Groups, and Coefficient of Variation (% C.V.) for Seed Cotton Yield and Plant Height in 2020, 2021, and Combined Years

Source of Variation	Seed Cotton Yield (kg da ⁻¹)			Plant Height (cm)		
	2020	2021	Mean	2020	2021	Mean
DAS	**	**	**	ns	*	*
K	**	**	**	ns	ns	ns
DAS × K	**	**	**	ns	ns	*
Year (Y)	-	-	**	-	-	**
Y × DAS	-	-	**	-	-	ns
Y × K	-	-	**	-	-	ns
Y × DAS × K	-	-	**	-	-	ns
Years (Y)	416,36 a	401,41 b		98,71 a	86,19 b	
Days After Sowing (DAS)						
DAS 30	458,0 a	416,8a	437,4 a	99,3	88,2 a	93,7 a
DAS 40	407,7 b	400,0b	403,9 b	99,2	87,0 ab	93,1 ab
DAS 50	383,4 c	387,4c	385,4 c	97,6	83,4 b	90,5 b
Potasyum Dose (K)						
K ₀ (Control)	415,4 b	409,8b	412,6 b	99,2	85,2	92,2
K ₁₀ (10 kg da ⁻¹)	386,5 d	366,1d	376,3 d	100,3	85,9	93,1
K ₂₀ (20 kg da ⁻¹)	466,8 a	452,6a	459,5 a	97,7	85,5	91,6
K ₃₀ (30 kg da ⁻¹)	397,2 c	377,2c	387,2 c	97,6	88,2	92,9
Interactions (DAS × K)						
DAS 30 × K ₀	481,7 b	461,1a	471,4 b	93,6	86,1	89,9 ab
DAS 30 × K ₁₀	416,2 c	356,7e	386,4 de	106,5	88,5	97,5 a
DAS 30 × K ₂₀	523,7 a	472,3a	498,0 a	101,7	87,0	94,3 ab
DAS 30 × K ₃₀	410,5 cd	377,2 cd	393,9 d	95,3	91,2	93,2 ab
DAS 40 × K ₀	371,2 fg	388,2c	379,7 e	104,3	85,5	94,9 ab
DAS 40 × K ₁₀	394,2cde	376,2cd	385,2 de	101,8	87,8	94,8 ab
DAS 40 × K ₂₀	485,6 b	453,6a	469,6 b	93,8	85,3	89,5 ab
DAS 40 × K ₃₀	379,9 ef	382,0cd	381,0 de	97,0	89,2	93,1 ab
DAS 50 × K ₀	393,1 def	380,1cd	386,6 de	99,8	83,9	91,9 ab
DAS 50 × K ₁₀	349,0 g	365,3de	357,2 f	92,6	81,3	86,9 b
DAS 50 × K ₂₀	390,2 def	431,9 b	411,0 c	97,8	84,3	91,0 ab
DAS 50 × K ₃₀	401,1cde	372,4cde	386,7 de	100,4	84,1	92,3 ab
%CV	12,35	10,02		6,59	4,50	

DAS: days after sowing

Plant Height (cm)

In 2020, none of the treatments had a statistically significant effect on plant height. However, in 2021, the number of days after sowing (DAS) had a significant effect at the $p \leq 0.05$ level, while other variations were found to be insignificant (Table 2). In the combined analysis of both years, DAS and DAS × K dose interactions were determined to be significant. The average plant height was measured as 98.71 cm in 2020 and 86.19 cm in 2021. Applications on the 30th, 40th, and 50th days after sowing did not have a statistically significant effect on plant height in the first year, but in the second year, they were significant at the $p \leq 0.15$ level. The average plant height values ranged from 90.53 cm to 93.74 cm, with the highest combined years' average (93.74 cm) recorded in the 30th-day

application.

Potassium application doses did not have a statistically significant effect on plant height, which ranged from 92.21 cm to 93.08 cm. When DAS and potassium dose interactions were analyzed, plant height values ranged from 81.33 cm to 106.53 cm, with the highest values recorded in the first year for the DAS30 × K10 and DAS30 × K0 interactions (104.26 cm). The findings regarding the effects of potassium applied at different doses and growth stages on plant height are consistent with studies by Hussain et al. (2021); Wang et al. (2012); Qi et al. (2019); and Bumguardner (2018, 2024). No studies in the literature contradict our results regarding plant height.

Number of fruiting branches (number plant⁻¹)

The number of days after sowing (DAS) and potassium doses had statistically significant effects on the number of fruiting branches in 2020, 2021, and the combined years' average (Table 3). The average number of fruiting branches per plant was 11.30 in 2020 and 10.18 in 2021. Applications on the 30th, 40th, and 50th days after sowing significantly influenced the number of fruiting branches, which ranged between 10.40 and 11.20 number plant⁻¹. The highest combined years' average (11.20 number plant⁻¹) was obtained from the 30th-day application. In 2020, the number of fruiting branches per plant was 11.85, 11.23, and 10.82 for the 30th, 40th, and 50th-day applications, respectively. In 2021, these values were 10.50, 10.01, and 9.98 number plant⁻¹, respectively. Delayed applications resulted in a decrease in the number of fruiting branches.

Potassium application doses also had a statistically significant effect on the number of fruiting branches, with values ranging from 10.99 to 11.90 number plant⁻¹ in 2020 and 9.56 to 10.81 number plant⁻¹ in 2021. The combined years' average showed values between 10.28 and 11.36 number plant⁻¹, with the highest value obtained from the 20 kg da⁻¹ potassium dose. When DAS and potassium dose interactions were analyzed, the number of fruiting branches ranged from 9.47 to 12.64 units/plant. In both years, the highest value was obtained from the DAS30 × K20 interaction. A decrease in the number of fruiting branches was observed with delayed applications. The results for the number of fruiting branches obtained in this study align with the findings of Wang et al. (2012), Ziaul Hassan et al. (2014), Yang et al. (2016), Hu et al. (2017), Qi et al. (2019).

Table 3. Variance Analysis Results, Mean Values, Tukey-HSD Test Groups, and Coefficient of Variation (% C.V.) for Number of Fruiting Branches and Number of Bolls in 2020, 2021, and Combined Years

Source of Variation	Number of Fruiting Branches			Number of Bolls		
	2020	2021	Mean	2020	2021	Mean
DAS	**	*	**	**	**	**
K	*	**	**	**	**	**
DAS × K	ns	ns	ns	ns	ns	ns
Year (Y)	-	-	**	-	-	**
Y × DAS	-	-	ns	-	-	ns
Y × K	-	-	ns	-	-	**
Y × DAS × K	-	-	ns	-	-	ns
Yıllar (Y)	11,30 a	10,18 b		14,63 a	14,03 b	
Interactions (DAS × K)						
DAS 30	11,85 a	10,50	11,20 a	15,29 a	14,61 a	14,96 a
DAS 40	11,23 b	10,01	10,62 b	14,39 b	14,16 b	14,27 b
DAS 50	10,82 b	9,98	10,40 b	14,21 b	13,32 c	13,76 b
Potasyum Dose (K)						
K ₀ (Control)	11,10 b	10,10 ab	10,60 b	14,84 a	14,18 b	14,51 b
K ₁₀ (10 kg da ⁻¹)	10,99 b	9,56 b	10,28 b	13,93 b	13,02 c	13,48 c
K ₂₀ (20 kg da ⁻¹)	11,90 a	10,81 a	11,36 a	15,22 a	15,21 a	15,22 a
K ₃₀ (30 kg da ⁻¹)	11,21 ab	10,26 ab	10,74 b	14,53 ab	13,70 b	14,12 b
Interactions (DAS × K)						
DAS ₃₀ × K ₀	11,86 ab	10,47	11,17	15,47	14,62	15,05
DAS ₃₀ × K ₁₀	11,37 ab	9,73	10,55	14,95	14,00	14,48
DAS ₃₀ × K ₂₀	12,64 a	11,20	11,92	15,76	15,62	15,69
DAS ₃₀ × K ₃₀	11,51 ab	10,82	11,17	14,99	14,21	14,60
DAS ₄₀ × K ₀	10,57 b	10,04	10,31	14,84	14,11	14,47
DAS ₄₀ × K ₁₀	11,06 ab	9,49	10,28	13,22	13,08	13,15
DAS ₄₀ × K ₂₀	11,80 ab	10,60	11,20	15,02	15,49	14,21
DAS ₄₀ × K ₃₀	11,48 ab	9,91	10,70	14,47	13,95	15,25
DAS ₅₀ × K ₀	10,85 b	9,78	10,32	14,20	13,81	14,00
DAS ₅₀ × K ₁₀	10,53 b	9,47	10,00	13,62	11,97	12,80
DAS ₅₀ × K ₂₀	11,27 ab	10,64	10,96	14,88	14,53	14,70
DAS ₅₀ × K ₃₀	10,64 b	10,04	10,34	14,12	12,95	13,54
%CV	6,70	6,83		6,13	7,50	

Number of Bolls (number plant⁻¹)

The number of days after sowing (DAS) and potassium doses had statistically significant effects on the number of bolls per plant in 2020, 2021, and the combined years' average (Table 3). The average number of bolls per plant was 14.63 in 2020 and 14.03 in 2021. Applications on the 30th, 40th, and 50th days after sowing significantly influenced the number of bolls, with averages ranging between 13.76 and 14.96 units/plant. The highest combined years' average was obtained from the 30th-day application. Specifically, the number of bolls per plant was 15.29, 14.39, and 14.21 *units plant⁻¹* for the 30th, 40th, and 50th-day applications, respectively, in 2020, and 14.61, 14.16, and 13.32 *units plant⁻¹* in 2021. The highest number of bolls was recorded for the 30th-day application, followed by the 40th and 50th-day applications. A decrease in the number of bolls was observed with delayed application timings.

Potassium doses also had a statistically significant effect on the number of bolls. In 2020, the number of bolls ranged from 13.93 to 15.22 *units plant⁻¹*, while in 2021, it ranged from 13.02 to 15.21 *units plant⁻¹*. Combined years' averages varied between 13.48 and 15.22 *units plant⁻¹*, with the highest value (15.22 *units plant⁻¹*) obtained from the 20 kg da⁻¹ potassium dose. When DAS and potassium dose interactions were analyzed, the number of bolls ranged from 11.97 to 15.76 *units plant⁻¹*. In both years, the highest values were obtained from the DAS30 × K20 interaction, while delayed applications resulted in a reduction in the number of bolls per plant. The results obtained for the number of bolls per plant in this study are in full or partial agreement with those reported by Faircloth et al. (2004), Pervez et al. (2004), Khalifa et al. (2012), Zia-ul Hassan et al. (2014), Yang et al. (2017), Bumguardner (2018), and Echera et al. (2020).

Boll Seed Cotton Weight (g)

The number of days after sowing (DAS), potassium doses, and DAS × K interactions had no statistically significant effect on boll seed cotton

weight in 2020, 2021, or the combined years' average (Table 5). The average boll seed cotton weight was 5.48 g in 2020 and 4.83 g in 2021. Applications on the 30th, 40th, and 50th days after sowing did not significantly influence boll seed cotton weight in either study year. The average weight ranged from 5.44 g to 5.50 g in 2020 and from 4.76 g to 4.88 g in 2021. Similarly, potassium doses had no statistically significant effect on boll seed cotton weight, which ranged from 5.39 g to 5.57 g in 2020 and from 4.77 g to 4.88 g in 2021. The DAS × K interactions also showed no significant differences.

The findings on boll seed cotton weight in this study align with the first-year results of Sawan et al. (2006) and Faircloth et al. (2004), but they contradict the second-year findings. Furthermore, they do not align with the results reported by Pervez et al. (2004), Khalifa et al. (2012), Zia-ul Hassan et al. (2014), and Yang et al. (2016). These discrepancies may stem from variations in ecological conditions of the experimental regions, genetic differences in the cotton varieties used, or differences in cultural practices applied during the trials.

100-Seed Weight (g)

In 2020, none of the treatments had a statistically significant effect on 100-seed weight, whereas in 2021, the number of days after sowing (DAS) had a significant effect at the $p \leq 0.05$ level. The 100-seed weight was recorded as 8.73 g in 2020 and 8.85 g in 2021 (Table 5). Applications on the 30th, 40th, and 50th days after sowing did not significantly affect 100-seed weight in 2020, but they were significant at the $p \leq 0.05$ level in 2021. The average 100-seed weight ranged from 8.66 g to 8.78 g in 2020 and from 8.61 g to 9.10 g in 2021, with the highest value (9.10 g) obtained from the 30th-day application. This was followed by the 40th and 50th-day applications.

Potassium doses did not have a statistically significant effect on 100-seed weight. Analysis of DAS × K interactions revealed that 100-seed weight values ranged between 8.36 g and 9.43 g, but these differences were not statistically

significant. The findings of this study are consistent with those reported by Krisnan et al. (2007). However, they do not align with the results of Sawan et al. (2006), Pervez et al. (2004), and Görmüş & Kanat (1998). These discrepancies

may be attributed to differences in ecological conditions of the experimental regions, genetic variations in the cotton varieties used, or diversity in cultural practices applied during the experiments.

Table 5. Variance Analysis Results, Mean Values, Tukey-HSD Test Groups, and Coefficient of Variation (% C.V.) for Boll Seed Cotton Weight and 100-Seed Weight in 2020, 2021, and Combined Years

Source of Variation	Boll Seed Cotton Weight (g)			100-Seed Weight (g)		
	2020	2021	Mean	2020	2021	Mean
DAS	ö.d.	ö.d.	ns	ns	*	ns
K	ns	ns	ns	ns	ns	ns
DAS × K	ns	ns	ns	ns	ns	ns
Year (Y)	-	-	**	-	-	ns
Y × DAS	-	-	ns	-	-	ns
Y × K	-	-	ns	-	-	ns
Y × DAS × K	-	-	ns	-	-	ns
Yıllar (Y)	5,48 a	4,83 b		8,73	8,85	
Days After Sowing (DAS)						
DAS 30	5,49	4,84	5,16	8,78	9,10 a	8,94
DAS 40	5,44	4,88	5,16	8,66	8,61 b	8,64
DAS 50	5,50	4,76	5,13	8,75	8,84 ab	8,79
Potasyum Dose (K)						
K ₀ (Control)	5,39	4,83	5,11	8,71	9,07	8,89
K ₁₀ (10 kg da ⁻¹)	5,48	4,88	5,18	8,92	8,66	8,79
K ₂₀ (20 kg da ⁻¹)	5,48	4,82	5,15	8,58	8,74	8,66
K ₃₀ (30 kg da ⁻¹)	5,57	4,77	5,17	8,71	8,92	8,82
Interactions (DAS × K)						
DAS ₃₀ × K ₀	5,49	4,90	5,20	8,70	9,43	9,06
DAS ₃₀ × K ₁₀	5,34	4,84	5,09	8,96	8,89	8,93
DAS ₃₀ × K ₂₀	5,36	4,70	5,03	8,36	8,82	8,59
DAS ₃₀ × K ₃₀	5,76	4,91	5,33	9,11	9,26	9,19
DAS ₄₀ × K ₀	5,30	4,82	5,06	8,62	8,62	8,62
DAS ₄₀ × K ₁₀	5,41	4,96	5,19	8,97	8,61	8,79
DAS ₄₀ × K ₂₀	5,42	4,92	5,17	8,62	8,49	8,56
DAS ₄₀ × K ₃₀	5,64	4,81	5,23	8,43	8,73	8,58
DAS ₅₀ × K ₀	5,36	4,76	5,06	8,82	9,16	8,99
DAS ₅₀ × K ₁₀	5,67	4,85	5,26	8,82	8,48	8,65
DAS ₅₀ × K ₂₀	5,67	4,83	5,25	8,75	8,92	8,84
DAS ₅₀ × K ₃₀	5,30	4,60	4,95	8,61	8,77	8,69
%CV	4,34	5,00		5,31	4,87	

Ginning Outturn (%)

In 2020, none of the treatments had a significant effect on ginning outturn, while in 2021, the number of days after sowing (DAS) showed a statistically significant effect at the $p \leq 0.01$ level. Potassium applications and DAS × K interactions were significant at the $p \leq 0.05$ level (Table 6). The ginning outturn was recorded as 44.25% in 2020 and 43.76% in 2021. Applications on the 30th, 40th, and 50th days after sowing had no significant effect on ginning outturn in 2020 but were significant at the $p \leq 0.01$ level in 2021. The average ginning outturn ranged from 43.88%

to 44.60% in 2020 and from 43.03% to 44.20% in 2021. The highest ginning outturn values were obtained from the DAS50 (44.20%) and DAS40 (44.06%) applications.

Potassium doses did not have a statistically significant effect on ginning outturn in 2020, but they showed a significant impact in 2021. In 2020, ginning outturn values ranged from 43.88% to 44.47%, while in 2021, they ranged from 43.45% to 44.22%, with the highest value (44.22%) obtained from the 30 kg da⁻¹ K application. Analysis of DAS × K interactions revealed that ginning outturn ranged from 42.43% to 45.31%,

but these differences were not statistically significant. The results on ginning outturn from this study are consistent with the findings of Phipps et al. (2007) and Yang et al. (2016a). However, they do not align with the results reported by Read et al. (2006), Faircloth et al.

(2004), and Pervez et al. (2004). These discrepancies may be attributed to differences in ecological factors of the experimental regions, genetic characteristics of the cotton varieties used, or variations in cultural practices applied.

Table 6. Variance Analysis Results, Mean Values, Tukey-HSD Test Groups, and Coefficient of Variation (% C.V.) for Ginning Outturn in 2020, 2021, and Combined Years

Source of Variation	Ginning Outturn (%)			Interactions (DAS × K)			
				Sources of variation	2020	2021	Mean
	2020	2021	Mean				
DAS	ns	**	ö.d.	DAS ₃₀ × K ₀	44,09	42,86 cd	43,47
K	ns	*	ö.d.	DAS ₃₀ × K ₁₀	44,53	42,43 d	43,48
DAS × K	ns	*	ö.d.	DAS ₃₀ × K ₂₀	44,11	43,51 abcd	43,81
Year (Y)	-	-	ö.d.	DAS ₃₀ × K ₃₀	42,80	43,31 bcd	43,06
Y × DAS	-	-	ö.d.	DAS ₄₀ × K ₀	44,49	44,55 ab	44,52
Y × K	-	-	ö.d.	DAS ₄₀ × K ₁₀	43,90	43,56 abcd	43,73
Y × DAS × K	-	-	ö.d.	DAS ₄₀ × K ₂₀	43,68	43,53 abcd	43,61
Years (Y)	44,25	43,76		DAS ₄₀ × K ₃₀	44,99	44,59 ab	44,79
				DAS ₅₀ × K ₀	44,84	43,68 abcd	44,26
Days After Sowing (DAS)							
DAS 30	43,88	43,03 b	43,46	DAS ₅₀ × K ₂₀	45,03	43,97 abc	44,50
DAS 40	44,26	44,06 a	44,16	DAS ₅₀ × K ₃₀	45,31	44,77 a	45,04
DAS 50	44,60	44,20 a	44,40	%CV	4,14	1,79	
Potasyum Dose (K)							
K ₀ (Control)	44,47	43,70 ab	44,08				
K ₁₀ (10 kg da ⁻¹)	43,88	43,45 b	43,67				
K ₂₀ (20 kg da ⁻¹)	44,27	43,67 ab	43,97				
K ₃₀ (30 kg da ⁻¹)	44,37	44,22 a	44,29				

Conclusion

This study has revealed the effects of different potassium fertilizer doses on cotton (*Gossypium hirsutum* L.). The findings indicate that potassium applications on the 30th, 40th, and 50th days after sowing statistically influenced yield parameters such as seed cotton yield, plant height, number of fruiting branches, and number of bolls. Moreover, fiber technological properties such as uniformity and short fiber content varied depending on potassium doses. The highest seed cotton yield and ginning outturn were achieved with 30 kg da⁻¹ potassium applied on the 50th day. However, some parameters, such as boll seed cotton weight and 100-seed weight, were found to be statistically unaffected by certain treatments, highlighting the complex effects of varying application timings and doses on plant growth and quality.

Based on the results, potassium applications of 20–30 kg da⁻¹ on the 30th and 50th days after sowing are recommended to improve yield and quality in cotton production. However, the findings also suggest that the effects of potassium applications can vary under different ecological conditions and among cotton varieties with genetic differences. Therefore, the development of specific fertilization programs based on soil and plant analyses for each region is essential. Such practices will not only support sustainable agriculture but also enhance the economic value of cotton production.

Declarations

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

Author Contribution: All authors contributed

equally to the manuscript. The final version of the manuscript was reviewed and approved by all authors.

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Development of *Meloidogyne chitwoodi* resistant potato lines

Meloidogyne chitwoodi'ye dayanıklı patates hatlarının geliştirilmesi

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ABSTRACT

Meloidogyne chitwoodi is an important root-knot nematode in some potato production regions of Türkiye. Crop rotation and some chemical and cultural control methods remain ineffective in controlling this nematode. Developing resistant varieties is an environmentally safe and most effective solution for *M. chitwoodi*. The current study was aimed at the development of *M. chitwoodi*-resistant potato lines and varieties adapted to different regions. Two breeding populations ('22-99: PP-89 X Maradonna', '22-108: PP-85 X Banba') and two offspring populations ('32-12: 22-99-33 X Provento'; '32-10: 22-108-24 X Saturna') were used as genetic material. *Meloidogyne chitwoodi* molecular marker and field pathogenicity tests were used for investigation of *M. chitwoodi*-resistant lines and varieties. The results of the molecular marker and field tests were compatible. A total of 112 potato breeding lines were evaluated in two populations and 81 were resistant to *M. chitwoodi*. Consequently, potato line of number 33 from the 22-99 population and potato line of number 24 from the 108-24 population, along with their superior agronomic and yield characteristics, were chosen as *M. chitwoodi*-resistant lines according to the marker and field test studies. Overall the results suggest that resistant varieties can be improved by large-scale molecular markers and field tests.

Key Words: Potato lines, Molecular marker, Root-knot nematode, *Solanum tuberosum* L., Nematode resistance

Öz

Meloidogyne chitwoodi Türkiye'nin bazı patates üretim bölgelerinde önemli bir kök ur nematodudur. Ekim nöbeti, bazı kimyasal ve kültürel uygulamalar bu nematodla mücadelede etkili olmamıştır. *Meloidogyne chitwoodi*'ye karşı çevre açısından güvenli ve etkili en iyi yöntem dayanıklı çeşitlerin geliştirilmesidir. Bu araştırmanın amacı, *M. chitwoodi*'ye karşı farklı bölgelere adapte olmuş dayanıklı patates hatları ve çeşitleri geliştirmek olmuştur. Denemede genetik materyal olarak iki ıslah popülasyonu (22-99: PP-89 X Maradonna, 22-108: PP-85 X Banba) ve ikinci döl popülasyonu ('32-12: 22-99-33 X Provento'; '32-10: 22-108-24 X Saturna') kullanılmıştır. Nematod dayanıklı hat ve çeşitlerin geliştirilmesinde *M. chitwoodi* moleküler markeri ve tarla patolojik testleri kullanılmıştır. Moleküler marker testi ve tarla testlerinin sonuçları uyumlu gözükmiştir. İki popülasyonda 112 patates ıslah hattı değerlendirilmiş olup bunların 81'i *M. chitwoodi* nematoduna dayanıklıdır. Araştırma sonucunda 22-99 popülasyonuna ait 33 numaralı patates hattı ve 108-24 popülasyonuna ait 24 numaralı hatlar, üstün agronomik ve verim özelliklerine sahip olmaları yanında, marker ve tarla deneme çalışmalarına göre *M. chitwoodi*'ye dayanıklı hat olarak seçilmiştir. Bu çalışmanın devamında, geniş çaplı moleküler marker test ve tarla denemeleri sonrasında dayanıklı çeşitler geliştirilebilecektir.

Anahtar Kelimeler: Patates hattı, Moleküler Marker, Kök-ur Nematod, *Solanum tuberosum* L., Nematod dayanıklılığı

Introduction

Meloidogyne chitwoodi is an important nematode in potatoes and some vegetables and field crops in western Europe, the Netherlands, the United States, Belgium and Germany (Nyczepir et al., 1982; Molendijk & Mulder, 1996; Karssen & Moens, 2006; Graebner et al., 2018; Bali et al., 2022). *Meloidogyne chitwoodi* was first reported from Türkiye during 2007 in Niğde and Nevşehir (Ozarslandan et al., 2007). *Meloidogyne* root-knot nematode species, including *M. chitwoodi*, were identified and determined using morphological and molecular analyses (Devran et al., 2009; Ozarslandan et al., 2009). *Meloidogyne chitwoodi* race 1 was detected only in Türkiye (Evlice & Bayram, 2019).

Meloidogyne chitwoodi is a major pest of potato crop in the countries having intensive potato production. It has been recorded from Türkiye recently and readily spread to 11 provinces in 4 regions of the country. These provinces constitute 48.6% of Türkiye's potato production areas and 56.8% of the total production indicating severe consequences of infestation (Evlice & Bayram, 2016). Infestation is rising in Türkiye since its first appearance, and producers continue to cultivate potato with intensive nematicide applications. Despite being one of the world's major potato-producing countries, Türkiye has no native commercial pest-resistant variety that can be used for commercial production. Considering the climate, soil and potato production regions in Türkiye, it is quite difficult to develop varieties resistant to race-specific pests in these regions (Avci et al., 2018).

Meloidogyne chitwoodi causes necrotic spotting under the tuber skin and vigorous galling formation on the tuber surface, which collectively reduce the quality of fresh and/or processed potato tubers (Castagnone-Sereno et al., 1999; Mitkowski & Abawi 2003; Devran et al., 2009; Graebner et al., 2018). Currently, growers/farmers have a limited number of management options against *M. chitwoodi*. There are many limitations of crop rotation to control

M. chitwoodi nematode. First, *M. chitwoodi* has a wide host range and low damage threshold. Even in the most effective rotation, unacceptable losses can occur in the long warm season in low populations at the residue level (Graebner et al., 2018; Bali et al., 2019; Bali et al., 2022; Hu et al., 2023).

Tolerance to *M. chitwoodi* may vary among potato varieties (Van Riel, 1993). Several studies have determined the resistance of *M. chitwoodi* in *Solanum* species, including *S. hougasii*, *S. bulbocastanum* and *S. fendleri* (Brown et al., 1989; 1991; Janssen et al., 1998; Brown et al., 2004). In addition, two resistance genes, i.e., RMc1 (blb) and RMctuber (blb) preventing root development and tuber infections, respectively have been identified (Brown et al., 1996; 2009). Candidate varieties resistant to *M. chitwoodi* race 1 have been improved in recent years (Brown et al., 1991; 2004; 2006; 2009; Norshie et al., 2011; Dinh et al., 2014). However, commercial production and distribution of candidate resistant potato varieties remain limited (Bali et al., 2019; Bali et al., 2022). The resistant gene RMc1 (blb) from *S. bulbocastanum* was transferred to the potato gene pool. This gene provides resistance against *M. chitwoodi* race 1, and enables the growth and development of potato roots. Thus, the tubers get rid of nematode damage. The RMc1 (blb) gene obtained from PA99N82–4 material has been genetically characterized and it provides resistance against *M. chitwoodi*. It was a single dominant gene and mapped on the 11th chromosome. The resistance mechanism is understood to a limited extent (Bali et al., 2019; Bali et al., 2022, Hu et al., 2023).

This study used *M. chitwoodi*-resistant and susceptible potato materials and populations. The study aimed to determine the resistance of the tested potato material to *M. chitwoodi* nematode using molecular marker under natural field infestation.

Material and Methods

Plant material

Two tetraploid *M. chitwoodi* resistant materials ('PP-85', 'PP-89'), two susceptible commercial varieties ('Granola' and 'Marabel'), other commercial varieties (Table 1 and 2), four population ('22-99: PP-89 X Maradonna', '22-108: PP-85 X Banba', '32-12: 22-99-33 X Provento'; '32-10: 22-108-24 X Saturna') were used as plant materials in the experiment.

Biological tests, field experiments and marker tests

22-99 (PP-89 X Maradonna) and 22-108 (PP-85 X Banba) Populations

Granola, Marabel, Maradonna and Banba commercial potatoes varieties were grown in the experiment field in 2015 in Niğde / Konaklı (38° 10' 33"N, 34° 50' 39"E, 1347 m above sea level). The field was determined as infected with *M. chitwoodi* and the varieties grown were susceptible (data not presented). This field was used for resistance testing experiment in 2017 only Konaklı location.

Resistant materials ('PP-89' and 'PP-85') were

crossed with 'Maradonna' and 'Banba' varieties during 2016 spring season. A total of 100 seeds from each population were sown and grown under greenhouse conditions during 2016 autumn season. A total of 60 genotypes in '22-99' population and 52 genotypes in '22-108' population were selected and used in the field experiment. The '22-99' and '22-108' populations were used in the *M. chitwoodi* breeding program. Tubers were planted on May 27, 2017, and harvested on October 25, 2017. Two resistant materials, two populations, and ten commercial varieties were used in the field experiment (Table 1). A total of 112 genotypes from two populations, commercial varieties, and resistant materials were planted in the *M. chitwoodi*-infected field. Breeding genotypes belonging to '22-99' and '22-108' populations were planted using one tuber for each genotype. Resistant materials and commercial varieties were planted in the field using 50 tubers. The tubers were planted without replication as 70 cm distance between rows and 30 cm among rows. Necessary irrigation, fertilization, and plant protection practices were carried out during the season.



Figure 1. *Meloidogyne chitwoodi* resistant potato lines (22-99 population lines: 2, 23, 48, 51 and 57; 22-108 population lines: 31, 36 and 49)

In genetic materials ('PP89', 'PP85', commercial varieties, '22-99' and '22-108' populations) samples were taken from the true

leaves of plants in the field and were screened with *M. chitwoodi* molecular marker test. The molecular marker developed by Zhang et al.

(2007) was used in the research. Potato genotypes, populations, commercial varieties were determined as resistant or susceptible according to the marker. Observations were

determined phenotypically for nematodes in the field at harvest time and compared with marker test results (Figure 1 and 2).



Figure 2. *Meloidogyne chitwoodi* susceptible potato lines (line 51 is belonging to 22-108 population)

32-10 (22-108-24 X Saturna) and 32-12 (22-99-33 X Provento) Populations

Commercial varieties used in potato breeding in the second phase of the research. Genetic materials, and *M. chitwoodi*-resistant materials determined by marker test in two offspring populations developed from 64 lines ('32-10', '32-12') were included in the second phase. The '32-10' and '32-12' breeding populations were planted in the non-nematode infected field as 400 single tubers in the spring season of 2018. A total of 64 lines selected according to their tuber characteristics, were grown in pots in the autumn season of 2018. One tuber in 64 lines, and two tubers of commercial varieties and other genetic materials were used for planting. Leaf samples were taken from plants and screened for molecular marker testing to determine whether they were resistant to *M. chitwoodi*.

Results and Discussion

The results obtained by planting tubers in the infected field are included in Table 1. Marker test results and the phenological observations were in

harmony with each other. Resistance was indicated as (+) and susceptible as (-) in the current study. Resistant potato genotypes provided resistance in field conditions and *M. chitwoodi* damage was not observed in tubers (Figure 1). All commercial varieties were found to be susceptible in both marker testing and field phenological observations. Out of 60 breeding lines, 43 were resistant (+, presence) and 17 were susceptible (-, absence) in '22-99' population. In '22-108' population, 38 lines were resistant and 14 were susceptible.

In the second phase of the study, commercial varieties were used in potato breeding studies, and advanced genotypes, '32-10', and '32-12' populations formed from resistant genotypes were screened with marker test. All commercial varieties were found susceptible. The first tuber generation plants (64 lines) of the 2 populations were screened by marker test. As a result of the screening, 40 lines in the '32-12' population and 37 lines in the '32-10' population were found resistant (+) (Table 2). The resistant/susceptible ratio was found to be approximately 1: 2 in both populations.

Table 1. *Meloidogyne chitwoodi* resistance of potato lines

22-99						22-108					
<i>R_{Mc1(blb)}</i>			Field			<i>R_{Mc1(blb)}</i>			Field		
1	+	+	31	+	+	1	+	+	33	+	+
2	-	-	32	+	+	2	+	+	34	+	+
3	+	+	33	+	+	3	+	+	35	-	-
4	+	+	34	+	+	4	+	+	36	+	+
5	+	+	35	+	+	5	+	+	37	+	+
6	+	+	36	+	+	6	+	+	38	+	+
7	+	+	37	+	+	7	+	+	39	-	-
8	+	+	38	+	+	8	+	+	40	+	+
9	+	+	39	-	-	9	+	+	41	-	-
10	+	+	40	+	+	10	+	+	42	-	-
11	-	-	41	+	+	11	+	+	43	+	+
12	-	-	42	-	-	12	-	-	44	+	+
13	-	-	43	-	-	13	+	+	45	+	+
14	-	-	44	+	+	14	+	+	46	+	+
15	+	+	45	-	-	15	+	+	47	+	+
16	+	+	46	-	-	16	+	+	48	+	+
17	+	+	47	+	+	17	+	+	49	+	+
18	+	+	48	+	+	18	+	+	50	-	-
19	+	+	49	+	+	19	+	+	51	-	-
20	-	-	50	-	-	20	-	-	52	-	-
21	-	-	51	+	+	21	+	+	Agata	-	-
22	+	+	52	-	-	22	-	-	Agria	-	-
23	+	+	53	-	-	23	-	-	Blondine	-	-
24	+	+	54	+	+	24	+	+	Granola	-	-
25	+	+	55	+	+	25	-	-	Madeline	-	-
26	+	+	56	+	+	26	-	-	Marabel	-	-
27	+	+	57	+	+	27	+	+	Melody	-	-
28	+	+	58	-	-	28	-	-	Natasha	-	-
29	+	+	59	-	-	29	+	+	Maradonna	-	-
30	+	+	60	+	+	30	+	+	Banba	-	-
						31	+	+	PP-89	+	+
						32	+	+	PP-85	+	+
Marker presence (+) : 43						Marker presence (+): 38					
Marker absence (-) : 17						Marker absence (-): 14					

R_{Mc1(blb)} : *Meloidogyne chitwoodi* molecular marker, PP-89 and PP-85: Resistant genotypes; 22-99: PP-89 X Maradonna, 22-108: PP-85 X Banba

Table 2. *Meloidogyne chitwoodi* resistance of commercial varieties and potato breeding lines

	$R_{Mc1(bl b)}$	32-12	$R_{Mc1(bl b)}$	32-12	$R_{Mc1(bl b)}$	32-10	$R_{Mc1(bl b)}$	32-10	$R_{Mc1(bl b)}$
-Cara	-	1	+	33	+	1	-	33	+
Hermes	-	2	+	34	-	2	+	34	+
Saturna	-	3	-	35	+	3	+	35	-
Safrane	-	4	+	36	+	4	-	36	+
Innovator	-	5	-	37	-	5	+	37	-
Markies	-	6	+	38	+	6	+	38	+
Alexia	-	7	-	39	-	7	+	39	+
Solist	-	8	+	40	+	8	-	40	+
Fontane	-	9	-	41	+	9	+	41	-
Actress	-	10	+	42	-	10	+	42	-
Musica	-	11	-	43	+	11	+	43	-
Doremi	-	12	+	44	-	12	-	44	+
Sante	-	13	+	45	+	13	-	45	-
Saline	-	14	+	46	+	14	+	46	+
Colomba	-	15	-	47	+	15	-	47	-
Van Gogh	-	16	-	48	+	16	-	48	-
Ivory Rus.	-	17	+	49	-	17	+	49	+
Orchestra	-	18	+	50	+	18	+	50	-
Nazca	-	19	+	51	-	19	-	51	+
Concordia	-	20	+	52	+	20	+	52	-
Victory	-	21	-	53	-	21	-	53	+
Triomphe	-	22	+	54	+	22	+	54	+
Touareg	-	23	+	55	-	23	-	55	+
Provento	-	24	-	56-	-	24	+	56	+
		25	+	57	+	25	-	57	+
		26	+	58	+	26	+	58	-
		27	-	59	+	27	-	59	+
		28	+	60	-	28	+	60	-
		29	-	61	+	29	-	61	+
		30	+	62	-	30	+	62	+
		31	-	63	+	31	-	63	-
		32	+	64	+	32	+	64	+
Marker presence (+): 40					Marker presence (+): 37				
Marker absence (-): 24					Marker absence (-): 27				

$R_{Mc1(bl b)}$: *Meloidogyne chitwoodi* molecular marker, 32-12: 22-99-33 X Provento; 32-10: 22-108-24 X Saturna

Root-knot nematode infestation is increasingly being reported from potato produced in Türkiye. Two strategies seem useful against *M. chitwoodi* nematode. The first strategy is using nematicides and the second is the use of resistant varieties. Today, chemical control is carried out against the nematode in the nematode-infected fields by spraying during the flowering period. This method is both costly and harms the environment and human health. Therefore, it is increasingly necessary to develop and utilize resistant varieties. For this purpose, breeding studies were initiated. Results of the preliminary studies indicated a large amount of nematode infection almost every part of the experiment field, due to the use of nematode-infected susceptible 'Granola' variety (unpublished data). Advanced

lines evaluated as nematode resistant were planted in infected field, and their resistance reactions were determined. In the study, the results of field resistance and marker test (presence/absence) were found to be compatible.

Norshie et al. (2011) concluded that the single resistance gene provides 97% partial resistance against *M. chitwoodi*. It has been demonstrated that at this tolerance level, yield losses decrease and tuber quality increases at high population density. *Meloidogyne chitwoodi* is distributed in Türkiye is almost all agriculture regions. Potato and tomato were the main host of *M. chitwoodi*, which is the most important agricultural product, to make damage in crops such as wheat and barley increased the importance of Türkiye even more (Evlice &

Bayram, 2016). In the potato, many studies have been conducted on obtaining varieties and variety candidates resistant to *M. chitwoodi* (Brown et al., 1989; Brown et al., 1991; Janssen et al., 1998, Brown et al., 1999; Brown et al., 2004). The results of these studies, resistance genes and markers were identified, hopeful field trial results were obtained (Brown et al., 2006, Zhang et al., 2007; Brown et al., 2009; Norshie et al., 2011; Dinh et al., 2014). However, there is no commercially produced resistant variety yet (Bali et al., 2022; Hu et al., 2023). Studies have shown that the injury caused by *M. chitwoodi* on potato tubers varies depending on the varieties (Evlice & Bayram, 2016). Van Riel (1993) reported that the symptoms on tubers varied between 3-21% at the end of the study comparing 20 different potato varieties. It has been stated by Suffert & Giltrap (2012) that tubers of some potato varieties have much less damage from *M. chitwoodi* than other varieties. For example, 'Agria' variety has been reported to be more tolerant than variety 'Hansa'. In the field trial conducted by Van Riel (1994) using 20 different varieties, symptoms on the tuber varied between 3.4-34.1% depending on the varieties, and the highest population density in the soil was 2778 and 4167 J2 / 100 cm³, respectively, in early and late varieties. The fact that 'Marabel' variety is early and susceptible to *M. chitwoodi* in the field experiment and marker test, refutes the concept that early varieties are resistant. The damage caused by *M. chitwoodi* on potato tuber skin varies depending on the varieties used (Evlice & Bayram, 2016; Van Riel 1993, 1994). While resistant lines were not shown any nematode symptoms, susceptible lines obtained lots of *M. chitwoodi* symptoms on the skin of tubers.

Crop rotation with the use of non-host plants is one of the most important methods used in controlling root-knot nematodes globally (Sikora et al., 2005; Bali et al., 2022). However, due to the wide host distribution, correct crop rotation is extremely difficult to implement. Commonly cultivated crops in Türkiye like tomato, potato, wheat, oats, barley, corn, sugar beets, sunflowers,

some of the ornamental plants and fruit trees are host for *M. chitwoodi* and there are no available resistant varieties of these plants. The crop rotation, which will be made using tolerant/resistant varieties, will be very important in the future, but current information on the subject is extremely limited. There are records of less damage and even unharmed products from early potato varieties (Van Riel, 1993). Bali et al. (2022) stated that the STS marker used by Zhang et al. (2007) for *M. chitwoodi* resistance gave false fingerprints across populations or in initial populations. They suggested that this situation may be due to these markers not being tightly linked with the *RMC1(blb)* locus and may lead to losses in recombination in advanced selections. In this study, the marker results were found to be compatible with the results in the infected field in initial and advanced selections.

Consequently, developing resistant varieties, crop rotation is necessary to avoid the spread of *M. chitwoodi*.

Conclusion

The root-knot nematode damage and infection of potato planted in Türkiye is increasing every year. Studies on the root-knot nematode continue in the form of chemically control and bean-wheat crop rotation. Potato planting will be eliminated in some regions due to nematodes in the coming years. In the current study, resistance was determined in some potato genotypes by using the marker developed by Zhang et al. (2007). Single resistance gene to *M. chitwoodi* resulted in high resistance, improved tuber quality and tolerance for yield reduction.

It has emerged that the resistant materials obtained, and the population created from them must be tested in the infected field and the resistance must be confirmed. The resistance determined by the marker test has been carried out in line with this purpose and has provided resistance in the field. *M. chitwoodi*-resistant commercial variety or candidates will be developed by choosing advanced lines among

durable materials. At the end of the research, the potato line of number 33 from the 22-99 population, along with its good agronomic, plant, and tuber characteristics, was determined as *M. chitwoodi*-resistant line according to molecular marker and field phenotypic test results. The '22-99-33' potato line was found successful in registration trials. However, it could not be registered because it was very susceptible to PVY (Potato Virus Y). It was used as a parent in the breeding of PVY and *M. chitwoodi* resistant varieties. The variety candidates developed from this line was applied for registration in 2025.

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Compliance with ethical standards and conflict of interest

The authors state in the article that no unethical studies were conducted on humans or animal.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's Contributions

The author declares that he has contributed to the article.

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Modeling quality changes in heat-processed orange juice: a comparative study of artificial neural network and multiple linear regression approaches

Isıl işlem uygulanmış portakal suyunda kalite değişimlerinin modellenmesi: yapay sinir ağı ve çoklu doğrusal regresyon yaklaşımlarının karşılaştırmalı bir çalışması

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ABSTRACT

The purpose of the study was to assess the prediction ability of multiple linear regression (MLR) and artificial neural network (ANN) models for the browning index, total carotenoid content, 5-hydroxymethylfurfural (HMF) and ascorbic acid of orange juice during storage after heat processing. For ANN models, the effect of neuron number of the hidden layer, epoch number, training algorithms and transfer functions are investigated using the trial-error method for selecting best design ANN models. The different methods (stepwise and enter) in multiple linear regression models were performed for detecting impact of independent variables on dependent variables. The performance of ANN and MLR models was determined through unseen data by means of statistical analysis. Regarding performance indices of ANN models for test data, overall R and R² were recorded as follows: 0.92 and 0.84 (browning index), 0.99 and 0.98 (HMF), 0.92 and 0.86 (ascorbic acid), 0.97 and 0.94 (total carotenoid content), respectively. R and R² values of MLR models for test data were 0.79 and 0.68 (browning index), 0.94 and 0.88 (HMF), 0.92 and 0.85 (ascorbic acid), 0.93 and 0.90 (total carotenoid content), respectively. Both models provided accurate predictions. However, the superior predictive power of ANN models is that they can learn directly from examples without calculating the parameters using statistical techniques. The results revealed that ANN models showed greater potential with high R and R² value, and the lowest error values when compared with the MLR model, but both ANN and MLR had almost same performance for prediction of ascorbic acid.

Key Words: Artificial neural network, Multiple linear regression, Orange juice, Color, modelling

Öz

Çalışmanın amacı, ısıtılma işleminden sonra depolama sırasında portakal suyunun esmerleşme indeksi, 5-hidroksimetilfurfural (HMF), askorbik asit ve toplam karotenoid içeriği için çoklu doğrusal regresyon (MLR) ve yapay sinir ağı (ANN) modellerinin tahmin yeteneğini değerlendirmektir. En iyi tasarım YSA modellerini seçmek için gizli katmandaki nöron sayısının, epok sayısının, eğitim algoritmalarının ve transfer fonksiyonlarının etkisi deneme-yanılma yöntemi kullanılarak araştırılmıştır. Tüm bağımsız değişkenlerin bağımlı değişkenler üzerindeki etkisini tespit etmek için çoklu doğrusal regresyon modellerindeki farklı yöntemler (stepwise ve enter) uygulanmıştır. ANN ve MLR modellerinin performansı istatistiksel analiz yoluyla test verileri kullanılarak değerlendirilmiştir. Test verileri için ANN modellerinin performans endekslerine ilişkin olarak, genel R ve R² sırasıyla; 0,92 ve 0,84 (esmerleşme indeksi), 0,99 ve 0,98 (HMF), 0,92 ve 0,86 (askorbik asit), 0,97 ve 0,94 (toplam karotenoid içeriği) belirlenmiştir. Test verileri için MLR modellerinin R ve R² değerleri sırasıyla 0,79 ve 0,68 (esmerleşme indeksi), 0,94 ve 0,88 (HMF), 0,92 ve 0,85 (askorbik asit), 0,93 ve 0,90 (toplam karotenoid içeriği) olarak tespit edilmiştir. YSA modellerinin üstün tahmin gücü, istatistiksel teknikler kullanarak parametreleri hesaplamaya gerek kalmadan doğrudan örneklerden öğrenebilmeleridir. Sonuçlar, ANN

modelinin yüksek R ve R² değeri, ve düşük hata değerleri ile MLR modellerine kıyasla daha büyük potansiyel göstermiştir. Askorbik asit için hem ANN hem de MLR'nin tahminleme performansı neredeyse aynı düzeyde olduğu belirlenmiştir.

Anahtar Kelimeler: Yapay sinir ağı, Çoklu doğrusal regresyon, Portakal suyu, Renk, Modelleme

Introduction

Orange juice is one of common juice manufactured by beverage industry (Tiwari et al., 2009; Ağçam et al., 2016). Orange juice includes valuable bioactive compounds such as carotenoids, phenolic compounds and ascorbic acid etc. (Lee and Coates, 2003; Zulueta et al., 2013; Ağçam et al., 2014). The color of orange juice is an essential factor of quality for determining juice quality and its acceptance. Its color is mainly due to carotenoid compounds such as α , β and ζ -carotene, lutein, antheraxanthin, zeinoxanthin, zeaxanthin, luteoxanthin and other carotenoid compounds (Meléndez-Martínez et al., 2007). It is also well known that orange juice is quite sensitive to different processing conditions (Shinoda et al., 2004; Tiwari et al., 2009; Ağçam et al., 2016). During heat treatment and storage, orange juice involves a number of deteriorating processes, including browning, ascorbic acid decomposition, clouding, microbiological spoiling, off-flavor, and viscosity (Zheng et al., 2011). It is stated that color change is a marker of chemical and biochemical reactions (Wibowo et al., 2015a). These changes may occur particularly as a result of the formation of non-browning or degradation of carotenoids during processing and storage. The aerobic as well as anaerobic degradation for ascorbic acid and Maillard reactions are two possible processes of non-enzymatic browning (Fustier et al., 2011; Ağçam et al., 2016). Furfural, 5-hydroxymethylfurfural (HMF), ascorbic acid levels, and furfural are some of the indicators of non-enzymatic browning (Shinoda et al., 2004; Burdurlu et al., 2006; Wibowo et al., 2015; Wibowo et al., 2015a). Heat processing is frequently employed method for long shelf life of orange juice because it inactivates spoilage bacteria and pectin methylesterase (PME), one of the endogenous enzymes that is resistant to heat

(Elez-Martinez et al., 2006; Timmermans et al., 2011; Vervoort et al., 2011). Despite these improvements, the studies has been stated that heat processing often causes undesirable changes such as nutrients, flavor and color by affecting the overall juice quality (Yeom et al., 2002; Cortés et al., 2006; Rivas et al., 2006; Vervoort et al., 2011; Ağçam et al., 2014). Therefore, prediction of critical parameters of foods during heat processing and storage conditions by models is very important stages (Agha and Alnahhal, 2012). The artificial neural network (ANN) model is one of the often-employed strategies. The function of ANN is to simulate the neuro-physiological structure of the human brain (Huang et al., 2007; Yasar et al., 2012; Khashei et al., 2012; Torkashvand et al., 2017; Deng et al., 2023). ANN model is commonly accepted as modeling technique to express linear or non-linear relationships of complicated input and output parameters in processes (Tehlah et al., 2016; Bhagya Raj and Dash, 2022). There are several neural network models. The most used of this model is feed-forward multi-layer perceptron (MLP) (Chegini et al., 2008; Tiryaki and Aydın, 2014; Huang et al., 2024). Three layers typically make up this network model: input, output and hidden layers. Numerous interconnected neurons, known as processing elements, are present in each layer (Zheng et al., 2011; Erzin and Cetin, 2013; Tiryaki and Aydın, 2014; Nejatdarabi and Mohebbi, 2023). The construction of these structure determines the success of the ANN (Asilturk and Cunkas, 2011). The statistical method of multiple linear regression (MLR) analysis is used to identify the correlations between variables. This approach has been characterized as a standard prediction technique. The MLR is less complex and allows the simulation of situation by changing the values of independent variables (Kolasa-Wiecek, 2015). MLR models consist of several methods which are backward elimination, enter, forward selection

and stepwise regression. To achieve the high coefficient of correlation and lower the mean squared error in linear regression, the stepwise multiple regression model has been utilized to identify the most effective independent variables. To maintain the best possible model, this strategy adds all independent variables and then progressively eliminates them as needed for each phase (Ghani and Ahmad, 2010; Shacham and Brauner, 2014; Kolasa-Wiecek, 2015). In the enter method, all independent variables are simultaneously entered into the equation regardless of whether they are statistically significant, and so the effect of all independent parameters upon dependent variables are assessed (Shacham and Brauner, 2014). Comparative studies of ANN and MLR have shown satisfactory results in several studies, including kiwifruit firmness (Torkashvand et al., 2017), the quality changes during storage of gouda cheese (Stangierski et al., 2019), rehydration process of mushroom powder (Nejatdarabi and Mohebbi 2023), drying of pineapple cubes (Meerasri and Sothornvit 2022). ANN models performed better in systems with nonlinear and complicated interactions. However, MLR had better potential than ANN. Additionally, these models may allow later prediction in similar situations without experimenting.

In the literature, a study about prediction of critical quality parameters of orange juice during storage after heat processing is not found. Therefore, the purpose of the presented study was to identify models based on the ANN and MLR model to predict the loss of ascorbic acid and total carotenoid content (TCC), formation of browning index (BI) and HMF of orange juice during storage after heat processing. We also compared the prediction performance of these models for each output or dependent variables using the common statistical parameters.

Material and Methods

Orange juice

Oranges of the Kozan variety were cleaned,

peeled, sliced in half, and then squeezed using an orange press (CANCAN, Turkey). To get rid of the seeds and tough pulps, orange juice was filtered into 1-mm stainless steel sieves. Using heat processing equipment, juice was processed quickly according to Ağçam et al. (2016). Three replications of the investigation were conducted.

Heat pasteurization treatment

Heat pasteurization system for using applications was designed by Ağçam et al. (2014). Heat processing at between 70-90°C for 15-120 s was applied. Following the procedures, the samples were placed in sterile and amber bottles. They stored in darkness at 4 and 25 °C for 180 days before being analyzed. The samples were examined at certain days (0, 60, 120 and 180) (Akyildiz et al., 2021).

Determination of browning Index, HMF, ascorbic acid and total carotenoid content

5 mL of orange juice and 5 mL of 95% ethyl alcohol were mixed with a high-speed vortex in teflon tubes to determine the browning index. The mixture was centrifuged (4000 rpm, 10 minutes, 4°C). The absorbance at 420 nm was measured and supernatant was filtered with a 0.45 µm teflon membrane filter (Tiwari et al., 2008).

HPLC method was utilized to determine the ascorbic acid concentration using extraction process suggested by Lee and Coates (2003). A calibration curve was obtained by comparing retention time with an external standard ($R^2=0.997$).

HMF analysis was performed using an HPLC (Shimadzu, Japan) system. HMF was identified at 285 nm by examining the UV spectra and retention time with the standard (Ağçam and Akyildiz, 2014; Akyildiz et al., 2021).

The total carotenoid content (TCC) was calculated with a few adjustments based on Lee and Castle (2001). TCC was determined using Equation (1) based on β -carotene:

$$TCC \left(\frac{mg}{100 mL} \right) = \frac{A \cdot DF}{E^{1/2}} \times 1000 \quad (1)$$

where A = Absorbance; DF = Dilution factor; $E^{1/2} = 2505$ (β -carotene extinction coefficient in hexane).

Artificial neural networks analysis

In present paper, the multilayer feed-forward networks structure based on backpropagation algorithm was used to find the best ANN network structure. The multi-layer perceptron (MLP) network is one of the most often used feedforward neural networks. The

primary benefit of MLP over other neural networks is its simplicity in construction and estimation of any input and output (Nejatdarabi and Mohebbi, 2023; Gonçalves Neto et al., 2021). This network architecture is consist of combination of various model parameters which have a significant effect on learning ability (Tripathy and Kumar, 2009; Chegini et al., 2008; Aghbashlo et al., 2012; Gonçalves Neto et al., 2021). The structure of neural networks used in this study was presented in Fig. 1.

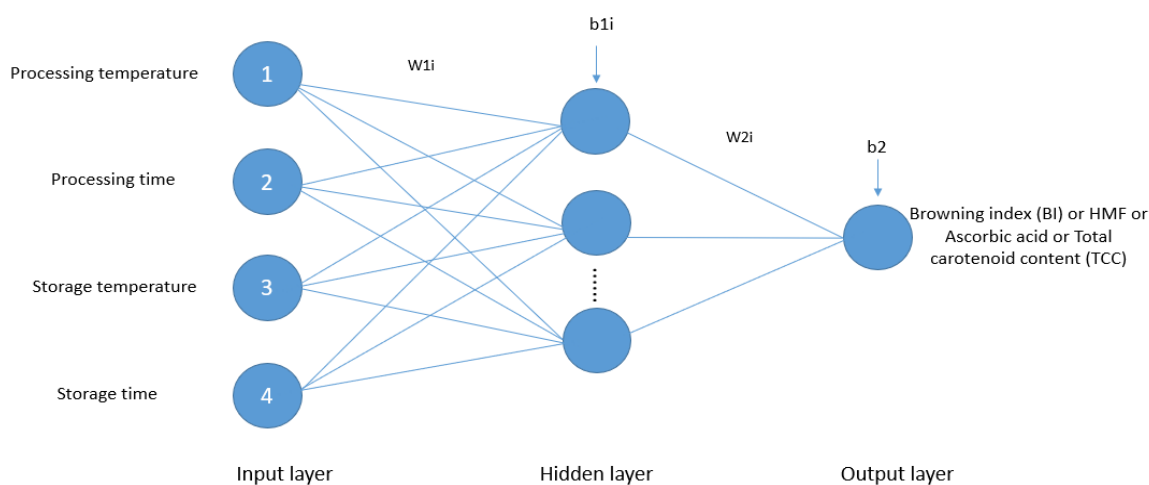


Figure 1. The structure of neural networks

Training of a network is an important point for high or good prediction in neural networks (Zou et al., 2007). At the training process, the weights and biases are initialized (Nikbakht et al., 2014). Training process was used to calculate the gradient and update the weights and biases of network. The training process was run until the validation process can avoid overfitting in the model. It was finished when the validation process resulted in a minimum of the performance goal (Mean Squares Error (MSE)) (Erbil et al., 2018). One of the problems during training is overfitting or memorization. The error of training is a very small value for the network that performed well on the training data, but not well on new data, and so the error of network is large. In other words, the network has memorized the training data, but it has not ability of generalization to unseen data (Sablani and Rahman, 2003; Matworks, 2018). Early stopping

or regularization are used to avoid this problem (Matworks, 2018). In this study, early stopping was used for preventing overfitting. The aim of validation process was to verify the efficiency of a network model, and to determine how it perform to a test data known as unseen (outside) data (Bocco et al., 2010). The epoch (iteration) is one training step when the ANN model demonstrates all training data. The error of validation was showed during the training phase. After validation, the models are tested with unseen data about the prediction accuracy of trained model (Sablani and Rahman, 2003; Zheng et al., 2011; Maghrebi et al., 2014). In other words, the basic principle of backpropagation is the use of gradient descent to find the point or points with the lowest degree of error on an error surface. Every iteration consists of two sweeps: forward activation to generate a solution and backward propagation of the calculated error to adjust the

weights of the neurons (Nejatdarabi and Mohebbi, 2023).

The inputs selected in the present study are process temperature, process time, storage temperature and storage time while outputs are HMF, BI, TCC and ascorbic acid. Data or variables are divided randomly three parts as training, validation and test. As mentioned above, 85% of all data (70%: training and 15%:validation) was used in multiple linear regression analysis. The last 15% of data was used to compare and test models (Erbil et al., 2018). MSE which is defined in Eq. 4 was used to determine performance of each network.

$$MSE = \frac{1}{n} \sum_{i=1}^n (xi - yi)^2 \quad (4)$$

where n is overall number of data; yi is the predicted value and xi is the actual value.

To obtain accurate and suitable models, the impact of various back propagation learning algorithms (*trainlm*, *trainscg*, *trainbr* and *trainrp* etc.) and transfer functions (*logsig*, *tansig* and *purelin*) were performed for all models (Tripathy and Kumar, 2009). The networks were trained with *trainlm* which accomplished minimization of error (Chia et al., 2012; Xu et al., 2014; Li et al., 2016; Yang et al., 2023; İnan-Çinkır et al., 2024). It provided the best results for all output because the second-degree derivatives of the function are used by the *trainlm* algorithm. Therefore, network output can get closer desired output (Nikbakht et al., 2014). The learning function was used *leargdm*. The transfer function was *logsig* for hidden layer layers, while *purelin* was suited best for the output layer. The learning rate is very important in achieving the minimum of error. The selection of too small or large learning rate may cause slow processing or obtain poor prediction results. The values of learning rate varied from 0.05 to 0.75 (Rai et al., 2005; Yang et al., 2023). The learning rate was selected 0.3 in our study. The other parameters as performance goal and coefficient of momentum were 0.001 and 0.6 for training respectively. The stopping criteria for *trainlm* includes a minimum gradient value of 10^{-7}

and a maximum total number of iterations (1000). *Trainlm* use μ as a stopping criterion (maximum value of 10^{10}) when it exceeds the MATLAB standard (Gonçalves Neto et al., 2021).

In neural networks, it is best practice to pre-process (normalization) input and output data before use to prevent impeding the learning process. Normalization is required when there are big differences in the ranges of all variables. It is helpful to facilitate the training of the network, provides memory efficient, improves predictive capabilities of the network, and so yields accurate predictive results (Basheer and Hajmeer, 2000; Bataineh and Marler, 2017). In this study, a total of 200 data were normalized or scaling between -1 and +1 before entering the network so as to avoid the overfitting in training process (İnan-Çinkır et al., 2024). The normalized variable x_N is given by Eq. 5:

$$x_N = \frac{(y_{max}-y_{min})*(x_R-x_{min})}{(x_{max}-x_{min})} + y_{min} \quad (5)$$

where; x_N is normalized data; x_R is actual data, x_{max} , x_{min} are minimum and maximum data, and y_{min} , y_{max} are normalizing limits (respectively (-1, +1)).

Multiple linear regression analysis

Multiple linear regression analysis is common method for investigating the best suitable connection between variables (Agha and Alnahhal, 2012; Tiryaki and Aydin, 2014; Erbil et al., 2018; Yu et al., 2018). This study used dependent variables (browning index, HMF, ascorbic acid and total carotenoid content) and independent variables (process temperature (T_p), process time (t_p), storage temperature (T_s) and storage time (t_s)). We were carried out based on stepwise, enter, forward and backward method in the multiple regression model to forecast the dependent variables (Torkashvand et al., 2017). Then, the optimum models, including all independent parameters were chosen with high R and R^2 . The analysis was carried out using SPSS version 20 statistical software (IBM Corporation). Multiple regression model can be designed (Eq.2) as;

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_K X_K + \varepsilon \quad (2)$$

where, $X_1, X_2, \dots, X_k$ are independent variables; Y is the dependent variable; $\beta_1, \beta_2, \dots, \beta_k$ are model coefficients; ε is a random element that influences the dependent variable.

The multicollinearity between the independent variables can lead to misleading results in selecting the most important predictors of regression equations. Therefore, a tolerance value (T) and variance inflation factor (VIF) values were used to determine multicollinearity between independent variables (Eq. 3). There is a multicollinearity issue when the tolerance value is not higher than 0.10 and the VIF is > 10 (Lin, 2008). The Durbin-Watson (DW) test which used to detect the presence of serial correlations (autocorrelation) between errors. The test statistics are changed from 0 to 4, DW values less than 1 or greater than 3 may generally show a problem (Mekanik et al., 2013; Niazi et al., 2018).

$$\begin{aligned} Tolerans (T) &= 1 - R^2 \\ VIF &= 1/t \end{aligned} \quad (3)$$

where R^2 is the coefficient of determination,

The data used in regression analysis was 85% (170) of all data. This data was used in training (140: 70% of all data) and validation (30: 15% of all data) of neural nets. The remaining 15% (30) of the data were employed to test and compare the models.

Comparison process

The performances capabilities of all models are compared with statistical methods. To determine prediction accuracy of proposed models, mean square error (MSE), root mean square error ($RMSE$), correlation coefficient (R), correlation of determination (R^2), mean absolute error (MAE), mean absolute percentage error ($MAPE$) and mean percentage error ($MPE\%$) were used (Eq. 6-11) (Erbil et al., 2018; İnan-Çinkır et al., 2024). The best trained network models should have the lowest error like MSE , $RMSE$, $MAPE$ etc. and high

R and R^2 . The statistical parameters are defined as:

$$R = \frac{n(\sum x_i y_i) - (\sum x_i)(\sum y_i)}{\sqrt{n \sum x_i^2 - (\sum x_i)^2 (n \sum y_i^2 - (\sum y_i)^2)}} \quad (6)$$

$$R^2 = 1 - \sum_{i=1}^n \frac{(x_i - y_i)^2}{(x_i - \bar{x})^2} \quad (7)$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - y_i)^2} \quad (8)$$

$$MPE = \frac{1}{n} \sum_{i=1}^n \frac{x_i - y_i}{x_i} \times 100 \quad (9)$$

$$MAPE = \frac{1}{n} \sum_{i=1}^n \left| \frac{x_i - y_i}{x_i} \right| \times 100 \quad (10)$$

$$MAE = \frac{1}{n} \sum_{i=1}^n |x_i - y_i| \quad (11)$$

where n is the total data number; y_i is predicted value for all models while x_i is the actual value.

Results and Discussion

According to our results, increasing processing temperature and processing/storage time lead to decrease concentration of ascorbic acid (AA) and carotenoid pigments, and accelerating the formation of HMF and BI (Table 1A). For 0 days, heat processing at 70°C for 15-120 s (HP1) had little deterioration or best quality compared to other heat treatments. Bull et al. (2004) reported the browning index of pasteurized orange juice as 0.096 abs. Ağçam et al. (2016) determined that BI was 0.223-0.355 in pasteurized orange juice for 60-180 days. Similar results were obtained in this study. Pham et al. (2019) found that HMF was not detected in pasteurized orange juice at the start of storage, its concentration increased noticeably with storage time. HMF was 25.91 mg L⁻¹ at 42°C for 15 weeks.

The quality deterioration of orange juice during storage conditions was determined based on AA concentration (Elez-Martinez et al., 2006). For 0 days, HP1 had little deterioration or best quality compared to other heat treatments. HP1 was also suitable for little quality deterioration during the 180 days at 4 and 25°C storage. The lowest ascorbic acid contents belonged to heat processing at 85°C for 120 s during the 180 days at 25°C storage. AA decreased from 666.91±6.95 to 78.69±5.55 mg L⁻¹ on 180th day (Table 1B). The European Fruit Juice Association (AIJN) stated

that AA as quality criterion has been identified 400-500 mg L⁻¹ in fresh orange juice. The amount was defined as 200 mg L⁻¹ until the last consumption (Elez-Martinez et al., 2006). Polydera et al. (2003) stated that shelf life of orange juice ends when 50% of ascorbic acid is lost. They determined that processing, temperature of storage, and packaging factors all had a significant impact on the degradation of ascorbic acid. Wibowo et al. (2015b) showed that

the amounts of AA and dehydroascorbic acid (DHA) decrease with temperature and storage duration. They observed that over 75% of AA degraded in 32 weeks at 20°C. Gomez Ruiz et al. (2018) determined that degradation of AA caused several undesirable compounds during storage. pH, oxygen and metal presence, AA concentration, thermal processing and storage temperature, light exposure, and citric acid are the most crucial elements for degradation of AA.

Table 1A. The value ranges of browning index, HMF during storage conditions (4°C and 25°C between 0 and 180 days) after heat processing (70-90°C and 15-120 s).

Symbol [†]	BI (abs)				HMF (µg L ⁻¹)			
	4°C		25°C		4°C		25°C	
	0 day	180 day	0 day	180 days	0 day	180 day	0 day	180 day
A	0.179-0.187	0.227-0.261	0.179-0.187	0.288-0.433	5.07-12.80	46.13-181.60	5.07-12.80	1760.80-2500.93
B	0.204-0.228	0.238-0.279	0.203-0.228	0.376-0.493	5.87-14.20	78.93-108.13	5.87-14.20	2217.63-2744.67
C	0.191-0.208	0.257-0.296	0.191-0.208	0.276-0.434	5.64-17.60	82.93-142.13	5.64-17.60	1227.60-2340.80
D	0.181-0.192	0.259-0.288	0.181-0.192	0.281-0.461	6.36-22.13	105.73-127.07	6.36-22.13	977.87-2304.67
E	0.180-0.189	0.239-0.286	0.180-0.189	0.290-0.382	7.33-31.74	88.80-146.27	7.33-31.74	1904.67-2652.93

[†] A: heat processing at 70°C for 15-120 s, B: heat processing at 75°C for 15-120 s, C: heat processing at 80°C for 15-120 s, D: heat processing at 85°C for 15-120 s E: heat processing at 90°C for 15-120 s, BI: browning index, ± values are standard deviation.

Table 1B. The value ranges of browning index and HMF during storage conditions (4°C and 25°C between 0 and 180 days) after heat processing (70-90°C and 15-120 s).

Symbol [†]	AA (mg L ⁻¹)				TCC (mg L ⁻¹)			
	4°C		25°C		4°C		25°C	
	0 day	180 day	0 day	180 day	0 day	180 day	0 day	180 day
A	635.09-678.59	361.61-427.63	635.09-678.59	257.89-310.12	7.39-8.03	2.72-3.63	7.39-8.03	3.44-4.62
B	577.31-607.27	361.97-427.63	571.33-607.27	195.62-311.81	6.80-6.97	3.51-4.18	6.76-7.43	3.41-4.82
C	585.48-628.81	263.03-370.09	585.48-628.81	159.58-308.69	6.11-6.75	3.00-3.85	6.27-6.67	3.03-3.70
D	608.16-666.91	288.36-385.51	608.16-666.91	78.69-296.25	6.33-6.70	3.66-4.46	6.33-6.71	2.92-3.48
E	611.51-685.07	289.52-401.76	611.51-685.07	134.06-330.15	6.92-7.64	3.62-4.32	7.04-7.34	2.16-2.88

[†] A: heat processing at 70°C for 15-120 s, B: heat processing at 75°C for 15-120 s, C: heat processing at 80°C for 15-120 s, D: heat processing at 85°C for 15-120 s E: heat processing at 90°C for 15-120 s, TCC: total carotenoid content, ± values are standard deviation.

The orange juice quality during heat processing and storage was imposed by two important changes which are off-flavor and browning. During the storage, BI indicates the color changes of orange juice depending on non-enzymatic browning (Tiwari et al., 2008). More than one of the mechanisms are related to formation of brown pigments. These mechanisms may be Maillard reactions and degradation of the ascorbic acid (Fustier et al., 2011). The brown

pigments also consist of the oxidation of phenolic compounds. AA acts as antioxidant and represses browning reaction but it may act formation of furfural and HMF because of easily oxidized and decomposed at high temperatures (Shinoda et al., 2004; Ağçam et al., 2016; Akyıldız et al., 2021).

TCC values were 2.16-8.03 mg L⁻¹ for 60-180 days. Gama and Sylos (2007) determined the changes in TCC in orange juice due to thermal pasteurization. They reported that TCC in fresh

orange juice, which was 12 mg/L, decreased by approximately 13% to 10.40 mg/L with pasteurization. Cortes et al. (2006) reported that TCC values decreased by %12.6 with pasteurization.

There are many studies about critical parameters of orange juice such as HMF and ascorbic acid treated by heat pasteurization and PEF (pulsed electric fields) Ağçam et al. (2016), the effect of (PEF) and thermal pasteurization on phenolic compounds of orange juice Ağçam et al. (2014), total phenolic content, total carotenoid content, antioxidant capacity, ascorbic acid and turbidity index (Zulueta et al. 2013), color and ascorbic acid in pasteurized blood orange juice (Remini et al., 2015). Researchers have stated that there is a progressive loss of nutritional content and a rise in undesirable elements because of heat processing and storage.

ANN models

Trial and error strategy were used to optimize the ANN models (Saraceno et al., 2012; Ding et al., 2017). The optimum number of neurons in the hidden layer, hidden layer number and epoch number were determined owing to this method. Because trial and error strategy demonstrate that two or more hidden layers and epoch number more than 1000 decrease the prediction efficiency of the non-linear function. Therefore, epoch number fixed at 1000 and one hidden layer was selected for all network models in the our study (Zheng et al., 2011; Torkashvand et al., 2017; Li et al., 2016). The neuron number in the hidden layer ranged from 2 to 20. Our results showed that the network construction included one hidden layer with 4, 3, 8, 4 neurons had the best structure for prediction of BI, HMF, AA and TCC, respectively. All networks had one neuron in the output layer. Selecting optimal neurons number in the hidden layer is very important for the development of the best ANN models. Using a lot of neurons in the network model may prolong the training time and may lead to overfitting. In the same way, using fewer neurons for hidden layer become trapped in a local minimum and so

it decrease the performance of model (Erzin et al., 2010).

The network with *logsig* transfer function and *trainlm* algorithm performed the best in terms of obtained minimum errors for each of models. The validation performance plot of the network is given in Fig. 2. All performance goals for validation processes were reached at 74, 52, 15 and 29 epochs for BI, HMF, ascorbic acid and TCC, respectively. According to performance plots, *MSE* converged minimum, and the learning ability for network increased as the number of epochs increased. However, increasing the epoch number may increase the problem of overfitting. To avoid overfitting, epoch numbers should be limited (Youssefi et al., 2009). This figure (Fig. 2) determined that the error of test and validation have same behavior and any significant over fitting did not occur.

Based on Fig. 3, the R values between actual and predicted BI values were higher than 0.90 for training, validation, test and all of data, respectively. For AA, R value of training, validation, test and all data were 0.91, 0.90, 0.92 and 0.91. Predicted values for HMF and TCC were positively correlated to the actual values. The correlation coefficients of training, validation, test and all data of HMF are 0.99, 0.97, 0.99 and 0.99, respectively. The correlation coefficients were 0.95, 0.95, 0.97 and 0.95 for TCC. These results stated that the ANN models were satisfactory, because the output data were as close as possible to the desired output data. For this reason, data points became intense around the line (Fig. 3).

ANN models for predicting and obtaining optimum model were applied by Fazaeli et al. (2013), Torkashvand et al. (2017), Yadav and Chandela (2017), Nikbakht et al. (2014), Anastacio et al. (2016), Deng et al. (2023), Nejatdarabi and Mohebbi (2023). As a result, it is stated that ANN is suitable for obtaining optimum models and calculating satisfactory results.

ANN Formulation

For formulation of ANN models, the following mathematical equations (Eq. 12-17) was used the

weights (w) and biases (b) values for selected the best ANN models are given in Table 2.

$$E_i = w_{1i} * T_p + w_{2i} * t_p + w_{3i} * T_s + w_{4i} * t_s + b_{1i} \quad (12)$$

where, E_i values are calculated using Eq. 11 includes processing temperature (T_p), processing time (t_p), storage temperature (T_s), storage time (t_s).

$$F_i = \frac{1}{1 + e^{-E_i}} \quad (13)$$

where, F_i values are determined using the *logsig* transfer function given in Eq.12.

The network output data for BI, HMF, AA and TCC were given Eq. 14,15,16 and 17, respectively.

$$BI = 9,698955 * F_1 - 0,12679 * F_2 + 0,472012 * F_3 + 3,866417 * F_4 - 4,74462 \quad (14)$$

$$\log(HMF) = -3,551471 * F_1 + 3,380717 * F_2 + 0,537706 * F_3 - 0,47159 \quad (15)$$

$$\log(AA) = -0,34178 * F_1 - 0,13789 * F_2 + 0,155299 * F_3 - 0,10878 * F_4 + 0,60083 * F_5 + 0,731891 * F_6 + 0,144765 * F_7 + 0,694978 * F_8 - 0,20744 \quad (16)$$

$$(TCC) = 2,117841 * F_1 - 3,84915 * F_2 - 1,48123 * F_3 - 1,81265 * F_4 + 2,639356 \quad (17)$$

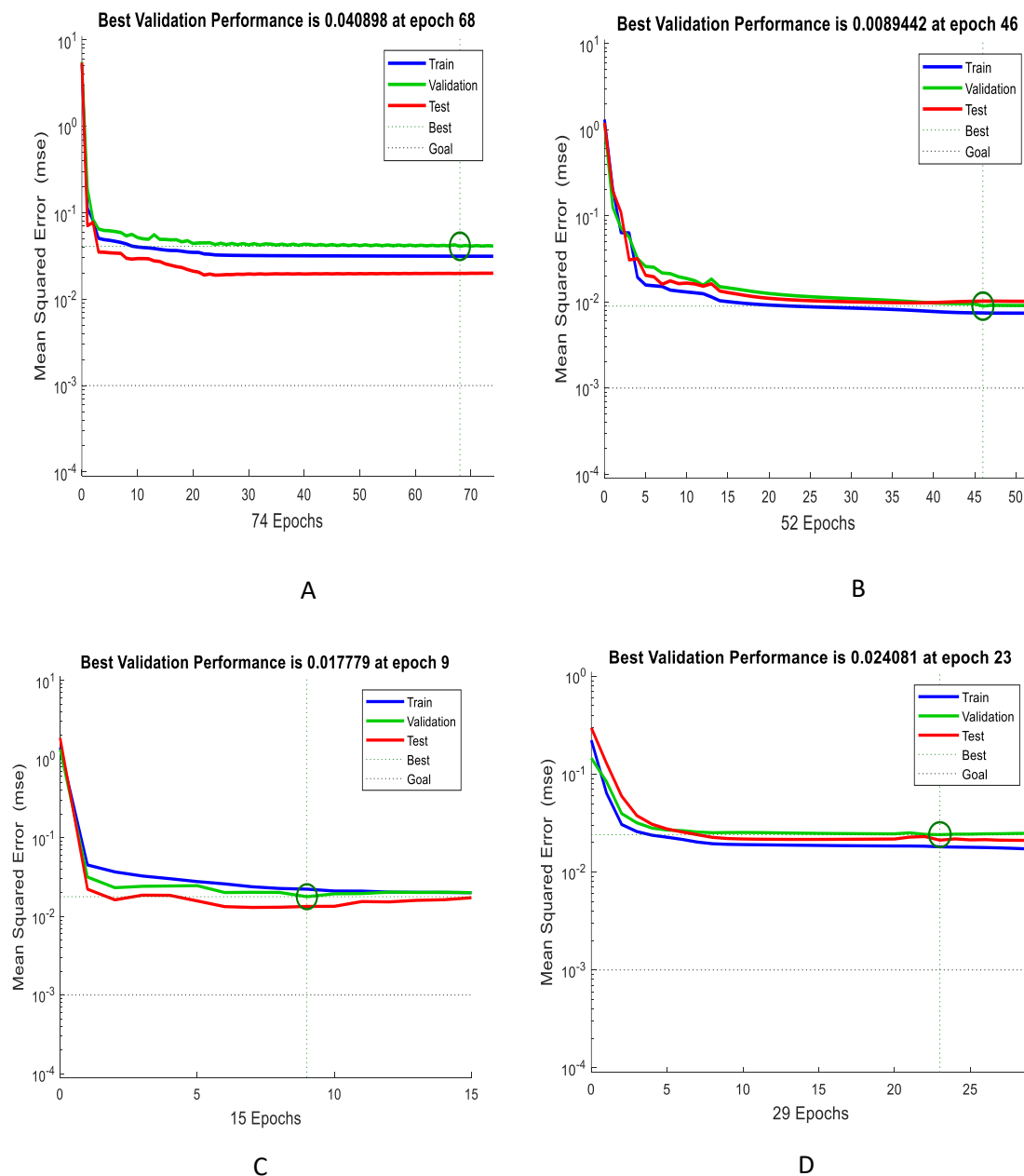


Figure 2. The validation performance plot of the network for A) BI, B) HMF, C) AA and D) TCC.

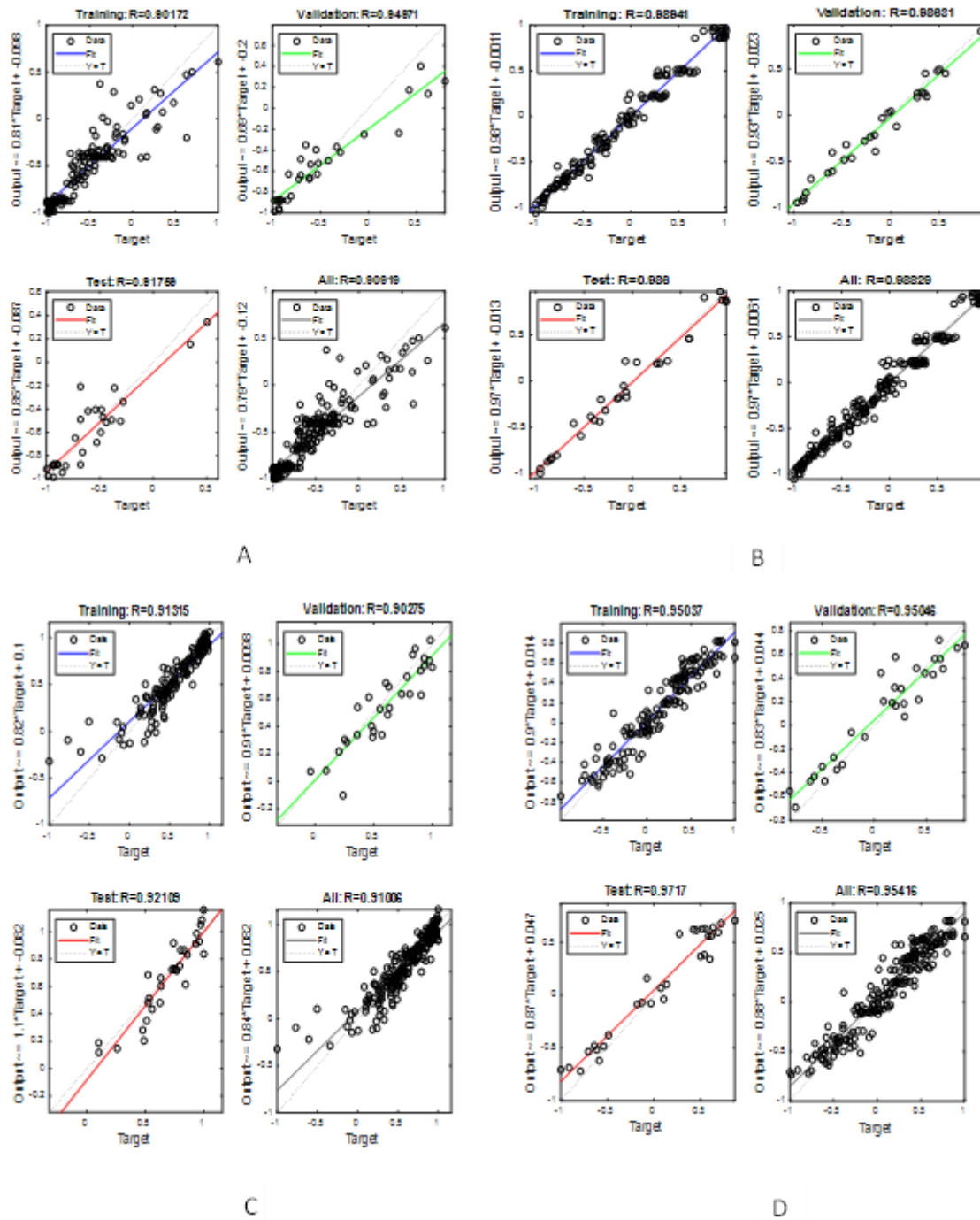


Figure 3. Relationship of the prediction and actual values of BI (A), HMF (B), AA (C) and TCC (D) for training, validation, test and all data, respectively.

Table 2. The weight and bias values of the best ANN models

ANN models	i	Weights				Bias		
		Input layer				Output layer	Hidden layer	Output layer
		w1i	w2i	w3i	w4i	w2j	b1i	b2j
BI	1	-0.3372	0.2235	2.0174	3.8000	9.6990	-8.3483	-4.7446
	2	13.8277	-3.1640	-3.0611	4.7539	-0.1268	-11.3891	
	3	-0.2329	0.7439	14.0288	7.5705	0.4720	3.2407	
	4	10.8565	-0.9146	-1.1401	-0.7883	3.8664	14.6206	
HMF	1	-0.1351	-0.2341	-5.3158	-7.4640	-3.5515	1.0845	-0.4716
	2	-0.0017	0.0870	1.2998	-2.9617	3.3807	0.5673	
	3	0.9656	1.0053	1.6905	3.0620	0.5377	3.9195	
Ascorbic acid	1	-3.6692	0.9634	0.1395	3.4246	-0.3418	5.0797	-0.2074
	2	1.4656	4.8249	2.4622	-2.5826	-0.1379	-3.3427	
	3	1.8019	2.6854	-2.8838	3.7548	0.1553	-0.9538	
	4	-1.9014	-3.1319	2.9213	-2.2445	-0.1088	-0.4960	
	5	-2.7970	0.0238	-2.2907	-1.3944	0.6008	-0.9294	
	6	-2.9236	-2.0830	-3.0205	5.3019	0.7319	-2.3076	
	7	0.6414	3.3304	2.1005	-3.2013	0.1448	3.0403	
	8	4.7526	-0.4884	-1.7770	-5.5692	0.6950	2.5743	
TCC	1	1.6294	0.0096	0.8085	-3.2545	2.1178	-4.0007	2.6394
	2	0.2901	-0.0647	1.0716	-1.3180	-3.8492	-1.3269	
	3	0.0818	0.1795	1.2368	-0.1039	-1.4812	-1.2501	
	4	1.2068	0.0603	-1.2278	2.6437	-1.8127	2.8522	

Multiple linear regression models

MLR models were performed by backward, forward, enter and stepwise regression methods. In statistical analysis, the presence of multi collinearity among independent variables was identified by calculating Variance Inflation Factors (VIF) and tolerance values indicates whether an independent variable has a strong linear relationship with other independent variable (Dormann et al., 2013; Nireesh and Velnampy, 2014). All VIF values were well below 10 and tolerance levels were not below 0.1. Thus, there was no multicollinearity between the independent variables. Durbin-Watson values were given Table 3. The presence of autocorrelation between errors was not detected due to these values are between 1 and 3.

In MLR analysis, our aim was detected the effect of processing temperature, processing time, storage temperature and storage time on BI, HMF, ascorbic acid and TCC at confidence level of 1%. MLR equation of each dependent variables

were presented in Table 3. We only applied logarithmic modification for HMF and AA data for constituting more reliable predictive equations. For HMF, stepwise regression model was chosen due to high R and R^2 . In a stepwise regression model, controllable variables are added and removed based on each step's statistical significance (Ghani and Ahmad, 2010). Therefore, it is found that the effect of processing temperature, processing time, storage temperature and storage time on HMF are statistically significant. According to the results of regression analysis, all models seemed to be good depending on R and R^2 values. However, the MLR models for HMF and TCC had a relatively higher predictability than BI and AA.

MLR is a well-known and practical method for determining a connection between the variables. As a limited approach to creating mathematical models, MLR models have the drawback of only being able to explain linear relationships between variables, ignoring other types of interactions.

Despite being considered unproductive, multiple linear regression models are frequently employed

in modeling processes and have been validated with success (Stangierski et al., 2019).

Table 3. Multiple linear regression model equation and statistic values of each output variables

MLR models	Methods	Equation	R	R ²	DW	VIF
BI	Enter	$y_1 = 8.728889 - 0.021634 * T_p - 0.000798 * t_p - 0.017825 * t_s + 0.010577 * T_s$	0.82	0.67	2.13	1.000
log HMF	Stepwise	$\log(y_2) = -0.378287 + 0.009511 * T_p + 0.002133 * t_p + 0.009124 * t_s + 0.039685 * T_s$	0.92	0.84	2.56	1.006
log AA	Enter	$\log(y_3) = 2.985761 - 0.001271 * T_p - 0.000532 * t_p - 0.001923 * t_s - 0.003716 * T_s$	0.83	0.68	1.43	1.001
TCC	Enter	$y_4 = 8.728889 - 0.021634 * T_p - 0.000798 * t_p - 0.017825 * t_s + 0.010577 * T_s$	0.92	0.85	1.69	1.007

BI: browning index. TCC: total carotenoid content. VIF: Variance Inflation Factors. DW: Durbin-Watson value

Performance comparison of ANN and MLR models

The prediction accuracy of proposed ANN and MLR models was assessed on basis of various statistical parameters to avoid establishing inconsistent findings on the data from the models. The results are listed in Table 4. It is stated that the models with lowest *MSE*, *RMSE*, *MAE*, *MAPE*, *MPE* and, highest *R* and *R*² are best suited for each condition.

According to Yadav and Chandel (2017), a model is considered to have very good predicting ability if its *MAPE* value is 10% or less, good prediction performance if it is 10%–20%, acceptable prediction quality if it is 20%–50%, and incorrect prediction efficiency if it is greater than 50%. For ANN models, the *MAPE* ranged from 1.57% to 8.16%, indicating a larger prediction

accuracy range. They range from 1.90% to 17.44% for MLR models. which shows good prediction efficiency.

The comparison of between the actual and prediction values of ANN and MLR for testing data of all output was given Fig. 4. As can be observed from Fig. 4, The curves of predicted values for the MLR and ANN models followed a similar pattern. The ANN model demonstrated a superior match. ANN is a more accurate predictor than MLR for all outputs with the highest *R* and *R*², and the lowest error values. ANN have a unique property that removes errors, making it a more effective prediction tool (Stangierski et al., 2019). MLR had an acceptable prediction performance for HMF, AA and TCC.

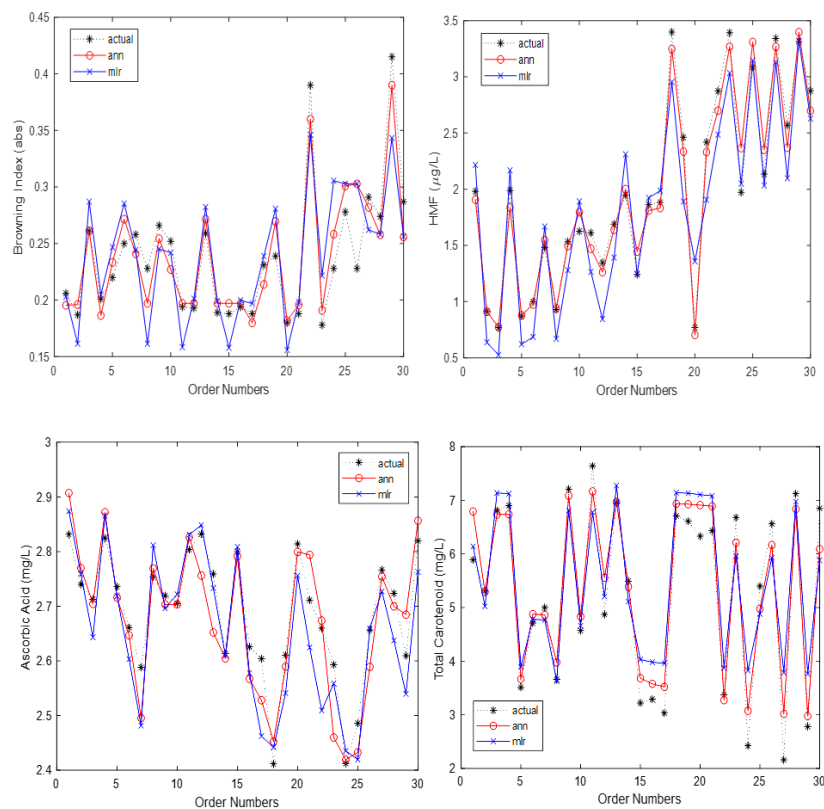


Figure 4. Comparison of the actual and prediction values of ANN and MLR for testing data of browning index (BI), HMF, AA and total carotenoid content (TCC).

ANN models are formed requiring lengthy iterative calculations. whereas the MLR models are generated only one step calculation. Therefore, ANN model has higher predictability because of a greater degree of fault tolerance and robustness. and shows better generalization capacity than the MLR. ANN models seem to be more appropriate method to approximate

nonlinearity systems (Erzin and Cetin, 2012; Prakash Maran et al., 2013; Nejatdarabi and Mohebbi, 2023). The comparison of key results/similarities/differences with recent related studies was given in Table 5. ANN had superior performance compared to MLR, but some studies were stated that MLR had greater potential for prediction than ANN.

Table 4. The statistical parameters of MLR and ANN for test data.

Statistical Parameters	BI		HMF		AA		TCC	
	MLR	ANN	MLR	ANN	MLR	ANN	MLR	ANN
R	0.79	0.92	0.94	0.99	0.92	0.92	0.93	0.97
R ²	0.68	0.84	0.88	0.98	0.85	0.86	0.9	0.94
MSE	0.00	0.00	0.1	0.02	0.00	0.00	0.44	0.18
RMSE	0.04	0.02	0.31	0.14	0.06	0.05	0.67	0.43
MAE	0.03	0.02	0.27	0.11	0.05	0.04	0.55	0.35
MPE	-0.92	-0.89	6.74	0.39	1.16	0.47	-8.5	-4.49
MAPE (%)	11.85	7.03	17.44	5.47	1.87	1.57	13.88	8.16

Table 5. The comparison of Key results/similarities/differences with recent related studies

Food- Applied Process	Models	Results	References
Orange juice quality parameters-heat process and storage	ANN-MLR	ANN models showed greater potential in comparison with MLR models for all variables, but ANN and MLR performed nearly identically for ascorbic acid prediction (R^2 : 0.86-0.98 and RMSE: 0.02-0.43 for ANN; R^2 : 0.68-0.94 and RMSE: 0.04-0.67 for MLR).	In this study
Red bayberry juice quality parameters- heat process and storage	ANN- Partial Least-Squares Regression (PLSR)	The PLSR model was sufficient for predicting antioxidant activity, and the ANN model could be used to predict changes in anthocyanin content and ascorbic acid in bayberry juice during storage.	Zheng et al. (2011)
Kiwifruit firmness with different mineral concentration	ANN-MLR	Modeling was made with four different model sets. MLR models evaluated the firmness of the fruit with more accuracy than the ANN models on three datasets (R^2 : 0.08-0.724 and RMSE: 0.481-1.37 for ANN; R^2 : 0.398-0.551 and RMSE: 0.809-3.11 for MLR).	Torkashvand et al. (2017)
Gouda cheese- quality changes during storage	ANN-MLR	Compared to the MLR models, the ANN model performed moderately better (R^2 : 0.98 and RMSE: 1.35 for ANN; R^2 : 0.94 and RMSE: 2.48 for MLR).	Stangierski et al. (2019)
Rice- Enzymatic Milling	ANN-MLR	The ANN approach performs better than MLR (R^2 : 0.97-0.99 and MSE:0.005-6.13 for ANN; R^2 : 0.87–0.90 and SSE: 0.008–8.25 for MLR).	Kothakota et al. (2021)
Pineapple cubes-drying process	ANN-MLR-mathematical models	When compared to other models, ANN models had superior performances (R^2 : 0.9975-0.9999 and RMSE: 0.001-0.0008).	Meerasri and Sothornvit (2022)
Mushroom powder - rehydration process	ANN-MLR	ANN showed fewer error values than MLR (R^2 : 0.98-0.99 and RMSE: 1.17-3.12 for ANN; R^2 : 0.96-0.98 and RMSE:1.53-4.24 for MLR).	Nejatdarabi and Mohebbi (2023)
Physicochemical Properties of Grape-Skin Compost	ANN-MLR-piecewise linear regression (PLR)	MLR and PLR models were suitable for prediction. ANN had potential for predicting some properties like dry-matter and moisture.	Sokač Cvetnić et al. (2024)

Conclusions

In present paper, ANN and MLR models to modeling the browning index, HMF, ascorbic acid and total carotenoid as output variables are examined and analyzed the most common statistical methods to determine performance of models. The developed back propagation ANN models were optimized by changing the number of neurons in the hidden layer and selecting appropriate training algorithm, transfer function and epoch number. The back-propagation training algorithms. The best results were obtained with 4, 3, 8 and 4 neurons in the hidden layer for BI, HMF, AA and TCC, respectively. It is found that both ANN models show good

predictions. For MLR model. several methods used to learn relationship between independent variables by eliminating data collinearity. To check the performance of the models, many performance criteria were calculated. Based on results of performance criteria. The ANN models are found to be more suitable with the highest R and R^2 value and the lowest MSE , MAE , $MAPE$ in comparison with MLR models. Lastly, it was found that proposed ANN models may seem a very useful method as an alternative for predictive modelling of quality parameters of orange juice. For quality parameters, using moderate pasteurization standards and selecting a storage temperature below 25 °C are crucial. Due to these results, less undesirable compounds caused by

heat processing may be produced, and juice products that are high in nutrients and minimally processed may be obtained. These models also may provide satisfactory knowledge to produce better quality orange juice for researchers and manufacturers. The ANN has higher accuracy, high error tolerance, efficient handling of non-linear functions, shorter calculating time and capability of learning compared to MLR. These advantages of ANN will lead to use more applications for predicting and optimization in food engineering.

Declarations

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Conflict of Interest

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

Author Contribution

Asiye Akyıldız: Conceptualization, Resources, Project administration, Supervision, Writing–review&editing, **Tuba Şimşek Mertoğlu:** Data curation, Investigation, Visualization, Writing–original draft, **Nuray Inan-Çinkır:** Formal analysis, Conceptualization, Resources, Supervision, Writing–review & editing, **Erdal Ağçam:** Formal analysis, Conceptualization, Resources, Supervision, Writing–review & editing.

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Marmara bölgesinde sert çekirdekli meyve ağaçlarında şarka hastalığının (plum pox virus=PPV) tespiti ve moleküler karakterizasyonu

Detection and partial molecular characterization of sharka disease (plum pox virus=PPV) on stone fruit trees in Marmara Region

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ÖZ

Türkiye’de Şarka hastalığı ilk kez 1969 yılında Trakya Bölgesi’nde bulunan Edirne ilindeki erik ağaçlarında tespit edilmiştir. Daha sonraki yıllarda Ege, Marmara, Doğu Anadolu, İç Anadolu, Akdeniz ve Karadeniz Bölgelerinde de rapor edilmiştir. Şarka hastalığına neden olan plum pox virus (PPV) ülkemizde iç ve dış karantina listesinde yer alan önemli bir patojendir. Bu çalışma, Marmara Bölgesi’nde Bursa, Edirne, Bilecik ve Kocaeli illerinde PPV’nin varlığını belirlemek amacıyla 2014 ve 2015 yıllarında yapılmıştır. Sert çekirdekli meyve ağaçlarından 220 yaprak örneği (90 erik, 1 kiraz, 129 şeftali ve nektarin) toplanarak serolojik (DAS-ELISA) ve moleküler (Real-time PCR) yöntemlerle analiz edilmiştir. Erik ağaçlarına ait örneklerin % 17.1’inin, şeftali/nektarin ağaçlarına ait örneklerin ise % 9.3’ünün PPV ile enfekteli olduğu tespit edilmiştir. Ayrıca seçilen izolatların filogenetik analizleri gerçekleştirilmiş olup nükleotid homolojilerinin %81,43 - %99,44 aralığında olduğu ve izolatların PPV’nin Marcus ırkına ait olduğu belirlenmiştir. Hastalık etmeninin belirlendiği bahçelerdeki ağaçların tümü sökülerek imha edilmiştir.

Anahtar Kelimeler: PPV-M, DAS-ELISA, Real-time PCR, Filogeni

ABSTRACT

Plum pox virus (PPV), the causal agent of sharka disease, was first detected in 1969 in plum trees in Edirne province, located in the Thrace region in Türkiye. In subsequent years, it was also identified in the Aegean, Marmara, Eastern Anatolia, Central Anatolia, Mediterranean, and Black Sea regions. PPV, which causes sharka disease, is a significant pathogen listed in both domestic and international quarantine lists in Türkiye. This study was conducted in 2014 and 2015 to determine the presence of PPV in the Marmara region, specifically in the provinces of Bursa, Edirne, Bilecik, and Kocaeli. A total of 220 leaf samples from stone fruit trees (90 plum, 1 cherry, and 129 peach/nectarine) were collected and analyzed using serological (DAS-ELISA) and molecular (Real-time PCR) methods. It was found that 17.1% of the plum tree samples and 9.3% of the peach/nectarine tree samples were infected with PPV. Additionally, phylogenetic analysis of selected isolates was performed, revealing nucleotide homologies ranging from 81.43% to 99.44%, and it was determined that the isolates belonged to the Marcus strain of PPV. All trees in the orchards where the pathogen was detected were uprooted and eradicated.

Key Words: Sharka disease, PPV-M, DAS-ELISA, Marmara Region, Phylogeny

Giriş

Türkiye, iklim koşullarının elverişliliği nedeniyle sert çekirdekli meyve üretimi ve ihracatında küresel ölçekte önemli bir konuma sahiptir. Ülkemizde kayısı (*Prunus armeniaca*), erik (*Prunus domestica*), şeftali (*Prunus persica*), nektarin (*Prunus persica* var. *nucipersica*), kiraz (*Prunus avium*) ve vişne (*Prunus cerasus*) gibi sert çekirdekli meyve türlerinin toplam üretimi 3.207.895 ton (TÜİK, 2024) olarak kaydedilmiş olup bu üretimin yaklaşık %20'sine karşılık gelen 632.615 tonu Marmara Bölgesi'nde gerçekleştirilmektedir. Ancak, üreticilerin sertifikasız ve kontrolsüz fidan kullanımıyla bahçe tesis etmeleri, viral patojenlerin yayılımını hızlandırmakta ve bitki sağlığını tehdit etmektedir. Özellikle, bu patojenlere karşı etkili bir kimyasal mücadele yönteminin bulunmaması, bitkilerde verim ve kalite kayıplarına neden olmakta, meyvelerin pazar değerini önemli ölçüde düşürmektedir. Sert çekirdekli meyve ağaçlarında Şarka hastalığına neden olan plum pox virus (PPV), moleküler bitki patolojisi alanında en kapsamlı çalışılan 10 bitki virüsü arasında yer almaktadır (Scholthof ve ark., 2011; Gürcan ve Ceylan, 2016). PPV'nin neden olduğu ekonomik kayıplar ve epidemiyolojik yayılım dinamikleri, bu patojene karşı entegre mücadele stratejilerinin geliştirilmesini zorunlu kılmaktadır. Bu bağlamda, sertifikalı fidan kullanımının yaygınlaştırılması, biyoteknolojik ve moleküler tanı yöntemleri viral hastalıkların tespiti ve kontrolünde kritik bir rol oynamaktadır.

Plum pox virus (PPV) enfeksiyonlarında belirti oluşumu, virüsün ırkı, enfeksiyonun gerçekleştiği fenolojik dönem, çevresel koşullar, konukçu bitkinin lokasyonu, mevsimsel değişkenler, konukçu türü, çeşidi ve yaşı gibi birçok faktöre bağlı olarak hafiften şiddetliye kadar geniş bir spektrumda değişkenlik gösterebilmektedir. Bu faktörlerin etkileşimi sonucunda, belirtiler bitkinin farklı organlarında (yaprak, meyve, çiçek vb.) ortaya çıkabileceği gibi, latent enfeksiyon

durumları veya belirtilerin maskelenmesi gibi olgular da gözlemlenebilmektedir. Yaprak belirtileri genel olarak kayısı, erik ve şeftali bitkilerinde belirgin olup yaprak damarlarının etrafında renk açılması, damar bantlaşması, sarıdan açık yeşile değişen halkalı lekeler ve çizgiler bu bitkiler için oldukça tipiktir. Kayısı ve erik meyvelerinde şiddetli deformasyonlar ile meyve etinde görülen kahverengileşme, meyve yüzeyindeki soluk sarı halka ve çizgi şeklindeki belirtilerin aynı zamanda izdüşümü şeklinde çekirdekte de görülmesi tipiktir (Birişik ve ark., 2021). PPV; meyve ağırlığı, büyüklüğü, verimi, şeker oranında azalma, asitliğin artması, erken meyve dökümü, nekroz, deformasyon, meyvelerde şekil bozukluğu ve buna benzer belirtiler meydana getirdiğinden dolayı ürünün ticari değerinin kısmen ya da tamamen yok olmasına neden olmaktadır (Anonim, 2017). Bu belirtiler, PPV'nin konukçu türüne ve çeşidine bağlı olarak gösterdiği patojenite varyasyonunu açık bir şekilde ortaya koymaktadır. Bu nedenle, virüsün teşhisi ve yönetimi için konukçuya özgü belirtilerin dikkate alınması büyük önem taşımaktadır. Özellikle serin iklim koşullarında viral enfeksiyonun neden olduğu karakteristik belirtiler (örneğin, yapraklarda klorotik halkalar, meyvelerde deformasyon ve renk değişiklikleri) daha belirgin hale gelmekteyken sıcak hava koşullarında bu semptomların maskelenmesi veya baskılanması sıklıkla rapor edilmektedir (Anonim, 2024a). Bu durum, PPV'nin teşhisini ve epidemiyolojik izlenmesini zorlaştıran önemli bir faktör olarak karşımıza çıkmaktadır.

PPV, sert çekirdekli meyve ağaçlarına bulaştığında %100'e varan ürün kaybına neden olabilmektedir. Şiddetli enfeksiyon durumlarında ise ağaçların fizyolojik fonksiyonlarını bozarak ömürlerini kısaltmakta ve hatta ölümlerine yol açarak tüm plantasyonların yok olmasına sebep olmaktadır. PPV'nin epidemiyolojik yayılımı incelendiğinde, virüsün Bulgaristan'dan başlayarak erik (*Prunus domestica*) üzerinden diğer ülkelere yayıldığı bilinmektedir. En fazla etkilenen konukçu türleri arasında erik, kayısı (*Prunus armeniaca*), şeftali (*P. persica*) ve nektarin

(*P. persica* var. *nucipersica*) bulunmaktadır (Nemeth, 1986). Bu nedenle, hastalığın yayılımının kontrol altına alınması için sertifikalı fidan kullanımı ve özellikle Türkiye gibi hastalığın sınırlı olduğu ülkelerde karantina önlemlerinin titizlikle uygulanması kritik öneme sahiptir. Son 30 yılda, dünya genelinde PPV'nin yönetimi ve kontrolüne yönelik yapılan çalışmaların tahmini maliyetinin 10 milyon euroyu aştığı rapor edilmiştir (Cambra ve ark., 2006; Saydam ve ark., 2018). Bu durum, PPV'nin ekonomik etkisinin yanı sıra, küresel ölçekte tarımsal üretim üzerindeki tehdidini de açıkça ortaya koymaktadır. Dolayısıyla, virüsle mücadelede entegre yönetim stratejilerinin geliştirilmesi ve uluslararası iş birliğinin artırılması büyük önem taşımaktadır.

PPV, Türkiye'nin bitki sağlığı mevzuatı kapsamında hem dış karantina yönetmeliğinin "*Ek 2B-Türkiye'de sınırlı olarak bulunan zararlı organizmalar*" listesinde hem de iç karantina ve sertifikasyon düzenlemelerinde yer alan önemli bir patojendir. PPV'nin Türkiye'deki varlığı ilk kez 1968 yılında Edirne'de tespit edilmiş (Şahtiyancı, 1969) ve ardından Marmara, Trakya, Ege, Akdeniz, Doğu Anadolu, Karadeniz ve Orta Anadolu Bölgelerindeki çeşitli illerde farklı araştırmacılar tarafından rapor edilmiştir (Elibüyük, 2004; Koç ve Baloğlu, 2006; Gümüş ve ark., 2007; Candresse ve ark., 2007; Akbaş ve ark., 2011; Ulubaş Serçe ve ark., 2011; Çelik ve Topkaya Kütük, 2013; Ceylan ve ark., 2014; Deligöz ve ark., 2015; Birişik ve ark., 2021). Bu raporlar, PPV'nin ülke genelinde yayılım gösterdiğini ve farklı coğrafi bölgelerdeki sert çekirdekli meyve üretim alanlarını tehdit ettiğini ortaya koymaktadır. Bu nedenle, virüsün epidemiyolojik yayılımının izlenmesi, karantina önlemlerinin sıkı bir şekilde uygulanması ve sertifikalı fidan kullanımının yaygınlaştırılması, hastalığın kontrolü ve yönetimi açısından büyük önem taşımaktadır.

(PPV)'nin uzun mesafelere taşınması, enfekteli üretim materyalleri (örneğin, fidanlar, aşı gözleri) ve kontamine bitki parçaları aracılığıyla gerçekleşirken, bahçe içinde ve yakın çevredeki bahçelere yayılımı ise vektör yaprak bitleri (*Aphididae*) tarafından sağlanmaktadır. Bu

süreçte, özellikle *Aphis fabae*, *A. craccivora*, *A. gossypii*, *A. spiraeicola*, *Myzus persicae*, *Brachycaudus cardui*, *B. helichrysi*, *B. persicae*, *Hyalopterus pruni* ve *Macrosiphum euphorbiae* gibi yaprak biti türleri, PPV'nin etkili vektörleri olarak tanımlanmıştır (Çağlayan ve ark., 2013; Levy ve ark., 2000). Bu vektörler, virüsü non-persistans (kalıcı olmayan) bir şekilde taşıyarak, bitkiler arasında hızlı bir yayılıma neden olmaktadır. Bu nedenle, PPV'nin epidemiyolojik yayılımının kontrol altına alınması için hem enfekteli bitki materyallerinin hareketinin kısıtlanması hem de vektör yaprak biti popülasyonlarının yönetimi büyük önem taşımaktadır.

PPV, genetik çeşitliliği yüksek bir virüstür. Farklı coğrafyalarda ve konukçularda farklı ırkları tanımlanmıştır. Bu ırklar, konukçu spesifitesi, patojenite ve genetik yapı açısından farklılıklar göstermekte olup PPV'nin bugüne kadar tespit edilmiş Dideron (-D), Marcus (-M), PPV-D ve PPV-M Recombinantı (-Rec), Cherry (-C), El Amar (-EA), Winona (-W), Türkiye (-T), Cherry Rusya (-CR), Albanian (-An) ve Chilean (-CV), olmak üzere 10 farklı ırkı rapor edilmiştir (Kerlan and Dunez, 1979; Glasa ve ark., 2004; Wetzel ve ark., 1992; James and Varga, 2005; Candresse ve ark., 2007; Ulubaş Serçe ve ark., 2009; Chirkov ve ark., 2018; Palmisino ve ark., 2012; Sertkaya ve ark., 2003; Glasa ve ark., 2013; Nemchinow and Hadidi, 1996). Bu ırklar arasında PPV-D ve PPV-M en yaygın olanlardır. PPV-M (Marcus) ırkı, başlıca Doğu, Güneydoğu ve Orta Avrupa Bölgelerinde endemik olarak bulunmakta ve bu bölgelerdeki belirli *Prunus* türleri üzerinde bildirilmiştir. Buna karşılık, PPV-D (Dideron) ırkı ise Batı Avrupa, Akdeniz havzası ve Orta Avrupa ülkelerinde yaygınlık göstermekte, özellikle bu coğrafyalarda yetiştirilen şeftali (*Prunus persica*) ve erik (*Prunus domestica*) gibi konukçularda rapor edilmiştir (Morca ve ark., 2021).

Şarka hastalığına neden olan PPV varlığının tespiti, yayılış alanlarının belirlenmesi, korunmuş/ari bölgelerin oluşturulması, hastalıkla mücadele stratejilerinin geliştirilmesi, yayılımının önlenmesi ve eradikasyonuna yönelik asgari

önlemlerin düzenlenmesi amacıyla, “11/6/2010 tarihli ve 5996 sayılı Veteriner Hizmetleri, Bitki Sağlığı, Gıda ve Yem Kanunu”nun 15. maddesi temel alınarak “Şarka Hastalığı İle Mücadele Hakkında Talimat” yayınlanmıştır (Anonim, 2017). Bu talimat kapsamında, PPV'nin çiçeklenme döneminden itibaren izlenmesi ve bulaşık olduğu tespit edilen tüm bitkilerin imha edilmesi kararlaştırılmıştır. Özellikle Bursa, sert çekirdekli meyve fidanı üretiminin yoğun olarak yapıldığı ve bu fidanların ülke genelinde yaygın bir şekilde dağıtıldığı bir il olması nedeniyle PPV'nin izlenmesi ve kontrolü büyük önem taşımaktadır. Virüsün enfeksiyon başladıktan sonra kontrol altına alınmasının oldukça zor olması (Cambra ve ark., 2006; Gürcan, 2017), erken teşhis ve önleyici tedbirlerin hayati önemini ortaya koymaktadır.

Bu kapsamda, 2013 yılından itibaren başlanarak Bursa başta olmak üzere Marmara Bölgesi'ndeki il ve ilçe Tarım ve Orman Müdürlüklerinin teknik ekipleri tarafından her yıl sert çekirdekli meyve ağaçlarından düzenli olarak alınan örnekler, Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü'ne gönderilerek analiz ettirilmektedir. Analizlerde, serolojik yöntemlerden DAS-ELISA ve moleküler yöntemlerden real-time RT-PCR gibi ileri teknikler kullanılmakta; ayrıca seçilen izolatların filogenetik analizleri gerçekleştirilerek virüsün genetik çeşitliliği ve yayılım dinamikleri incelenmektedir. Hastalık etmeninin tespit edildiği bahçelerdeki ağaçlar, ilgili talimat doğrultusunda il müdürlüklerinin teknik elemanları gözetiminde sökülerek imha edilmekte ve böylece virüsün yayılımı engellenmeye çalışılmaktadır. Bu sürveyler kapsamında yapılan analizler, bölgedeki PPV varlığının izlenmesi ve kontrolüne yönelik önemli veriler sağlamakta olup hastalığın yönetiminde etkili bir strateji olarak öne çıkmaktadır.

Materyal ve Yöntem

Sürvey çalışmaları

Bursa, Bilecik, Kocaeli ve Edirne illerinde görev yapan Tarım ve Orman İl Müdürlükleri'ne bağlı

teknik personel tarafından 2014 ve 2015 yıllarının mayıs ve haziran aylarında, “Şarka Hastalığı ile Mücadele Hakkında Talimat” doğrultusunda sürvey çalışmaları yürütülmüştür (Anonim, 2024b). Çalışmalar kapsamında, sert çekirdekli meyve bahçelerinde bulunan ağaç popülasyonu dikkate alınarak temsili örnekleme yöntemi uygulanmış ve toplamda 129 şeftali/nektarin, 90 erik ve 1 kiraz ağacından yaprak ve meyve örnekleri toplanmıştır. Bu örnekler, analiz ve detaylı değerlendirme için soğuk zincir koşullarında Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü'ne gönderilmiştir.

DAS-ELISA yöntemi ile PPV'nin tanısı

Araziden toplanan örnekler, PPV tespiti için özgül poliklonal antiserumlar kullanılarak Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) yöntemi ile analiz edilmiştir. Analizler, üretici firmanın önerdiği protokole uygun şekilde gerçekleştirilmiştir. DAS-ELISA sonuçları, 405 nm dalga boyunda absorbans değerleri ölçülerek elde edilmiş ve bu değerler, ELISA okuyucusu kullanılarak kaydedilmiştir. Örneklerin enfeksiyon durumunun belirlenmesinde, negatif kontrolün kolorimetrik değerlerinin 2 katı veya daha yüksek absorbans değerine sahip olan örnekler, PPV ile enfekteli olarak kabul edilmiştir (Clark and Adams, 1977).

DNA dizileme ve Filogenetik analiz çalışmaları

DNA dizileme için örneklerin hazırlanması

DNA dizileme ve filogenetik analizleri gerçekleştirebilmek için PPV ile enfekteli olduğu DAS-ELISA ile tespit edilmiş 2 nektarin örneğinin total RNA ekstraksiyonu, RNeasy Plant Mini Kit ile (Qiagen, Almanya) firmanın sağladığı standart protokol kullanılarak gerçekleştirilmiştir. Elde edilen RNA'lardan PPV amplifikasyonu, PPV'ye özgü P1: 5' CAG ACT ACA GCC TCG CCA GA 3' ve P2: 5' ACC GAG ACC ACT ACA CTC CC 3' primer çifti (Wetzel ve ark.,1991) ile SYBR Green One-Step qRT-PCR (Qiagen, Almanya) kiti kullanılarak Rotor Gene (Qiagen, Almanya) real-time PCR sisteminde kit protokolüne uygun olarak gerçekleştirilmiştir. Real-time RT-PCR çalışmaları sonucunda çoğaltılan ampikonlar DNA dizileme

verilerinin elde edilebilmesi amacıyla Genmar (İzmir) firmasına gönderilerek çift yönlü kısmi genom dizileme hizmeti alınmıştır.

Filogenetik analiz çalışmaları

Filogenetik analizler için PPV izolatlarına ait coat protein geninin çift yönlü kısmi dizilenmesiyle elde edilen 533 bp'lık sekans verisi kullanılmıştır. Elde edilen sekans verilerinin kalite kontrolleri ve filogenetik analizlerde kullanılacak tüm izolatlara ait sekans verilerinin hizalama analizleri Geneious Prime 2024.0.7 programı ile gerçekleştirilmiştir. Filogenetik analizler için NCBI veri tabanında yer alan ve farklı ülkelerden ve konukçulardan elde edilen PPV'ye ait toplam 35 sekans verisi kullanılmıştır. Ayrıca sweet potato virus G (MT270404) dış grup olarak filogenetik analizlere dahil edilmiştir. Filogenetik analizlerde kullanılan tüm PPV izolatlarına ait bilgiler Çizelge 1'de yer almaktadır. Çizelge 1'de yer alan tüm PPV izolatlarına ait nükleotid dizilerinin homoloji düzeylerini belirlemek amacıyla diziler Sequence Demarcation Tool V1.3 programının CLUSTALW modülü ile analiz edilmiştir (Muhire ve ark., 2014).

Filogenetik analizler maximum likelihood (ML)

metodu kullanılarak Galaxy Europe platformu üzerinde IQ-TREE programı ile gerçekleştirilmiştir (Minh ve ark., 2020). ML analizi sırasında en uygun modeli belirlemek için ModelFinder programı kullanılmıştır (Kalyaanamoorthy ve ark., 2017). ML dendogramı, nükleotid ikamesi için -TIM2 (HKY: Hasegawa-Kishino-Yano ve GTR: General Time Reversible modelleri arasında ara bir model olarak yer almaktadır) modelini ve baz frekansları için -F (gözlemlenmiş nükleotid frekansları) modeliyle birlikte -I (veride değişime uğramayan sabit bölgeler) ile -G4 (varyasyonu modellemek için gamma dağılımı)'nın bir kombinasyonu olan TIM2+F+I+G4 modeli kullanılarak oluşturulmuştur. Bu model, DNA dizilimlerinde farklı bölgelerin farklı evrim hızlarına sahip olabileceğini dikkate alarak daha gerçekçi bir filogenetik analiz sağlamakta olup Bayesian Information Criterion (BIC) skor ve ağırlık (w-BIC) değerleri dikkate alınarak belirlenmiştir. Dendogramın güvenilirliğini değerlendirmek için analiz 1000 ultrafast bootstrap tekrarı ile yapılmıştır (Hoang ve ark., 2018).

Çizelge 1. Filogenetik analizlerde kullanılan PPV izolatlarına ait bilgiler.

Table 1. Information about PPV isolates that used for phylogenetic analyses.

Sıra No Serial number	Genbank Erişim Numarası Genbank accession number	İzolat adı Isolate name	Lokasyon Location	Konukçu Host	Strain İrk
1	KX423953	BrPc87	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
2	KX423954	BrPc88	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
3	KX423944	BrPc74	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
4	MZ835418	PZ697	Türkiye, Zonguldak	<i>Prunus domestica</i>	Marcus (M)
5	PQ497637	TR-N224*	Türkiye, Bursa, Kestel	<i>Prunus persica</i> <i>nucipersica</i>	Marcus (M)
6	PQ497636	TR-N223*	Türkiye, Bursa, Gürsu	<i>Prunus persica</i> <i>nucipersica</i>	Marcus (M)
7	EF626532	BG1	Bulgaristan	<i>Prunus domestica</i>	Marcus (M)
8	KX423905	BrPc11	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
9	KX423897	BrPc2d	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
10	MN877409	Brd424	Türkiye, Burdur	<i>Prunus persica</i>	Marcus (M)
11	KX423928	BrPc48	Türkiye, Bursa	<i>Prunus persica</i>	Marcus (M)
12	OK157498	P840 ANK	Türkiye, Ankara	<i>Prunus persica</i>	Marcus (M)
13	OK157483	P109 ANK	Türkiye, Ankara	<i>Prunus armeniaca</i>	Türkiye (T)
14	OK157492	P458 ANK	Türkiye, Ankara	<i>Prunus armeniaca</i>	Türkiye (T)
15	OK157496	P699 ANK	Türkiye, Ankara	<i>Prunus armeniaca</i>	Türkiye (T)
16	OK157502	P1001 ANK	Türkiye: Ankara	<i>Prunus armeniaca</i>	Türkiye (T)
17	OK376028	P575 ANK	Türkiye: Ankara	<i>Prunus armeniaca</i>	Türkiye (T)
18	HF674399	AL11pl	Arnavutluk	<i>Prunus domestica</i>	Albanian (An)
19	HQ452364	Savnik 1	Karadağ	<i>Prunus domestica</i>	Recombinant (Rec)
20	HQ452395	Bijelo Polje 12	Karadağ	<i>Prunus domestica</i>	Recombinant (Rec)
21	HQ452386	Kolasin 1	Karadağ	<i>Prunus domestica</i>	Recombinant (Rec)
22	KX423946	BrPI76	Türkiye, Bursa	<i>Prunus domestica</i>	Dideron (D)
23	KX423948	BrPI78	Türkiye, Bursa	<i>Prunus domestica</i>	Dideron (D)
24	AY847269	-	Mısır	<i>Prunus armeniaca</i>	El Amar (EA)
25	FN424180	APR II/4	Mısır, El Amar	<i>Prunus armeniaca</i> cv. <i>Amar</i>	El Amar (EA)
26	MH346284	Bg10	Rusya	<i>Prunus cerasus</i>	Cherry (C)
27	MH346312	Pav2	Rusya	<i>Prunus cerasus</i>	Cherry (C)
28	MH346286	Ka1	Rusya	<i>Prunus cerasus</i>	Cherry (C)
29	MW650877	Tat-102	Rusya	<i>Prunus cerasus</i>	Chilean (CV)
30	MW650878	Tat-NK6/2	Rusya	<i>Prunus cerasus</i>	Chilean (CV)
31	MG736813	Kp8-1	Rusya	<i>Prunus cerasus</i>	Cherry Rusya (CR)
32	MG736815	Pul-1	Rusya	<i>Prunus cerasus</i>	Cherry Rusya (CR)
33	KU359733	Pulk-1	Rusya	<i>Prunus domestica</i>	Winona (W)
34	KU359734	Vlad-4	Rusya	<i>Prunus domestica</i>	Winona (W)
35	KU359730	Karm	Rusya	<i>Prunus domestica</i>	Winona (W)
36	MT270404	19-2123**	Güney Afrika	<i>Ipomoea batatas</i>	-

* Çalışma kapsamında elde edilen izolatlar

** Filogenetik analizlerde dış grup olarak kullanılan izolat.

Araştırma Bulguları ve Tartışma

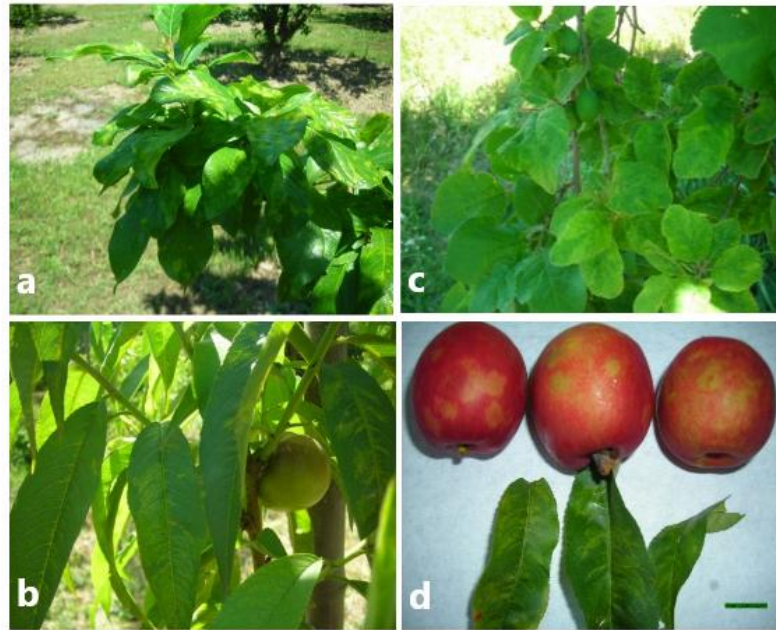
Semptomatolojik gözlemler

Gerçekleştirilen sürey çalışmasında erik ağaçlarına ait yaprak örneklerinde nekrotik halkalı lekeler (Şekil 1a) ve mozaik oluşumlarla damar bantlaşması (Şekil 1b), nektarin/şeftali yapraklarında açık yeşil lekeler ve orta-yan damarlar boyunca renk açılmaları (Şekil 1c) ayrıca

nektarin meyvelerinde halkalı sarı leke oluşumları (Şekil 1d) gözlenmiştir. Şarka hastalığına özgü belirtiler bitki çeşidine, virüs ırkına, iklim faktörlerine, bitkinin yaşına ve beslenmesine göre farklılık gösterebilmektedir (Desvignes 1999). Üretim materyallerinin yasal olmayan yollarla ülkelere girmesi ve latent enfeksiyonların tespit edilememesi virüsün uzun mesafelere

taşınmasında etkin olmaktadır (Cambra ve ark., 2006). Meyve bahçelerinde virüs rastgele dağılım göstermekte ve enfekteli bitkilerde 2-3 yıl sonra enfeksiyon yayılmaya başlamaktadır (Rimbaud ve

ark., 2015a, 2015b). Bu durum fidanlarda virüsün tespitini zaman zaman zora soktuğundan dolayı sertifikalı fidan kullanılmalıdır.



Şekil 1. a: Erik yapraklarında nekrotik halkalı lekeler; b-Erik yapraklarında mozaik ve damar bantlaşması semptomu; c-Nektarin/şeftali yapraklarında açık yeşil lekeler ve orta-yan damarlar boyunca renk açılması semptomu; d: Nektarin meyvelerinde halkalı sarı lekele oluşumları.

Figure 1. a: Necrotic ring spots on plum leaves; b- Mosaic and vein banding symptoms on plum leaves; c- Light green spots on nectarine/peach leaves and discoloration along mid-lateral veins; d- Circular yellow spots on nectarine fruits.

DAS-ELISA analizleri

“Şarka Hastalığı ile Mücadele Hakkında Talimat” çerçevesinde 2014-2015 yıllarında Bursa’dan 102, Kocaeli’nden 39, Bilecik’ten 40 ve

Edirne’den 39 olmak üzere alınan toplam 220 yaprak örneği (Çizelge 2) DAS-ELISA testine tabi tutulmuştur.

Çizelge 2. 2014-2015 yıllarında Marmara Bölgesi’nde gerçekleştirilen survey çalışmasına ait bilgiler.

Table 2. Information on survey studies conducted in the Marmara Region in 2014-2015.

il Province	ilçe District	Şeftali / Nektarin Peach / Nectarine	Erik Plum	Kiraz Cherry
		T.Ö.S./E.Ö.S. T.S.N./I.S.N.	T.Ö.S./E.Ö.S. T.S.N./I.S.N.	T.Ö.S./E.Ö.S. T.S.N./I.S.N.
Bursa	Gürsu	18 / 4	11 / 4	-
	İnegöl	11 / --	9 / 6	-
	Kestel	15 / 5	-	-
	Orhangazi	5 / --	-	-
	İznik	8 / --	10 / 3	-
	Karacabey	7 / 2	-	-
	Mudanya	-	8 / --	-
	Osmaneli	22 / 1	18 / --	-
Kocaeli	Gölcük	24 / --	15 / 1	-
Edirne	Merkez	19 / --	19 / 2	1 / --
TOPLAM		129 / 12	90 / 16	1 / --

T.Ö.S.: Toplam örnek sayısı, **E.Ö.S.:** Enfekteli örnek sayısı

T.S.N.: Number of total sample, **I.S.N.:** Number of Infected sample

DAS-ELISA sonuçlarına göre toplam 28 örnek PPV ile enfekteli bulunmuştur. Toplanan şeftali örneklerinde enfeksiyon oranı %9.3, erik örneklerinde %17.7 olarak tespit edilmiştir. Sert çekirdekli meyve üretiminin en çok yapıldığı il olan Bursa'dan alınan şeftali/nektarin örneklerinde enfeksiyon oranı %17.1, erik örneklerinde %34.2 olarak tespit edilmiştir. Bilecik'ten alınan şeftali/nektarin örneklerinde %4.5 enfeksiyon tespit edilmiş, erik örneklerinde ise enfeksiyon tespit edilmemiştir. Kocaeli'den alınan şeftali/nektarin örnekleri sağlıklı bulunurken, erik örneklerinde enfeksiyon %6.6 olarak tespit edilmiştir. Edirne'den alınan şeftali/nektarin örnekleri sağlıklı bulunurken erik örneklerinde enfeksiyon %10.5 olarak belirlenmiştir. Her ne kadar PPV ilk defa Trakya Bölgesinde tespit edilmiş olsa da üretim materyali ile yayılması oldukça hızlı olduğu için ülkemizin farklı bölgelerde de rapor edilmiştir. Bugüne kadar yapılan en kapsamlı çalışma 2007-2010 yılları arasında gerçekleştirilmiş olup Ege, Orta Anadolu, Marmara ve Akdeniz Bölgelerinde yer alan 56 ilde sert çekirdekli meyve ağaçlarında PPV enfeksiyonu tespit edilmiştir (Akbaş ve ark., 2011). Bu çalışmadan sonra enfekteli ağaç ve fidanlarda eradikasyon çalışmaları gerçekleştirilmiştir. PPV enfeksiyonu 2013-2018 yılları arasında Bursa'da %22.76, Edirne'de %9.13, Kocaeli'de %28.57 olarak tespit edilmiştir (Birişik ve ark., 2021). 2017 yılında Bursa'da şeftali ve erik örneklerinde PPV enfeksiyonu %54 (Gürcan ve Ceylan, 2016) olarak saptanmıştır. Benzer şekilde Saydam ve ark. (2018) Bursa'dan topladıkları erik,

nektarin ve şeftali örneklerinde enfeksiyon oranlarının sırasıyla %41.6, %40 ve %16.5 olduğunu belirlerken, kiraz ve badem ağaçlarında PPV enfeksiyonu saptamamışlardır. Gerek çalışmamızda gerekse farklı çalışmalarda tespit edilen farklı enfeksiyon oranları; analiz edilen örnek sayısı, konukçu çeşidi, örneklerin tekniğine uygun alınması gibi faktörlere bağlı olarak yıldan yıla değişiklik gösterebilmektedir. Serolojik analizler sonucu PPV ile enfekteli bulunan 12 adet şeftali/nektarin ve 16 adet erik numunesi ilgili il tarım ve orman müdürlüklerine resmi yazı ile bildirilmiştir. İlgili müdürlükler tarafından imhası gerçekleştirilen enfekteli ağaçlara ait görseller Şekil 2'de yer almaktadır. Epideminin engellenmesinde hiç şüphesiz ki hastalık kaynaklarının ortadan kaldırılması büyük önem taşımaktadır. Çalışmamızla benzer olarak 2013 ve 2018 yılları arasında Şarka hastalığından dolayı Türkiye'de 6.461 adet ağaç, 72.408 adet fidan imha edilmiştir (Birişik ve ark., 2021). Yine Amerika Birleşik Devletleri'nde şarka hastalığından dolayı 10 yılda yaklaşık 6778 dekada (Welliver et al. 2014; Birişik ve ark., 2021) ve İtalyada 15 yılda 47 bahçede sert çekirdekli meyve ağaçlarında eradikasyon gerçekleştirilmiştir (Myrta et al. 2006). Eradikasyon, özellikle yeni başlayan hastalığın yok edilmesinde faydalı olmaktadır, hastalığın yaygın olduğu yerlerde ise alınacak tedbirler daha sınırlı olup hastalığın görüldüğü alanlarda yaprak biti mücadelesi yapılması hastalığın birbirine yakın bahçelere bulaşmaması açısından çok önemlidir (Conti ve ark. (1985).



Şekil 2. Bursa ilinde eradikasyon işlemi gerçekleştirilen sert çekirdekli meyve ağaçları.
Figure 2. Eradication process of stone fruit trees in Bursa province.

Filogenetik analizler

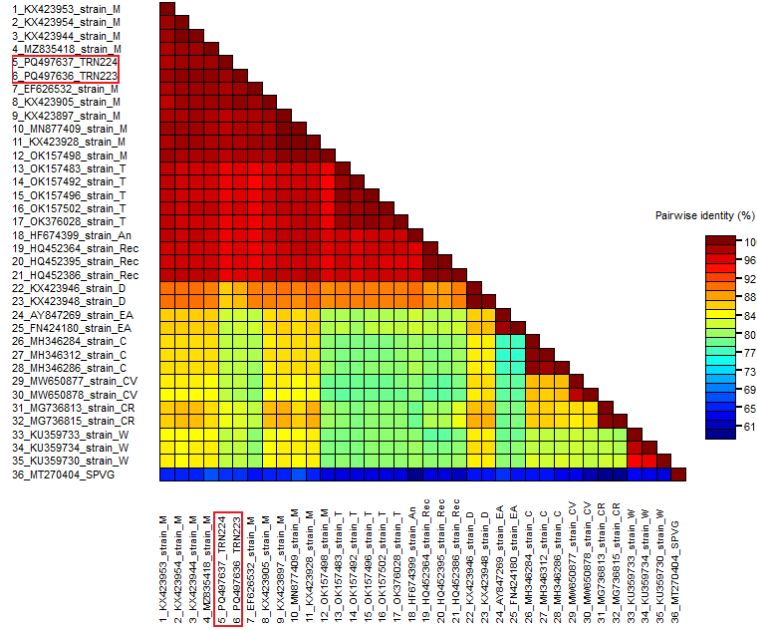
DAS-ELISA sonuçlarına göre PPV ile enfekteli olduğu belirlenen iki izolatin kısmi coat protein gen bölgesinin dizilenmesiyle elde edilen 533 bp'lik sekans verisi TR-N223 izolatu için PQ497636 erişim numarasıyla, TR-N224 izolatu için ise PQ497637 erişim numarasıyla 2024 yılında NCBI gen bankasına kaydedilmiştir.

PPV izolatlarına ait nükleotid dizilerinin homoloji düzeylerini belirlemek amacıyla Sequence Demarcation Tool V1.3 programının CLUSTALW modülü kullanılarak oluşturulan nükleotid homoloji matrisi sonucuna göre tüm PPV ırklarının birbirleriyle %75 (strain EA-AY847269 ve strain C-MH346312) - %99.86 (strain M- MN877409 ve KX423928) aralığında homoloji gösterdiği belirlenmiştir. Çalışmadan elde edilen sonuçlara yakın bir şekilde Gürcan ve Ceylan (2016) tarafından 2012-2013 yılları arasında Türkiye'nin farklı bölgelerinden toplanan 314 örnek üzerinde yapılan bir çalışmada PPV-T'nin - An, -M, -Rec, -D, -EA, -CR, -W ve -C ırkları ile sırasıyla %96.9, %96.3, %85.8, %85.1, %69.2, %68.1, %65.3 ve %65,0 düzeyinde homoloji gösterdiği bildirilmiştir.

Çalışma kapsamında elde edilen TR-N224 (PQ497637) nolu PPV izolatının analiz edilen diğer PPV izolatlarıyla nükleotid düzeyinde minimum %81.61 maksimum %98.69 düzeyinde homoloji gösterdiği; TR-N223 (PQ497636) nolu PPV izolatının ise minimum %81.43 maksimum %99.44 düzeyinde homoloji gösterdiği tespit edilmiştir. Bursa ilinin Kestel ilçesinden elde edilen TR-N224 (PQ497637) nolu PPV izolatının maksimum homoloji gösterdiği izolat Bursa ilinin Gürsu ilçesinden elde edilen TR-N223 (PQ497636) nolu PPV izolatu olurken TR-N223 (PQ497636) nolu PPV izolatının maksimum homoloji gösterdiği PPV izolatu bir başka çalışmada yine Bursa ilinden elde edilen BrPc74 (KX423944) olmuştur. Analiz edilen PPV ırklarının tür içi homoloji seviyeleri (minimum ve maksimum) ise PPV-M (%97.37-%99.86), PPV-T (%98.60-%99.40), PPV-Rec (%99.30-%99.50), PPV-D (%99.69), PPV-EA (%98.39), PPV-C (%99.00-

%99.50), PPV-CV (%97.47), PPV-CR (%99.62) ve PPV-W için (%92.08-97.68) olarak tespit edilmiştir (Şekil 3). Çalışmaya dahil edilen genom bölgeleri ve bölgelerin büyüklüklerine göre bazı farklılıklar olmakla birlikte Gürcan (2017) tarafından Bursa ilinden elde edilen ve 64 örnekle gerçekleştirilen bir çalışmada İstanbul'dan elde edilen PPV-M izolatlarının, Avrupa'dan elde edilmiş PPV-M izolatları ile P3-6K1 gen bölgesi için %98 ve CP-3'UTR gen bölgesi için 97.7 homoloji gösterdiği; PPV-M gurubu örnek sayısı en fazla olan grup olmasına rağmen genetik varyasyonun P3-6K1 gen bölgesi için % 1.3 ve CP-3'UTR gen bölgesi için % 1.4 olarak hesaplandığı bildirilmiştir. Ayrıca Bursa'daki kapama şeftali bahçelerinin Avrupa tipi PPV-M ile yaygın olarak bulaşık olduğu ve bu izolatların Aydın, Denizli, Çanakkale, Isparta ve Kayseri gibi yeni enfekte olmuş bölgelerdeki izolatlarla yüksek genetik benzerlik gösterdiği tespit edilmiştir. Bu durum, PPV-M'nin Türkiye'de hızla yayıldığını ve genetik çeşitlilik gösterdiğini ortaya koymaktadır. Gürcan ve ark. (2019) tarafından gerçekleştirilen bir çalışmada ise PPV-Mıs ile PPV-M, PPV-T, PPV-An, PPV-Rec, PPV-D ve PPV-EA izolatları arasındaki ortalama nükleotid uzaklıklarının sırasıyla 0.030 ± 0.001 , 0.058 ± 0.002 , 0.083 ± 0.003 , 0.107 ± 0.003 , 0.126 ± 0.003 ve 0.257 ± 0.006 olarak hesaplandığı bildirilmiş olup 21 PPV-Mıs, 4 PPV-M izolatu ve tüm izolatlar (21 Mıs + 4 PPV-M) için grup içindeki nükleotid dizi çiftleri üzerinden hesaplanan ortalama nükleotid farklılıklarının sırasıyla 0.015 ± 0.0005 , 0.016 ± 0.001 ve 0.020 ± 0.0007 olarak tespit edildiği bildirilmiştir. Coşkan ve ark. (2022) tarafından 250 PPV izolatının tam ORFs, tam CP, P3-6K1-CI ve kısmi Nıb-CP genom bölgeleriyle gerçekleştirilen bir diğer çalışmada ise PPV-T ırkı popülasyonlarının -D ve -M (Mıs hariç tutulduğunda) ırklarına kıyasla nükleotid düzeyinde daha yüksek bir genetik farklılık gösterdiği ortaya konmuştur. Aynı ayrı analiz edildiğinde PPV-M ve -Mıs ırklarına ait popülasyonların çok düşük genetik farklılık düzeyleri sergilediği belirlenmiş ancak PPV-Mıs

izolatlarının, PPV-M popülasyonuna dahil edilen sonuçlar önceki çalışmalardan elde edilen edildiğinde genetik çeşitliliğin önemli ölçüde sonuçlarla benzerlik göstermektedir. arttığı bildirilmiştir. Homoloji açısından elde

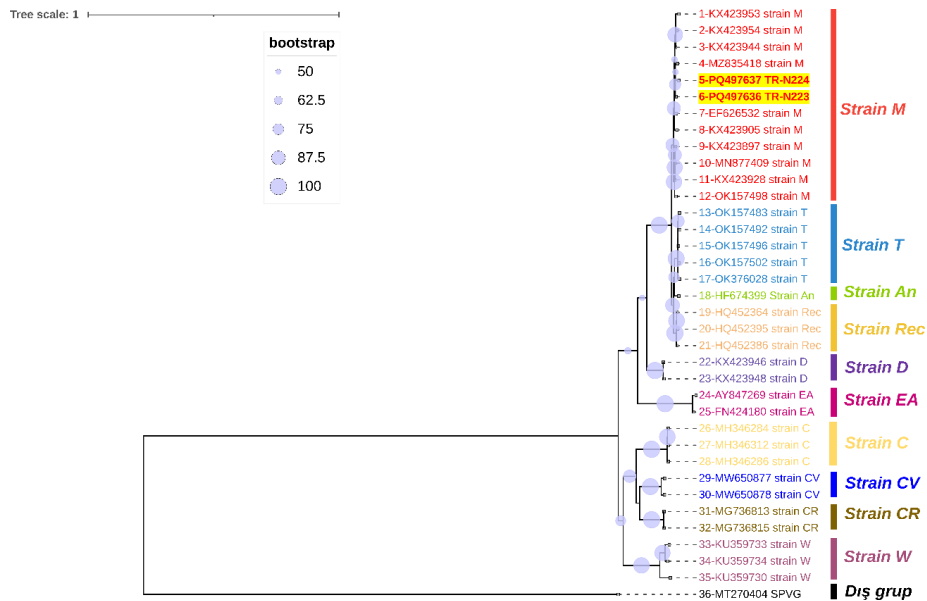


Şekil 3. PPV izolatlarının kısmi coat protein (CP) gen bölgelerine ait nükleotid dizilerinin Sequence Demarcation Tool V2.3 programının CLUSTALW modülü ile oluşturulmuş homoloji matrisi (n=36).

Figure 3. Homology matrix of nucleotide sequences of partial coat protein (CP) gene regions of PPV isolates generated by CLUSTALW module of Sequence Demarcation Tool V2.3 (n=36).

Filogenetik analizler için NCBI gen bankası veri tabanında yer alan ve farklı ülkelerden ve konukçulardan elde edilen PPV'ye ait toplam 35 sekans verisi ile oluşturulan dendrogram incelendiğinde PPV'nin -M, -T, -An, -Rec, -D, -EA, -C, -CV, -CR ve -W ırklarına ait izolatların farklı alt gruplarla birbirlerinden anlamlı bootstrap

değerleriyle (>50) ayrıldığı; çalışma kapsamında elde edilen TR-N223 (PQ497636) ve TR-N224 (PQ497637) numaralı PPV izolatlarının Türkiye'nin Bursa, Zonguldak, Burdur, Ankara illeri ile Bulgaristan'dan elde edilen ve NCBI gen bankasında yer alan PPV strain M izolatlarıyla aynı alt grupta yer aldığı belirlenmiştir (Şekil 4).



Şekil 4. PPV izolatlarının kısmi coat protein (CP) gen bölgelerine ait nükleotid dizileriyle oluşturulmuş Maximum likelihood (ML) dendrogramı (n=36). Dendrogram sweet potato virus G (MT270404) kullanılarak köklendirilmiştir. ML ağacı 1000 bootstrap değeri ile çizilmiş olup 50'nin altında olan bootstrap değerleri gösterilmemiştir.

Figure 4. Maximum likelihood (ML) dendrogram constructed with nucleotide sequences of partial coat protein (CP) gene regions of PPV isolates (n=36). The dendrogram was rooted using sweet potato virus G (MT270404). The ML tree was constructed with 1000 bootstrap values and bootstrap values below 50 are not shown.

Filogenetik analizler sonucunda elde ettiğimiz dendrogram, Morca ve ark. (2021) tarafından elde edilen sonuçlarla karşılaştırmalı olarak incelendiğinde Bursa'dan elde edilen izolatların PPV-M ırkına ait olduğunu ve farklı PPV ırklarını içeren grupların çalışmamızla benzer şekilde gruplandığını göstermiştir. Gürcan (2017) tarafından gerçekleştirilen bir başka çalışmada ise çalışmamız sonucunda elde ettiğimiz gruplamaya benzer şekilde Bursa izolatlarının bir kısmının Avrupa PPV-M izolatları ile grup oluşturduğunu ancak bulgularımızdan farklı olarak diğer bir kısmının ise Türkiye PPV-D izolatları ile grup oluşturduğu belirlenmiştir. Çelik ve ark. (2022) tarafından Bursa, Bilecik ve Bolu'dan toplanan 24 PPV izolatı üzerinde yapılan bir diğer çalışmada ise Bursa'dan 6 izolatın PPV-D, 12 izolatın ise PPV-M ile gruplandığı, Bolu izolatlarının ise PPV-M'is alt grubunda yer aldığı tespit edilmiştir. Bu, elde ettiğimiz sonuçlardan farklı olarak hem PPV-M'nin farklı alt gruplar halinde yayıldığını ve genetik çeşitlilik gösterdiğini hem de Gürcan (2017)'ye benzer şekilde PPV-D'nin Bursa'da varlığını ortaya koymaktadır. Son olarak, Teber ve ark. (2023) tarafından Türkiye'nin farklı bölgelerinden elde edilen 69 PPV-M izolatının genetik çeşitliliğinin incelendiği bir çalışmada ise izolatların yüksek bir genetik çeşitlilik gösterdiği belirtilmiştir. Bu bulgu, çalışmamızdakine benzer şekilde PPV-M'nin Türkiye'de hızla yayıldığını desteklemekle birlikte PPV-M'in genetik olarak farklılaştığına da işaret etmektedir.

Türkiye'de PPV'nin yayılımı ve farklı ırklarının dağılımı üzerine yapılan çeşitli çalışmalar, PPV-M'nin Türkiye'deki varlığının erken dönemlerde tespit edildiğini göstermektedir. Elibüyük (2003) tarafından 1995-2002 yılları arasında Ankara'da yürütülen bir çalışmada, enfekteli olduğu tespit edilen tüm örneklerin bulgularımıza benzer şekilde PPV'nin Marcus (PPV-M) ırkına ait olduğu bildirilmiştir. Yine benzer şekilde Dallot ve ark. (2004) ile Barba ve ark. (2011), PPV-M'nin Avrupa'daki en sorunlu PPV ırkı olduğunu ve hızla

yayılan salgınların çoğunlukla bu ırkla ilişkili olduğunu vurgulamışlardır. Bu durum, PPV-M'nin Türkiye'de de benzer bir tehdit oluşturduğunu düşündürmektedir. Araştırma bulgularımızla benzer sonuçlara sahip Çelik ve Topkaya Kütük (2013) tarafından 2008-2010 yılları arasında Antalya Döşemealtı'nda yapılan bir çalışmada, tek bir nektarin bahçesinden alınan 20 örneğin PPV-M ile enfekteli olduğu ve bu bahçedeki fidanların Bursa'dan temin edildiği bildirilmiştir. Gürcan ve Ceylan (2016) tarafından 2012-2013 yılları arasında Türkiye'nin farklı bölgelerinden toplanan 314 örnek üzerinde yapılan bir başka çalışmada ise Bursa'da PPV-D ve PPV-T'nin çoğunlukla konut bahçelerinde, PPV-M'nin ise kapama meyve bahçelerinde yaygın olduğu bildirilmiş olup bu bulgular, çalışmamız kapsamında elde edilen PPV enfekteli ağaçların kapama meyve bahçesinden elde edildiği sonucuyla ilişkilendirildiğinde PPV-M'nin özellikle ticari meyve bahçelerinde önemli bir tehdit oluşturduğunu göstermektedir.

Sonuçlar

Bu çalışmanın amacı, Tarım ve Orman Bakanlığı Gıda ve Kontrol Genel Müdürlüğü (GKGM) tarafından 2013 yılında başlatılan ve halen düzenli olarak devam eden sert çekirdekli meyve ağaçlarında PPV'nin sürvey ve eradikasyon programının uygulanması esnasında elde edilen verilerin ortaya konmasıdır. Çalışma kapsamında Bursa, Bilecik, Kocaeli ve Edirne illerinden gelen örnekler analiz edilmiş ve Bursa ilinden elde edilen nektarin izolatlarının filogenetik analizleri gerçekleştirilerek izolatların PPV'nin Marcus ırkına ait olduğu belirlenmiştir. PPV, Avrupa Birliği ülkeleri içerisinde çok önemli olarak gösterilen 16 zararlı organizmadan biridir. Ülkemiz dış karantina yönetmeliğinin "Ek 2B-Türkiye'de Sınırlı Olarak Bulunan Zararlı Organizmalar" listesinde ayrıca iç karantina ve sertifikasyon listelerinde yer almaktadır. Bu sebeple Bursa ili ülkemiz meyve

fidanı üretiminde üçüncü sırada yer alması (Anonim, 2022) ve ülke geneline yoğun bir fidan arzı sağlaması nedeniyle PPV açısından oldukça önemli bir konumda yer almaktadır. Türkiye'de yapılan çeşitli çalışmalar, PPV'nin farklı ırklarının ülke genelinde yaygın olduğunu ve araştırma bulgularımızda uyumlu şekilde, özellikle PPV-M'nin ticari meyve bahçelerinde önemli bir tehdit oluşturduğunu ortaya koymaktadır. Ayrıca, PPV-M'nin genetik çeşitliliği ve farklı alt gruplara ayrılabilmesi, bu virüsün yayılım dinamiklerinin daha iyi anlaşılması için özellikle fidan üretiminin, satışının ve kapama meyve bahçelerinin yoğun olduğu Bursa ilinde daha fazla araştırma yapılması gerektiğini göstermektedir. Bu çalışmanın sonucunda, PPV'nin tespit edildiği bölgedeki bitkiler imha edilerek, 1 km yarıçapında bir tampon bölge oluşturulmuştur. Bunun yanı sıra, bu tampon bölge içerisinde 3 yıl süreyle PPV'nin bilinen konukçuları olan kültür bitkilerinin yetiştirilmesi yasaklanmıştır. Hastalığın enfekteli fidanlarla uzak bölgelere yayılabildiği düşünüldüğünde, mücadelenin en kritik adımları olan sertifikalı fidan kullanımı, düzenli sürveyler ve analizler, enfekteli fidanların imhası ile patojenin temiz bölgelere bulaşmasının önlenmesi, bugün de geçerliliğini koruyan en güçlü önlemlerdir.

Ekler

Bu çalışma Antalya'da gerçekleştirilen "3rd International Symposium on Plum Pox Virus" etkinliğinde poster bildiri olarak sunulmuş ve sempozyum kitapçığında özet metin olarak yer almıştır.

Çıkar Çatışması Beyanı

Yazarlar aralarında herhangi bir çıkar çatışması bulunmadığını beyan ederler.

Yazarların Katkısı

Yazarlar tarafından katkılar; 'NU: Örneklerin serolojik ve moleküler yöntemlerle analizi, analiz

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Evaluation of Malatya province soils for apricot cultivation

Malatya ili topraklarının kayısı yetiştiriciliği bakımından değerlendirilmesi

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ABSTRACT

Malatya is the world's leading region in terms of dried apricot production. Understanding the optimal soil conditions for apricot growth is crucial for enhancing production. This study evaluated the soil fertility status of apricot orchards in Malatya province by analyzing key physicochemical soil properties and mapping their spatial variability using Geographic Information Systems (GIS). Malatya soils generally have high lime content (26.79%), neutral to slightly alkaline pH (7.25–8.13), high levels of Ca (5534.40 mg kg⁻¹) and K (633.25 mg kg⁻¹), moderate Fe (3.26 mg kg⁻¹), and low levels of organic matter, Zn (0.48 mg kg⁻¹), Mn (5.72 mg kg⁻¹), and B (0.33 mg kg⁻¹). While high levels of Ca and K improve fruit flavor, aroma, vitamin, and mineral content, limited availability of micronutrients restricts yield. Therefore, reducing soil pH with sulfur or acidic materials, improving soil properties by organic matter addition, and supplementing deficient micronutrients with foliar or soil applications are important for sustainable apricot production and yield improvement.

Key Words: Apricot, soil properties, nutrient elements, sustainable cultivation

Öz

Malatya, kuru kayısı yetiştiriciliğinde dünyanın önde gelen bölgesidir. Kayısı yetiştiriciliği için en uygun toprak koşullarının anlaşılması, üretimin artırılması için çok önemlidir. Bu çalışma, temel fizikokimyasal toprak özelliklerini analiz ederek ve Coğrafi Bilgi Sistemleri (CBS) kullanarak mekansal değişkenliklerini haritalayarak Malatya ilindeki kayısı bahçelerinin toprak verimlilik durumunu değerlendirmeyi amaçlamaktadır. Çalışma verilerine göre, Malatya toprakları genel olarak yüksek kireç içeriği (%26,79), nötr ile hafif alkali pH (7,25-8,13), yüksek Ca (5534,40 mg kg⁻¹) ve K (633,25 mg kg⁻¹), orta düzeyde Fe (3,26 mg kg⁻¹) ve düşük organik madde, Zn (0,48 mg kg⁻¹), Mn (5,72 mg kg⁻¹) ve B (0,33 mg kg⁻¹) seviyelerine sahiptir. Yüksek Ca ve K seviyeleri meyve tadını, aromasını, vitamin ve mineral içeriğini artırırken, mikro besin maddelerinin sınırlı bulunabilirliği kayısı verimini kısıtlamaktadır. Bu nedenle, kükürt veya asit karakterli materyaller uygulanarak toprak pH'sının düşürülmesi, organik madde ilavesiyle toprak özelliklerinin iyileştirilmesi ve yapraktan veya topraktan uygulamalar yoluyla eksik mikro besin maddelerinin takviye edilmesi, sürdürülebilir kayısı yetiştiriciliği ve verim artışı için çok önemlidir.

Anahtar Kelimeler: Kayısı, toprak özellikleri, besin elementleri, sürdürülebilir yetiştiricilik

Introduction

The economic and technological advancement era has highlighted the need for sustainable and environmentally friendly soil management to optimize productivity per unit area because of the increasing consumption of agricultural inputs. Cultivating the most suitable crops is essential based on key parameters such as physical, chemical, and biological soil properties, topography, climate, and altitude. Identifying soil variability and preserving soil health is crucial for managing natural resources (Demir et al., 2019). Soil water cycles and soil organic matter (SOM) retention in terrestrial ecosystems have been subjects of significant research because of their beneficial impacts on climate change and ecosystems (Gwak & Kim, 2017).

Plants require different types of nutrients, which can be classified into macronutrients and micronutrients. These include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), zinc (Zn), iron (Fe), boron (B), sulfur (S), and magnesium (Mg), etc. Taha and Sherif (2015) found that Ca, Zn, B, and amino acids play a significant role in flowering, and fruit setting and development, leading to increased yield and quality. Clay-textured soils contribute to improved fruit set, yield, leaf chlorophyll content, increased Ca, Zn, and B contents, and higher total soluble solids (TSS) in fruits. Soil pH is a vital factor influencing nutrient availability, either enhancing or restricting nutrient uptake. Lower pH reduces the availability of secondary macronutrients, while higher pH limits the

presence of micronutrients. Moreover, maintaining an optimum pH is essential for the successful application of herbicides, organic and inorganic fertilizers, consequently maximizing plant benefits (Toor et al., 2021).

Apricot (*Prunus armeniaca* L.) belongs to the Rosaceae family. Originating from Central and Western China, apricots have been consumed over 5,000 years and cultivated commercially for over 3,500 years (Manolopoulou & Mallidis, 1997; Karataş, 2021). Apricots have various uses as they can be consumed fresh as well as a significant portion is preserved by drying (Faust et al., 1998). Apricots play an important role in human nutrition due to their rich contents of sugar, organic acids, anthocyanins, phenolics, carotenoids, flavonoids, dietary fiber, minerals, and vitamins, making them a functional food beneficial for consumer health and disease prevention (Leccese et al., 2008; 2012; Milošević et al., 2013). Apricot flowering occurs before leaf development and relies on reserves accumulated from the previous season. Flowering phase often last one to two weeks, during which pollination and fruit development occur (Julian et al., 2010).

Turkey remains the world's leading apricot producer despite a decrease in production compared to previous years (Table 1). Turkey produced 750,000 tons of apricots during 2023 which was highest in the world. However, the production was lesser than 833,000 tons apricots produced during 2020 in the country. The area under cultivation (144,941 hectares) increase from previous years, indicating continued investment in apricot orchards.

Table 1. Global apricot production, cultivation area, and yield by country in 2023.

Rank	Country	Production (tons)	Rank	Country	Cultivation Area (ha)	Rank	Country	Yield (kg ha ⁻¹)
1	Türkiye	750000	1	Türkiye	144941	1	Albania	15751
2	Uzbekistan	500545	2	Iran	56137	2	USA	13519
3	Iran	318475	3	Uzbekistan	42936	3	Egypt	13485
4	Italy	207190	4	Algeria	28965	4	Jordan	13338
5	Algeria	200566	5	Afghanistan	22008	5	Turkmenistan	12426
6	Afghanistan	155429	6	China	21209	6	Romania	12093
7	Pakistan	135062	7	China, mainland	18046	7	Italy	11934
8	France	127780	8	Spain	18010	8	Uzbekistan	11658
9	Armenia	114494	9	Italy	17360	9	Argentina	11564
10	Spain	108450	10	Pakistan	13830	41	Türkiye	5174.5

Source: FAOSTAT, 2025

Turkey, with a yield of 5,174.5 kg ha⁻¹, ranks 41st globally, demonstrating that while it is the largest producer of apricots, its per hectare productivity is significantly lower to that of other countries, such as Albania (15,751 kg ha⁻¹) and the USA (13,519 kg ha⁻¹). Although Turkey is the leading producer, its per hectare yield is significantly lower than several countries, highlighting the need for improvements in soil management practices, organic matter improvement, and micronutrient supplementation to improve yields on sustainable basis.

Spatial mapping and geographic information systems (GIS) in soil fertility assessments are essential tools increasingly being used to optimize agricultural production. These methods visualize the regional variability of soil properties in agricultural areas, enabling farmers and researchers to make more informed decisions (Kılıç et al., 2021). Determining spatial distribution of nutrients is essential for effectively planning fertilization programs and enhancing yield of soil quality-sensitive crops like apricot (Demir et al., 2019). The GIS-based analyses help to develop soil management strategies specific to apricot agriculture by determining the regional distribution of factors such as pH, organic matter, macro- and micronutrients. This study was aimed at enhance the efficiency and sustainability of apricot agriculture by spatial mapping of soil characteristics in Malatya province.

This study used a Geographic Coordinate System (GCS) based on WGS84 for the initial data collection to ensure compatibility with GPS-acquired soil sample locations. The data were converted into a Projected Coordinate System (PCS) using the Universal Transverse Mercator (UTM) projection to reduce distortions in distance and area computations for spatial mapping and interpolation. The use of a PCS ensures improved precision in geographical analysis, interpolation, and visualization, especially in large-scale agricultural evaluations (Snyder, 1987).

The physical and chemical characteristics of

agricultural soils play a critical role in productivity and quality of crops. Apricot cultivation is directly affected by soil fertility, pH balance, organic matter content and nutrients (Karlıdağ et al., 2019). Malatya province is one of the most important apricot production centers in Turkey. It has high quality and yield potential due to its soil and climate conditions (Asma and Kan, 2007).

However, studies have revealed that apricot orchard soils in Malatya are deficient in some nutrients. Akin and Aygöl (2022) analyzed 579 apricot orchard soils in Malatya and reported that 88.9% of the soils were slightly alkaline and 10.9% were neutral; thus, high pH limited the uptake of micronutrients. In the same study, it was emphasized that organic matter content was low in 93.8% of the soils, and iron (36.4%), zinc (46.1%) and manganese (91.7%) deficiencies were prevalent.

Existing literature shows that high lime content (CaCO₃) reduces the availability of micronutrients, and chlorosis is observed in apricot trees, especially due to Fe deficiency (Mengel & Kirkby, 1982; Udo et al., 1970). Akin and Aygöl (2022) emphasized the importance of foliar Fe fertilization applications and organic matter addition to overcome this problem.

This study offers an extensive investigation of the chemical and physical characteristics of Malatya's soils concerning apricot cultivation, with the objective of assessing soil fertility in depth, identifying current macro- and micronutrient levels, and formulating effective soil management strategies. This study geographically maps nutrient availability across districts and provides region-specific suggestions to improve productivity and promote sustainable apricot cultivation, differing from previous studies.

Materials and Methods

Soil sampling was conducted between 2021 and 2022 across multiple districts in Malatya province, covering diverse ecological and agronomic conditions. A total of 30 soil samples

were collected from different apricot orchards, ensuring spatial representation across the study area.

At each sampling location, three replicate samples were taken at a depth of 0-30 cm, following standard soil sampling protocols (Jackson, 1958). These samples taken within a 2-meter radius were later homogenized to form a composite sample, minimizing small-scale variability and enhancing statistical robustness. The selection of sampling points was based on the spatial distribution criteria. Sampling locations

were systematically distributed across Akçadağ (Bölüklü (38°15'42.09"N 37°55'12.76"E), Ören (38°14'45.23"N 37°56'0.33"E)), Yeşilyurt (Bindal (38°25'14.92"N 38°10'33.59"E)), Battalgazi (Karahana (38°24'56.66"N 38°22'42.27")), Kuluncak (Karaçayır (38°52'17.85"N 37°44'52.30"E), Alvar (38°54'28.47"N 37°37'19.36"E), Çayköy (38°50'57.19"N 37°38'59.33"E), and Sofular (38°56'23.43"N 37°33'23.67"E)), Hekimhan (Kurşunlu (38°37'50.04"N 37°51'55.66"E)), and Yazihan Dedekargın (38°28'50.49"N 38°19'48.41"E)) to ensure comprehensive coverage of the region.

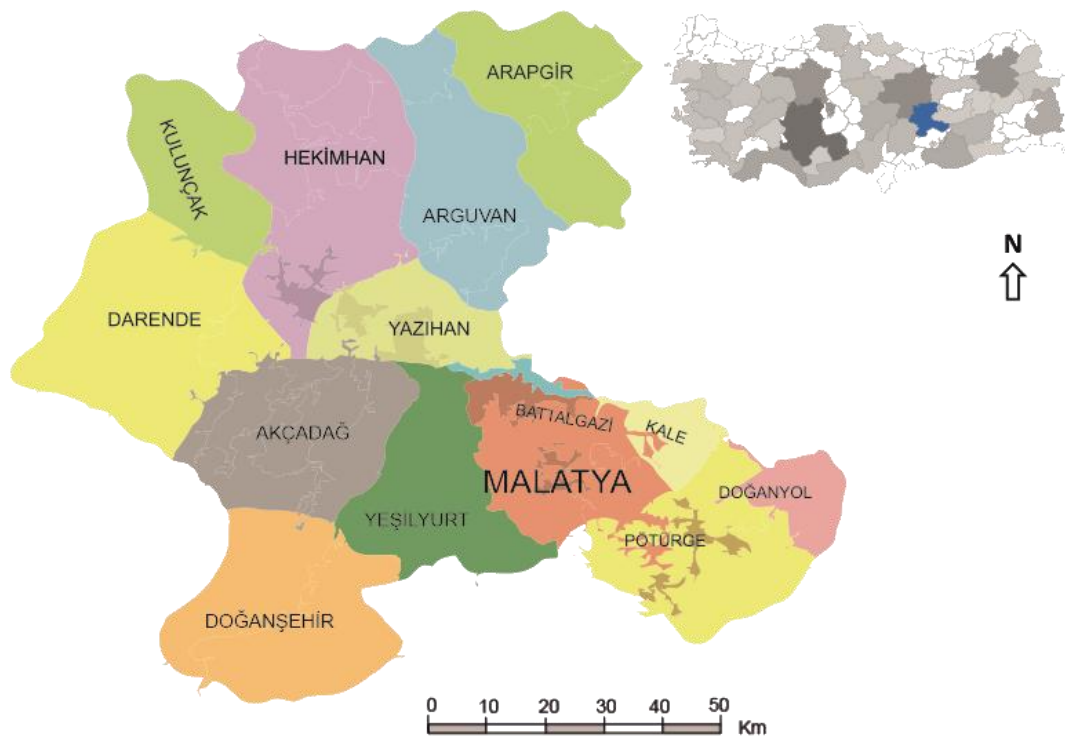


Figure 1. Map of Malatya province

Malatya province spans an area of 12,313 km², situated between 35° 54' and 39° 03' north latitude and 38° 45' and 39° 08' east longitude (Figure 1). Located in the Upper Euphrates region of Eastern Anatolia, Malatya does not fully exhibit

the typical continental climate that prevails in many parts of the region. Instead, it experiences a transition between a subtropical and continental climate, making it a distinct microclimatic zone.

Table 2. Malatya climate data (Meteorology General Directorate, 2025)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Temperature (°C)	-0.2	1.6	6.8	13	18	23.1	27.1	27.1	22.5	15.6	8	2.2	13.7
Highest Temperature (°C)	3.2	5.6	11.7	18.5	24	29.7	34	34	29.2	21.4	12.6	5.6	19.1
Lowest Temperature (°C)	-3.2	-2	2.3	7.6	11.9	16.3	19.9	20	15.6	10	4	-0.7	8.5
Precipitation (mm)	42.4	41.1	48.8	55	45.6	17.4	3.9	3.5	8.1	35.7	41.7	40.4	383.6

The annual average number of cloudy days is 77 days in the province. Additionally, the province experiences 152 days of partly cloudy weather

and 136 days of clear skies per year. The average number of foggy days is 13 per year, mostly occurring in December and January. Cloudy days

are primarily observed during the winter and spring months.

Long-term climate data for the province, recorded between 1929 and 2024, is presented in Table 2. According to MGM (2025) data, the highest average temperature is recorded in July at 34.0°C, while the lowest average temperature occurs in January at -3.2°C.

Soil Analyses

Soil samples were collected from 0–30 cm depth, with three replications per location. Soil samples were collected to ensure a homogeneous representation of the study area. In the laboratory, the samples were air-dried, sieved through a 2 mm mesh, and prepared for analysis. Soil pH was measured using the Peech method (1965) (1:25 w/v), while electrical conductivity (EC) was determined in deionized water following the Bower and Wilcox method (1965) (1:5 w/v). Exchangeable cations were analyzed using CH₃COONH₄ (Ammonium Acetate, pH 7.0) according to Knudsen et al. (1982). Organic carbon content was determined using the wet oxidation method with potassium dichromate (Nelson & Sommers, 1982). Total nitrogen (N) was measured using the Kjeldahl method (Mulvaney & Page, 1982), while plant-available metals (Fe, Cu, Mn, Zn) were extracted using DTPA and analyzed via Atomic Absorption Spectroscopy (AAS) (Pueyo et al., 2004). Available phosphorus (P) was determined through NaHCO₃ extraction and spectrophotometric analysis (Olsen & Dean, 1965). Plant-available boron (B) was extracted with 0.01 M mannitol + CaCl₂, and its concentration was determined based on the color intensity of the complex formed with carmine (anthraquinone dye) (Hatcher & Wilcox, 1950).

Soil Property Mapping

The analyzed soil properties were mapped using ArcGIS 10.8 (Figure 2). During the mapping process, the analyzed parameters were first matched with district-specific coordinate data and integrated into a spatial database. In this study, we used a PCS for spatial mapping and

interpolation. Specifically, we employed UTM coordinate system, Zone 37N, based on the WGS84 datum. Ordinary Kriging was used for the soil parameters because it accounts for spatial autocorrelation, making it suitable for soil properties that exhibit a strong regional trend (Goovaerts, 1997). This method was chosen based on their effectiveness in modeling different soil properties and their proven application in agricultural soil mapping studies.

Ordinary Kriging is a geostatistical interpolation method that works with spatial data and is defined as BLUE (Best Linear Unbiased Estimator). This method provides a "best" (minimum error variance), "linear" (uses weighted linear combinations of available data) and "unbiased" (mean error equal to zero) estimate. The basic principle of Ordinary Kriging is to estimate the value of a regionalized variable ($Z(s_0)$) at a location to be estimated (1) (Mesic Kis, 2016):

$$Z(s_0) = \sum_{i=1}^n \lambda_i Z(s_i) \quad (1)$$

(Webster & Oliver, 2007)

λ_i : Weight coefficients,

$Z(s_i)$: Observations at available data points,

n : The number of data points used.

These weights, the no bias condition (2) and the Minimum Variance Condition (3) are determined to satisfy the following conditions (Journel & Huijbregts, 1978) :

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

$$\min \text{VAR} \left[Z(s_0) - \sum_{i=1}^n \lambda_i Z(s_i) \right] \quad (3)$$

To find the weights, the equation in matrix form in equation 3 is used (Malvić & Balić, 2009) :

$$\begin{bmatrix} \gamma(s_1, s_1) & \gamma(s_1, s_2) & \cdots & \gamma(s_1, s_n) & 1 \\ \gamma(s_2, s_1) & \gamma(s_2, s_2) & \cdots & \gamma(s_2, s_n) & 1 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ \gamma(s_n, s_1) & \gamma(s_n, s_2) & \cdots & \gamma(s_n, s_n) & 1 \\ 1 & 1 & \cdots & 1 & 0 \end{bmatrix} \cdot \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \vdots \\ \lambda_n \\ \mu \end{bmatrix} = \begin{bmatrix} \gamma(s_1, s_0) \\ \gamma(s_2, s_0) \\ \vdots \\ \gamma(s_n, s_0) \\ 1 \end{bmatrix} \quad (3)$$

here:

- $\gamma(s_i, s_j)$: refers to the semivariogram value between points s_i and s_j ,
- μ : Lagrange multiplier.
- *Statistical Analysis*

- To assess the differences in soil properties, data were analyzed using one-way analysis of variance (ANOVA). A p-value of <0.05 was considered statistically significant, and Tukey's test was applied for multiple comparisons. Relationships among the investigated properties were examined using Pearson correlation and linear regression analyses. All statistical analyses were conducted using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA).

- Hierarchical clustering analysis (HCA) was performed to determine the similarities among the soil properties of Malatya's districts. All analyses were carried out using Python's Pandas, NumPy, Seaborn, and SciPy libraries. Additionally, data normalization and statistical tests were conducted using SPSS and Python-supported analyses. The relationships between soil properties were assessed through Pearson correlation analysis, and the silhouette score was calculated to evaluate clustering accuracy.

Results and Discussion

Soil pH and Soil Salinity

Soil pH plays a crucial role in nutrient uptake and directly affects the availability of essential elements. The analysis of Malatya's soils revealed that pH levels were generally neutral to slightly alkaline, though some areas exhibited pH values >8 . Elevated pH levels significantly reduce the solubility of micronutrients such as Zn and Fe, making them less available for plant uptake. This deficiency is a common issue in apricot orchards and can lead to reduced fruit quality and yield.

Globally, low soil pH is recognized as a major factor contributing to crop yield losses. Milosevic et al. (2010) identified excessively dry, shallow, sandy-loam, and acidic soils as the most limiting edaphic factors for apricot cultivation. In this study, the average soil pH was 7.78, indicating a slightly alkaline nature. The lowest pH (7.25) was recorded in Arguvan, while the highest (8.13) was observed in Kuluncak (Figure 2). While slightly alkaline soils can be beneficial for apricot growth, excessively high pH levels restrict the availability

of essential nutrients, particularly micronutrients.

Soil pH directly affects the solubility of nutrients in the soil solution, which determines how easily plants can absorb them. The optimum pH range for most crops is 6.0-7.5. Outside this range, particularly above pH 7.5, the availability of Fe, Zn, and Mn decreases, making nutrient uptake more difficult.

Apricot is one of the fruit crops that is highly sensitive to salinity. According to Maas and Grattan (1999), the threshold soil salinity level for apricot yield reduction is 1.6 dS m^{-1} . Beyond this threshold, increased salinity levels lead to progressive yield losses.

However, the results of this study indicate that the average soil salinity in Malatya is 0.16%, which is classified as non-saline. The lowest salinity level (0.01%) was recorded in Doğanyol district, while the highest salinity level (0.44%) was observed in Darende district. These results suggest that soil salinity is not a significant limiting factor for apricot cultivation in Malatya.

The analysis showed that the average total lime content was 22.91%, with the lowest values recorded in Arguvan (1.26%), Kale (2.91%), Doğanşehir (3.24%), and Doğanyol (3.76%) (Figure 2). The highest lime content was found in Darende (51.19%), indicating that lime management is crucial for improving nutrient uptake in apricot orchards.

Lime and Organic Matter content

High calcium carbonate (CaCO_3) content contributes to increased soil pH, leading to alkaline soil conditions. One of the most common consequences of high lime levels is iron deficiency-induced chlorosis, which manifests itself as leaf yellowing of leaves and stunted plant growth. In calcareous soils, reducing pH or applying chelated iron fertilizers is recommended to increase Fe availability.

Soil organic matter is essential for improving soil structure, water retention, and nutrient availability. The soils of Malatya had a low content of organic matter, which has a negative impact on soil health, nutrient retention, and

water infiltration. A lack of organic matter leads to weaker soil structure, faster water drainage, and increased nutrient loss, all of which can negatively impact apricot growth and yield.

The analysis revealed that the average organic matter content was 1.59%, with the lowest level

recorded in Doğanşehir (0.61%) and the highest in Hekimhan (2.61%) (Figure 2). To improve soil fertility, it is essential to incorporate organic amendments such as green manure, compost, and organic fertilizers into the soil.

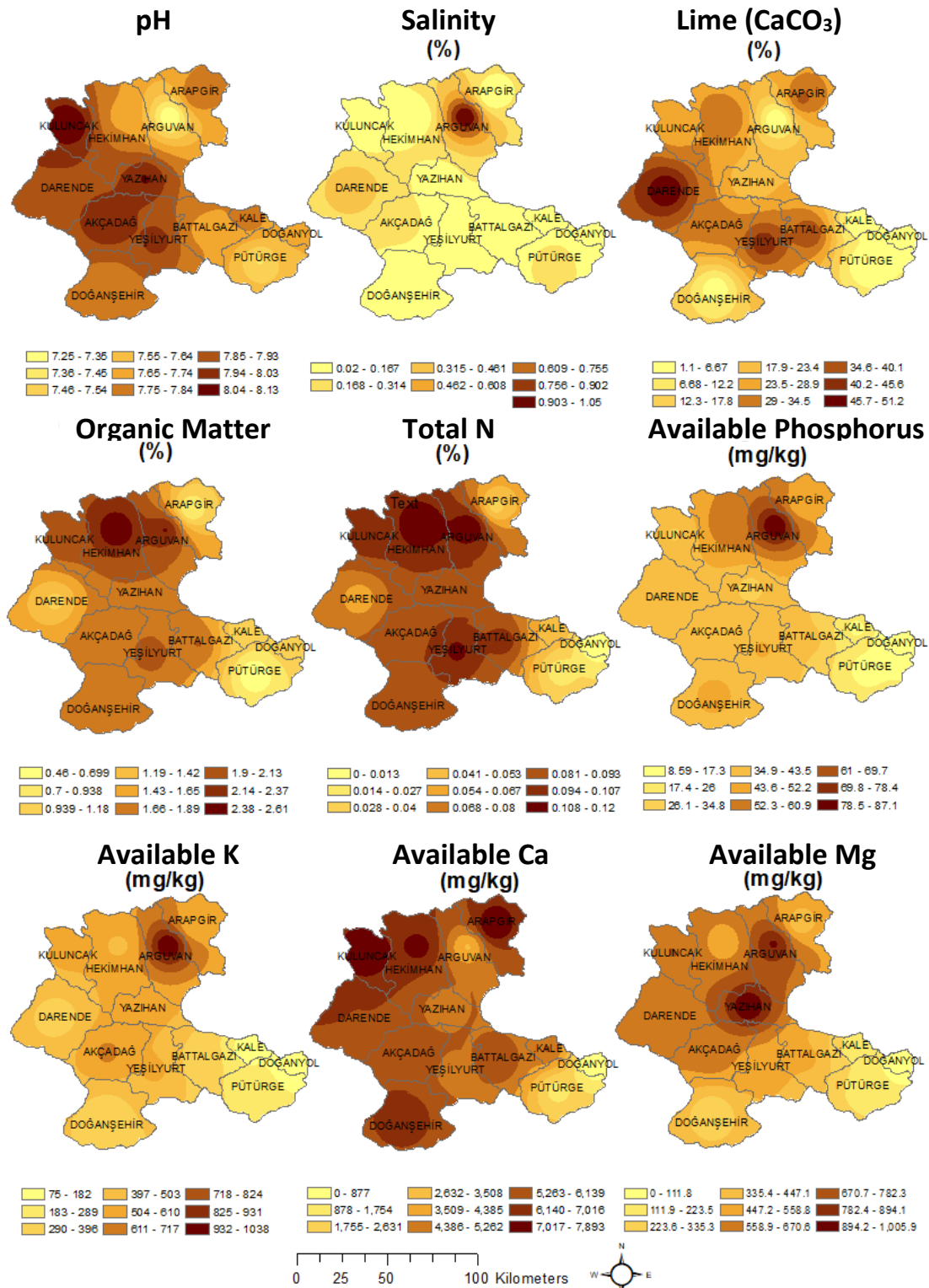


Figure 2. Some soil chemical properties and macronutrients of Malatya province soils

Soil pH Management and Fertilization Strategies

Effective soil pH management is key to optimizing nutrient availability and improving plant health and productivity. In high pH soils, nutrient uptake is limited and requires targeted interventions to maintain balanced soil conditions.

Lowering high soil pH: The application of elemental sulfur or organic matter amendments can help reduce soil alkalinity and improve nutrient availability. **Regular soil testing:** Conducting routine soil analysis allows for the development of site-specific fertilization programs that address nutrient deficiencies. **Chelated micronutrient applications:** In alkaline soils, the use of chelated Fe, Zn, and Mn fertilizers can significantly improve plant nutrient uptake. High soil pH and excessive lime content can negatively affect plant nutrient uptake, ultimately leading to yield and quality losses. Therefore, implementation of soil amendments, targeted fertilization strategies, and regular soil monitoring are essential to ensure sustainable apricot production in Malatya.

Macronutrients

Nitrogen is essential for plant growth, development, and flower formation. Its deficiency reduces flower production and negatively affects fruit yield (Karlıdağ et al., 2017). Additionally, nitrogen significantly influences dehydrogenase and urease activities, which are key indicators of total microbial activity in soil (Dick, 1994).

In this study, the average total nitrogen content was 0.08%, with the lowest values (0.02%) recorded in Doğanyol and Pütürge, while the highest (0.12%) was found in Hekimhan and Arguvan (Figure 2). According to Bussi et al. (2003), high nitrogen fertilization enhances vegetative growth, yield, and average fruit weight, while increasing K fertilization improves soluble solids and fruit color. However, Maas and Grattan (1999) reported that excessive nitrogen applications can promote excessive vegetative growth, leading to shading, poor fruit color, and

reduced sugar accumulation. Similarly, Radi et al. (2003) found that optimum nitrogen levels were the most influential factor in improving fruit quality. Additionally, Bussi and Smith (1998) noted that increasing nitrogen fertilization increases the nitrogen content of the fruit while decreasing the Ca concentration, which may affect fruit firmness and shelf-life.

Phosphorus plays a vital role in plant metabolism and energy transfer processes, as it is a key element in the nitrogen cycle, nitrate uptake, amino acid conversion, and protein synthesis during photosynthesis (Marschner, 1995). It also facilitates protein transfer to seeds during flowering and seed maturation through ATP-driven mechanisms. The average available phosphorus content was 37.68 mg kg⁻¹, with the lowest value (7.26 mg kg⁻¹) recorded in Pütürge and the highest (87.15 mg kg⁻¹) in Arguvan. The differences between the regions are probably due to differences in fertilization practices and the phosphorus content of the parent soil materials.

Potassium is considered a quality factor in plants, as it contributes to growth and high agricultural yields (Usherwood, 1985). It is essential to produce ATP, which supports transpiration, photosynthesis, and general metabolic processes. Potassium plays a vital role in stimulating photosynthesis, improving oil, starch, protein, and vitamin synthesis, and enhancing fruit quality (Nitin et al., 2017). In this study, potassium availability in Malatya soils was generally high, with an average of 439.25 mg kg⁻¹. The lowest value (117 mg kg⁻¹) was found in Kale, while the highest (1,038 mg kg⁻¹) was found in Arguvan. The high potassium content of Malatya's soils explains why apricots grown in the region are superior in flavor, aroma, mineral content, and vitamins compared to apricots from other regions.

Calcium is the most abundant alkali earth metal in the Earth's crust, comprising 2.94% of the upper continental crust. Calcareous or Ca²⁺-rich soils cover over 30% of the Earth's surface, while basic soils account for at least 12% of global

soil resources (Bertrand et al., 2007; Rowley et al., 2018). One of the best-known function of Ca in plants is stabilize cell membranes and maintain cellular integrity (Kirkby & Pilbeam, 1984). In addition, calcium has been shown to inhibit pathogen activity in jujube fruits (Guo et al., 2016). Due to the cross-linking between Ca^{2+} and carboxyl groups in pectin, calcium is widely used to extend the shelf life of apricots by preventing cell wall degradation (Liu et al., 2017). In this study, the average available Ca content was 5,127.04 mg kg⁻¹. The lowest value (75 mg kg⁻¹) was recorded in Doğanyol, while the highest (7,893 mg kg⁻¹) was found in Arapgir.

Magnesium is involved in many physiological and biochemical processes that are essential for plant growth and development. It plays a key role in plant defense mechanisms against abiotic stress and is most widely known as the central atom in the chlorophyll molecule, which is essential for photosynthetic carbon fixation (Şenbayram et al., 2015). The average available magnesium content was 490.20 mg kg⁻¹, with the lowest value (122 mg kg⁻¹) in Doğanyol and the highest value (1,006 mg kg⁻¹) in Yazıhan. This variation suggests that soil amendments may be necessary in certain regions to ensure optimal magnesium availability for plant growth.

Micronutrients

Micronutrients are essential for plant growth and metabolic processes. Soil analyses in Malatya have shown variations in micronutrient levels, particularly in elements such as Zn, Mn, B, Cu, and Fe, which play a critical role in apricot cultivation (Figure 3). Deficiencies in these micronutrients can result in poor tree growth, reduced fruit set, and poor fruit quality in apricots. This section examines micronutrient deficiencies, their relationship to soil pH, and their effect on apricot fruit production.

Zinc is required for enzymatic reactions, metabolic processes, and redox reactions in

plants, animals, and humans. It is essential for nitrogen metabolism, energy transfer, and protein synthesis by activating various enzymes (Hafeez et al., 2013). Increased soil pH promotes Zn adsorption onto soil components (e.g., clay minerals and metal oxides), significantly reducing its availability. The soluble Zn content in soil solution decreases by 30 to 45 times for each unit increase in soil pH (Gupta et al., 2016; Gondal et al., 2021). In Malatya, critical Zn deficiencies were detected in Kale (0.17 mg kg⁻¹) and Battalgazi (0.82 mg kg⁻¹) (Figure 3). Due to high soil pH and lime content, the solubility of Zn decreases, making it difficult for plants to absorb. Zinc deficiency in apricot trees causes stunted growth, bronzing of leaves, and small leaf formation (Alloway, 2008). Moreover, Zn deficiency reduces fruit size, delays fruit ripening, and lowers fruit sugar content, affecting overall fruit quality and marketability (Erdal et al., 2004). To correct zinc deficiency, zinc sulfate or chelated zinc fertilizers should be applied through both foliar sprays and soil amendments.

Manganese plays a vital role in photosynthesis and oxidative stress regulation and is essential for carbohydrate and lipid metabolism in plants (Alloway, 2008). As soil pH increases, manganese oxidizes into forms that are less available to plants, making it one of the primary limiting factors for Mn uptake (Fageria, 2001). The analysis showed that Mn levels were high in Pütürge (37.33 mg kg⁻¹) and Arguvan (11.78 mg kg⁻¹) but low in Kale (1.37 mg kg⁻¹) and Battalgazi (1.46 mg kg⁻¹). Manganese deficiency is commonly observed in high-pH soils, leading to chlorosis (leaf yellowing) and leaf spotting (Figure 3). In apricot trees, Mn deficiency reduces fruit firmness and affects skin color, leading to paler, less vibrant fruit with reduced shelf life (Tisdale et al., 1993). In low-Mn areas, manganese sulfate foliar sprays should be applied, and pH-lowering soil amendments should be considered.

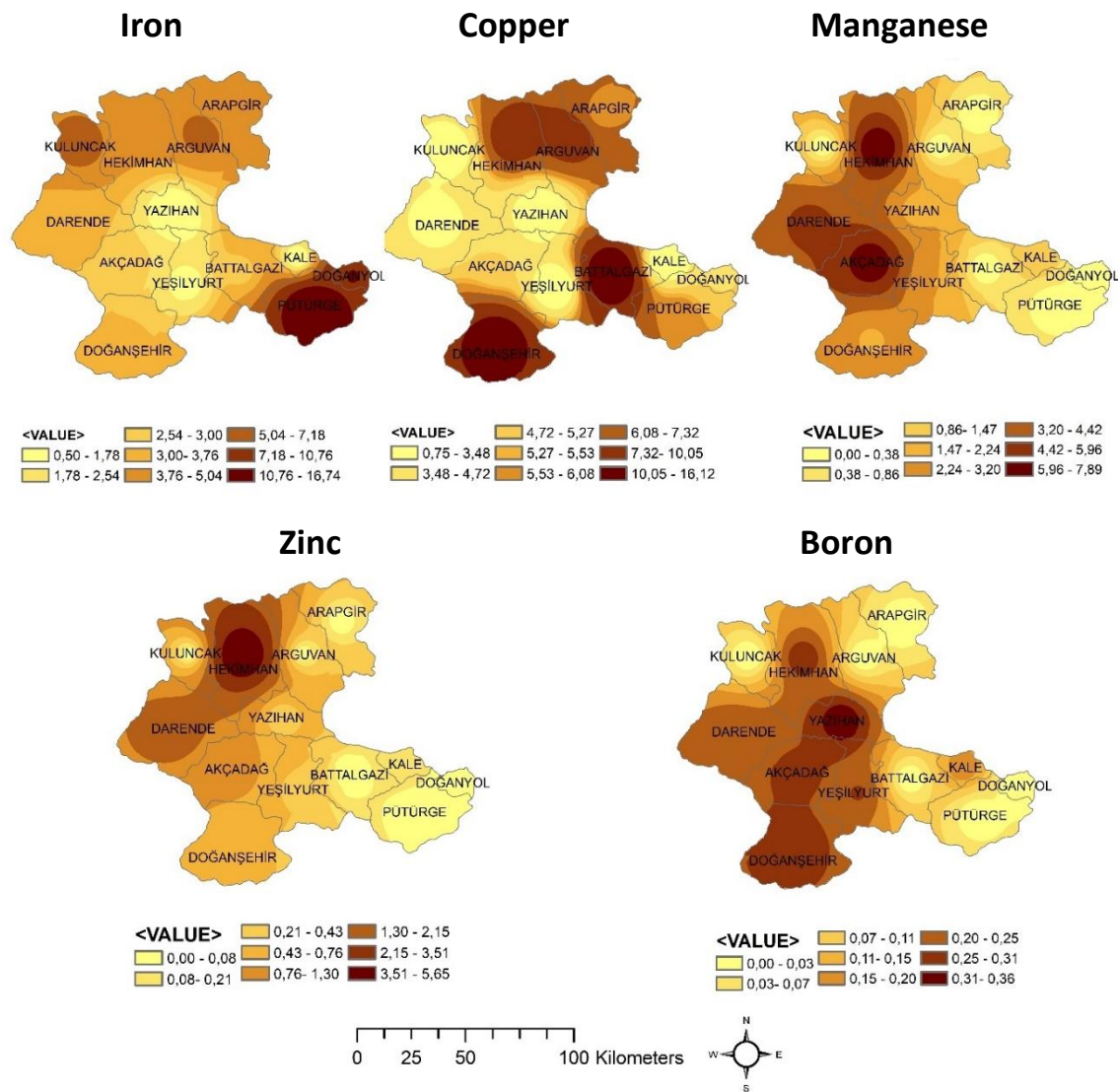


Figure 3. The micronutrient content of soils in Malatya province.

Boron is critical for cell wall development and flowering (Shorrocks, 1997). Boron deficiency adversely affects pollen germination and fruit set, resulting in reduced apricot yields (Brown et al., 2002). In soils with low B content, cell division and growth processes are disrupted (Sotiropoulos et al., 2019). Soil analysis showed that B levels ranged from 0.17 mg kg⁻¹ in Kale to 0.47 mg kg⁻¹ in Kuluncak (Figure 3). Boron deficiency during flowering significantly reduces fruit set, resulting in small, misshapen, or cracked fruits (Wójcik & Treder, 2006). Apricot trees suffering from boron deficiency often show hollow or spongy fruit centers and premature fruit drop (Nyomora et al., 1997). In low-B regions, B-fertilizers (e.g., borax) should be applied carefully to avoid toxicity due to over-application.

Copper is essential for lignin synthesis and plays a role in disease resistance, chlorophyll

synthesis, and respiratory enzymes (Marschner, 2012). However, excessive copper accumulation can lead to phytotoxicity (Kabata-Pendias, 2010). Soil analysis in Malatya indicated that copper levels were generally adequate (Figure 3). However, in high lime soils, Cu can be tightly bound, limiting plant uptake (Yadav et al., 2019). In apricots, Cu deficiency reduces fruit firmness and color intensity while increasing fruit susceptibility to cracking and fungal infection (Reuter & Robinson, 1997). In areas of low Cu availability, copper sulfate or copper-based fungicides should be applied carefully to avoid toxicity.

Iron is essential for chlorophyll synthesis and photosynthesis. High soil pH significantly reduces Fe bioavailability, as Fe precipitates in forms that plants cannot absorb when pH exceeds 7 (Lindsay & Schwab, 1982). Additionally, high lime content

promotes the formation of iron phosphates, further restricting Fe availability (Mengel & Kirkby, 2001). The study found that Fe-deficiency is widespread in the high pH soils of Malatya, with Kuluncak (5.82 mg kg⁻¹) and Arguvan (5.87 mg kg⁻¹) showing particularly low levels. Reduced Fe solubility in high pH soils limits plant uptake, leading to chlorosis (leaf yellowing) in apricot

trees (Figure 3). Iron deficiency is known to reduce fruit size, lower sugar content, and delay fruit ripening, significantly affecting fruit quality and economic value (Álvarez-Fernández et al., 2006). To address Fe deficiency, foliar or soil applications of chelated iron (EDDHA-Fe) should be used.

Table 4. Pearson correlation analysis of soil properties in Malatya province

Parametre	EC	pH	CaCO ₃	OM	Total N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn	B
Saturation	0.34	0.326	0.616*	0.22	0.282	0.445	0.324	0.598*	0.408	0.015	-0.3	-0.02	-0.318	0.137	0.023
EC		-0.5	-0.158	0.29	0.309	0.678*	0.713**	-0.131	0.541	-0.027	0.072	0.078	0.085	0.259	0.151
pH			0.532	0.07	0.142	-0.236	-0.126	0.547	0.222	0.579*	-0.58	-0.49	-0.561	-0.18	0.243
CaCO ₃				0.18	0.275	0.158	0.095	0.548	0.255	0.025	-0.306	-0.12	-0.318	0.002	0.01
OM					0.974**	0.705**	0.658*	0.421	0.627*	0.515	-0.342	0.3	-0.362	0.501	0.617*
Total N						0.757**	0.673*	0.522	0.634*	0.497	-0.514	0.275	-0.543	0.361	0.567
P							0.856**	0.435	0.587*	0.111	-0.254	0.342	-0.281	0.429	0.384
K								0.237	0.786**	0.373	-0.119	0.035	-0.081	0.404	0.543
Ca									0.305	0.276	-0.685*	0.104	-0.760**	0.196	0.348
Mg										0.716**	-0.313	-0.21	-0.262	0.254	0.661*
Na											-0.333	-0.37	-0.32	0.279	0.841**
Fe												0.119	0.981**	0.358	-0.055
Cu													-0.006	0.069	0.038
Mn														0.301	-0.117
Zn															0.597

Significant at *P < 0.05, **P < 0.01 and ***P < 0.001; ns= not significant (P > 0.05).

Understanding the relationships between soil properties and nutrient availability is critical for optimizing soil fertility and improving apricot production. The correlation analysis in this study provides valuable insights into the interactions between macronutrients, micronutrients, and soil properties, which are essential for developing effective soil management and fertilization strategies. These findings are compared with previous studies to highlight the significance of nutrient interactions in different agricultural systems.

Soil pH and Micronutrient Deficiencies

Soil pH plays a fundamental role in nutrient availability. The positive correlation between pH and Ca (r = 0.547) and Mg (r = 0.579) suggests that higher pH conditions favor the solubility of these macronutrients, which is consistent with Lindsay & Schwab (1982), who reported that alkaline soils increase Ca and Mg availability but significantly decrease Fe, Mn, and Zn solubility.

This study confirms these findings, as Fe (r = -0.58), Mn (r = -0.561), and Zn (r = -0.183) showed negative correlations with pH. A similar pattern

was observed by Mengel & Kirkby (2001) in Mediterranean soils, where high pH levels in calcareous soils caused severe Fe and Mn deficiencies in fruit crops, resulting in leaf chlorosis and reduced yield.

Furthermore, Fageria et al. (2018) demonstrated that Fe solubility decreased by nearly 1000-fold when pH increased from 5.5 to 7.5, further supporting the need for acidifying amendments such as elemental sulfur or organic matter applications to improve Fe bioavailability in apricot orchards.

Lime (CaCO₃) Content and Nutrient Solubility

Lime content significantly affects nutrient interactions in soils. The positive correlation between CaCO₃ and Ca (r = 0.548) is expected, because calcareous soils naturally contain high levels of Ca. However, the negative correlations with Fe (r = -0.306) and Mn (r = -0.318) confirm that excess lime limits the solubility of these micronutrients, in agreement with Marschner (2012).

Studies by Rombolà & Tagliavini (2020) indicate that Fe and Mn deficiencies are

widespread in high lime soils due to the precipitation of these elements as insoluble oxides, reducing their uptake by plants. This suggests that chelated Fe and Mn fertilizers (EDDHA-Fe) are necessary in apricot orchards to counteract lime-induced deficiencies.

Organic Matter and Nutrient Retention

Organic matter plays a crucial role in nutrient retention and microbial activity. The strong positive correlation between organic matter and total N ($r = 0.974$, $p < 0.01$), P ($r = 0.705$, $p < 0.01$), and K ($r = 0.658$, $p < 0.05$) suggests that higher organic matter enhances soil fertility and nutrient availability.

This is consistent with the findings of Kabata-Pendias (2010), who reported that organic matter increases Zn availability by forming stable organic complexes that prevent Zn from being adsorbed to soil particles. The positive correlation between organic matter and Zn ($r = 0.617$, $p < 0.05$) further supports this mechanism.

Additionally, Havlin et al. (2005) found that organic matter application in orchards significantly improved micronutrient uptake, enhanced root growth, and increased fruit yield, reinforcing the importance of compost and green manure application in Malatya's apricot orchards.

Soil Saturation and Nutrient Availability

Soil saturation reflects the water holding capacity of soil particles, which is critical for nutrient solubility and root uptake. The positive correlation of saturation with CaCO_3 ($r = 0.616$, $p < 0.05$) and Ca ($r = 0.598$, $p < 0.05$) suggests that calcareous soils retain more water, increasing the availability of Ca. However, negative correlations with Fe ($r = -0.3$) and Mn ($r = -0.318$) indicate that excess soil moisture reduces the solubility of these micronutrients, potentially leading to iron and manganese deficiency.

Similar findings were reported by Singh et al. (2017), who found that high soil saturation in calcareous soils reduced Fe and Mn availability, leading to chlorosis in fruit trees. In contrast, non-calcareous soils with lower water retention

showed improved Fe and Mn uptake, supporting the idea that drainage and aeration management are crucial for micronutrient solubility in apricot orchards.

Soil Salinity and Nutrient Uptake

Soil salinity has a significant effect on nutrient mobility and plant uptake. In this study, salinity showed a strong positive correlation with P ($r = 0.678$, $p < 0.05$) and K ($r = 0.713$, $p < 0.01$), suggesting that higher salinity may increase the availability of these macronutrients. However, the negative correlation with pH ($r = -0.502$) indicates that saline soils tend to be more acidic, a finding consistent with Hussain et al. (2020), who observed that salinity-induced soil acidification increases phosphorus solubility but limits the availability of micronutrients like Fe and Zn.

Römheld and Marschner (1991) further support these results, showing that high salt concentrations in soil solution enhance P and K solubility while simultaneously reducing Fe and Zn availability, leading to nutrient imbalances in fruit orchards. This highlights the need for site-specific fertilization strategies to compensate for salinity-induced nutrient deficiencies.

Interactions Between Macro and Micronutrients

Phosphorus and potassium (P-K relationship): A strong positive correlation ($r = 0.856$, $p < 0.01$) was observed between P and K, indicating that both nutrients complement each other in plant development. Similar results were reported by Alloway (2008), who found that P-K interactions are critical for improving fruit quality and resistance to abiotic stresses.

Calcium and magnesium (Ca-Mg relationship): A moderate positive correlation ($r = 0.305$) was observed between Ca and Mg. However, excess Ca can interfere with Mg uptake, a phenomenon well documented by Rombolà & Tagliavini (2020).

Iron and Manganese (Fe-Mn relationship): A strong positive correlation ($r = 0.981$, $p < 0.01$) suggests that both elements are influenced by oxidation-reduction processes in the soil, similar to the findings of Fageria (2001) in calcareous

orchard soils.

This logarithmic scale clustered heatmap visualizes the similarities and differences in soil parameters among Malatya's districts (Figure 4). In the graph, the districts are listed on the vertical axis, while the soil parameters are displayed on the horizontal axis. The clustering algorithm groups districts with similar soil properties, enabling a more systematic analysis of regional soil characteristics. Color intensity, ranging from light green to dark green, represents different levels of soil parameters, with light green indicating low values and dark green representing

high values.

The heat map analysis reveals that Arguvan, Hekimhan, and Kuluncak have similar soil properties, characterized by high lime (CaCO_3), Ca, and organic matter content. In contrast, Pütürge and Yazıhan form a distinct cluster, significantly differing from other districts in their soil characteristics. Darende and Akçadağ show moderate similarity, sharing some common soil characteristics but remaining distinct from the other major clusters.

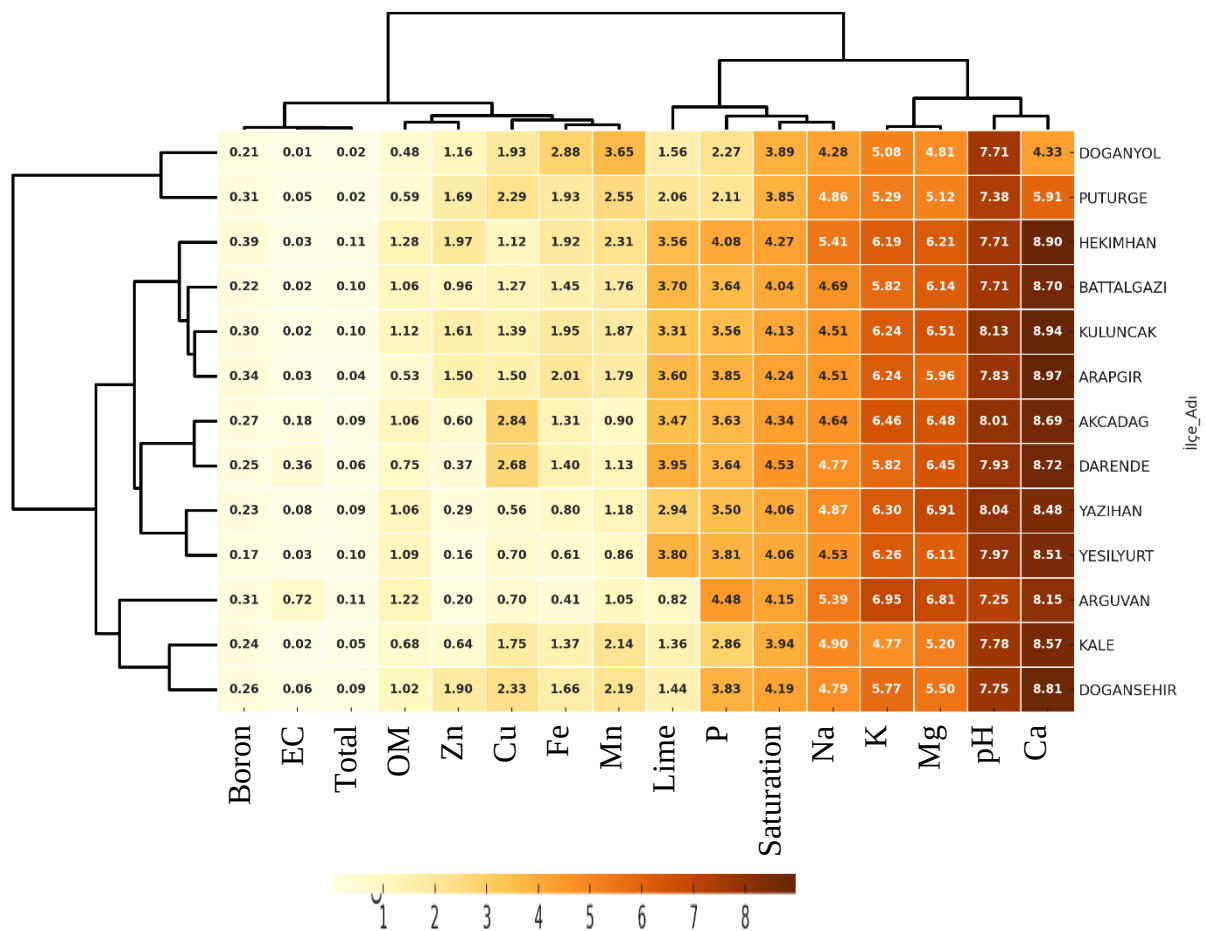


Figure 4. Clustered heatmap of soil parameters in Malatya districts

Examining the relationships between soil parameters, it is evident that pH, lime (CaCO_3), and Ca are closely clustered together, reinforcing the well-documented relationship that high pH soils tend to have elevated lime and calcium contents. Similarly, P, K, and Mg appear in the same cluster, suggesting that these elements play complementary roles in plant nutrition and tend to coexist in similar soil conditions. Additionally,

organic matter and N are grouped together, highlighting that organic matter content directly influences nitrogen availability, which aligns with previous studies on soil fertility dynamics.

Based on these findings, district-specific soil management strategies should be developed to enhance apricot cultivation in Malatya. Districts within the same cluster can benefit from common fertilization and soil management programs,

while distinct clusters require customized strategies. For example, in Hekimhan, Kuluncak, and Arguvan, where similar micronutrient deficiencies are likely, joint fertilization programs focusing on micronutrient supplementation can be implemented. Conversely, in Pütürge and Yazihan, which have different soil characteristics, targeted soil amendments and pH management strategies should be applied to optimize soil fertility.

In areas with high pH levels, deficiencies of Fe, Zn, and Mn are more prevalent, requiring foliar applications or soil amendments rich in these micronutrients. Similarly, regions with low potassium levels should be supplemented with potassium sulfate or potassium nitrate fertilizers to maintain optimal soil conditions.

Apricot is one of the most important fruit crops due to its high nutritional value and long history of cultivation (Karlıdağ et al., 2019). Apricot trees require significant amounts of mineral nutrients for proper growth and fruit production. To maintain optimal nutrient levels and sustain high yields, it is essential to supplement deficient nutrients either through soil fertilization or foliar applications (Medan, 2020).

Overall, this analysis underscores the importance of scientific data-driven agricultural management strategies for improving apricot production in Malatya. Clustering districts based on soil characteristics allow for more efficient agricultural policies, contributing to the sustainable enhancement of apricot yields in the region.

Conclusion

The soils of Malatya province are predominantly calcareous and alkaline, with neutral to high pH values throughout the soil profile. The high levels of Ca and K in these soils contribute positively to the flavor, aroma, vitamin, and mineral composition of apricots grown in the region. However, the alkaline nature of the soil poses a significant challenge to the availability of essential micronutrients such as Fe,

Zn, B, and Mn. Additionally, a considerable portion of applied P binds with Ca, forming insoluble compounds, which limits its availability to plants.

To enhance soil fertility and apricot productivity, targeted soil management strategies should be implemented. The following recommendations are essential for improving soil conditions and sustaining high apricot yields in Malatya:

Regular soil testing to monitor pH, salinity, and organic matter levels, ensuring informed decision-making for soil amendments and fertilization. Acidifying soil amendments, such as elemental sulfur or organic matter applications, to lower soil pH and enhance the availability of Fe, Mn, and Zn. Organic material incorporation, including compost and agricultural residues, to improve soil productivity by enhancing water retention capacity, cation exchange capacity, organic matter content, and nitrogen fractions while increasing the availability of P, K, Fe, Zn, and Mn. Balanced fertilization programs, considering the interaction between P and K, as well as the competition between Ca and Mg, to optimize nutrient uptake and minimize antagonistic effects. Chelated micronutrient fertilizers (EDDHA-Fe, Mn, Zn) to counteract lime-induced deficiencies and ensure optimal nutrient absorption by apricot trees.

By adopting these science-based soil management practices, apricot farmers in Malatya can enhance soil fertility, improve nutrient availability, and sustain high-quality fruit production despite the challenges posed by calcareous soil conditions.

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Assessment of soil organic carbon stock in pistachio orchards established under semi-arid climates of Siirt province, Türkiye

Siirt ili yarı kurak iklim koşullarında tesis edilen fıstık bahçelerinde toprak organik karbon stokunun değerlendirilmesi

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ABSTRACT

The management of agricultural lands plays a crucial role in the global carbon (C) cycle. Intensive soil tillage is one of the primary factors contributing to the mineralization of soil organic carbon (SOC), leading to its release into the atmosphere as CO₂. This study determined the soil organic carbon stock (SOCS) in pistachio-growing areas, which have expanded significantly in recent years in Kurtalan district, Siirt province. Spatial variation of SOCS was also mapped within the study area to ensure its long-term monitoring over time. For this purpose, soil samples were collected from a depth of 0–20 cm in 72 pistachio orchards. The soil organic C (SOC) content and bulk density (Bd) of the collected soil samples were determined, and SOCS values were calculated to generate spatial distribution maps. The SOC content ranged from 2.85 g kg⁻¹ to 26 g kg⁻¹, while SOCS varied between 8.97 t ha⁻¹ and 73.74 t ha⁻¹. Significant variability in SOC and SOCS was observed among soil samples belonging to different texture groups. Additionally, SOC and SOCS values significantly decreased as orchard age increased. The SOC and SOCS values decreased by 20.3% and 22.7%, respectively in pistachio orchards older than 20 years compared 5 years old or younger orchards. This decline in SOC content was also reflected in the SOC:Clay ratio, a key indicator of soil structure stability, which dropped from an average of 0.32 in younger orchards to 0.20 in older orchards. This suggests that agricultural practices in pistachio orchards disrupt soil aggregation, accelerating organic C mineralization and reducing the soil's ability to retain organic matter over time. These results show the urgent need for sustainable soil management strategies to preserve soil integrity, enhance C retention, and support long-term agricultural sustainability in semi-arid pistachio cultivation areas.

Key Words: Soil Organic Carbon, Sustainable Soil Management, Spatial Distribution, Pistachio Orchard

Öz

Tarım arazilerinin yönetimi, küresel karbon (C) döngüsünde çok önemli bir rol oynamaktadır. Yoğun toprak işleme, toprak organik karbonunun (SOC) mineralizasyonunu artıran ve CO₂ olarak atmosfere salınmasına yol açan başlıca faktörlerden biridir. Bu çalışmada son dönemlerde Siirt iline bağlı Kurtalan ilçesinde yetiştiriciliği önemli oranda artan Siirt Fıstık bahçelerinde toprak organik C stoku (TOCS)'nın belirlenmesi ve yersel değişimlerinin haritalanarak izlenebilirliğinin sağlanması amaçlanmıştır. Bu amaçla, 72 farklı fıstık bahçesinden 0-20 cm derinlikten toprak örnekleri alınmıştır. Alınan toprak örneklerinin toprak organik C (TOC) içeriği ve

hacim ağırlığı belirlenerek TOCS değerleri hesaplanmış ve yersel değişimleri haritalanmıştır. TOC içeriği 2.85 g kg^{-1} ile 26 g kg^{-1} arasında değişirken, SOCS değerleri 8.97 t ha^{-1} ile 73.74 t ha^{-1} arasında değişmiştir. Çalışma alanındaki farklı tekstür gruplarına ait toprak örnekleri arasında TOC ve TOCS değerlerinde önemli düzeyde değişkenlik gözlenmiştir. Ayrıca, meyve bahçesi yaşı arttıkça, SOC ve SOCS değerleri önemli ölçüde azalmıştır. 20 yaş ve üzeri Siirtfıstığı bahçelerinde TOC ve TOCS değerleri 5 yaş ve altı bahçelere göre sırası ile %20.3 ve %22.7 oranında azalmıştır. TOC içeriğindeki bu düşüş, toprak agregat stabilitesinin önemli bir göstergesi olan SOC:Kil oranına da yansımış ve genç bahçelerde ortalama 0.32 iken yaşlı bahçelerde 0.20'ye düşmüştür. Bu durum, Siirtfıstığı bahçelerindeki tarımsal uygulamaların toprak agregasyonunu bozarak organik karbon mineralizasyonunu hızlandırdığını ve toprağın zaman içinde organik madde tutma kabiliyetini azalttığını göstermektedir. Bu sonuçlar, yarı kurak iklime sahip Siirtfıstığı ekim alanlarında toprak bütünlüğünü korumak, karbon tutma oranını artırmak ve uzun vadeli tarımsal sürdürülebilirliği desteklemek için sürdürülebilir toprak yönetimi stratejilerine acil ihtiyaç olduğunu göstermektedir.

Anahtar Kelimeler: Toprak Organik Karbonu, Sürdürülebilir Toprak Yönetimi, Yersel Değişim, Siirt Fıstık Bahçeleri

Introduction

Soil organic carbon (SOC) is a fundamental component for the sustainability of agricultural ecosystems. The SOC plays a role in processes related to climate change by affecting the carbon cycle in the atmosphere (Yadav et al., 2017). Similarly, it is critically important in regulating nutrient and water cycles in the soil, as well as creating habitats for biodiversity (Padarian et al., 2022). The SOC also plays a significant role in increasing agricultural productivity by enhancing soil aggregate stability, increasing erosion resistance, regulating bulk density, improving water retention capacity to reduce drought stress, and supporting soil health by promoting microbial activity (Ahmad et al., 2025; Chowdhury et al., 2021; Francaviglia et al., 2023).

The SOC represents ~70-75% of the terrestrial carbon pool, meaning that even small changes in this pool can lead to significant and widespread impacts on the global carbon cycle (Muñoz-Rojas et al., 2017). In this context, the accumulation of SOC in the soil and its ability to maintain stability over long periods are crucial for combating climate change and ensuring the sustainability of soil health. The accumulation of SOC significantly varies depending on land use and the soil management strategies (Beillouin et al., 2022; Lessmann et al., 2022). Human activities such as deforestation, intensive tillage, inadequate crop rotation, excessive use of fertilizers and pesticides, and biomass burning lead to losses in the SOC pool (released into the atmosphere), which negatively impacts soil fertility and disrupts the ecosystem services provided by terrestrial

ecosystems (Abdulkadir et al., 2021; Adekiya et al., 2023; Kumar et al., 2020).

Approximately 11-30% of anthropogenic greenhouse gas emissions originate from agricultural lands (Acosta et al., 2024). The primary sources of CO_2 emissions from agricultural lands are excessive soil tillage and the use of fossil fuels in agricultural machinery (Sørensen et al., 2014; Hussain et al., 2021). Microbial activities occurring in the soil are the main source of CO_2 production, and the release of greenhouse gases into the atmosphere is directly related to factors such as soil pore volume, moisture content, and aggregation. Traditional tillage methods disrupt soil structure, leading to significant changes in moisture and temperature conditions. These changes, particularly the reduction of soil moisture and the formation of oxygen-rich environments, create conditions for the rapid decomposition of organic matter by microorganisms. As a result, SOC is converted to CO_2 and released into the atmosphere (de Oliveira Silva et al., 2019).

The role of SOC in determining soil structure and workability has gained increasing attention, particularly in relation to its balance with clay content. The SOC:clay ratio, proposed by Johannes et al. (2017), serves as a key criterion for assessing soil physical quality. This ratio helps determine whether the SOC content is sufficient relative to the clay fraction, classifying soil structural conditions based on threshold values. In light of these processes, this study focuses on the semi-arid region of Kurtalan, Siirt, where

intensive soil tillage is prevalent in pistachio orchards. The objective is to assess the SOC, SOCS levels and OC:Clay ratio and map their spatial variation within these orchards, as such practices may significantly influence the carbon dynamics and sustainability of the soil. Mapping these variations could provide critical insights into how soil management strategies in this region affect SOC stocks and contribute to broader understanding of carbon sequestration potential in semi-arid agricultural landscapes. Additionally, given its potential as an indicator of soil health and sustainable land management, this study considers the SOC:clay ratio as a key criterion for evaluating the effects of pistachio orchard age on soil degradation and identifying strategies for improving soil structure.

Material and Methods

Study Area

The study area encompasses ~2208 ha of pistachio orchards located in the Kurtalan district of Siirt province, between the coordinates 37°52'48" - 37°57'53" north latitude and 41°26'59" - 41°35'18" east longitude (Figure 1). Situated in the southeastern region of Türkiye, the study area exhibits a transitional climate between the continental and Mediterranean climates, with an average elevation of 1125 meters above sea level. The surrounding terrain is mountainous, with less rugged areas in the southern parts, and these geographical characteristics significantly influence the region's climatic conditions. The long-term average annual precipitation in the area is 572.9 mm, with a relative humidity of 52.8% and a mean temperature of 17.5°C (Karaman and Turan, 2019). Geologically, the region is underlain by the Germik and Şelmo formations (Yeşilova and Helvacı, 2013). The Germik formation is primarily composed of gypsum, anhydrite, dolomite, and limestone, while the Şelmo formation consists of continental sediments, which are believed to have originated in an ancient lake or sea basin.

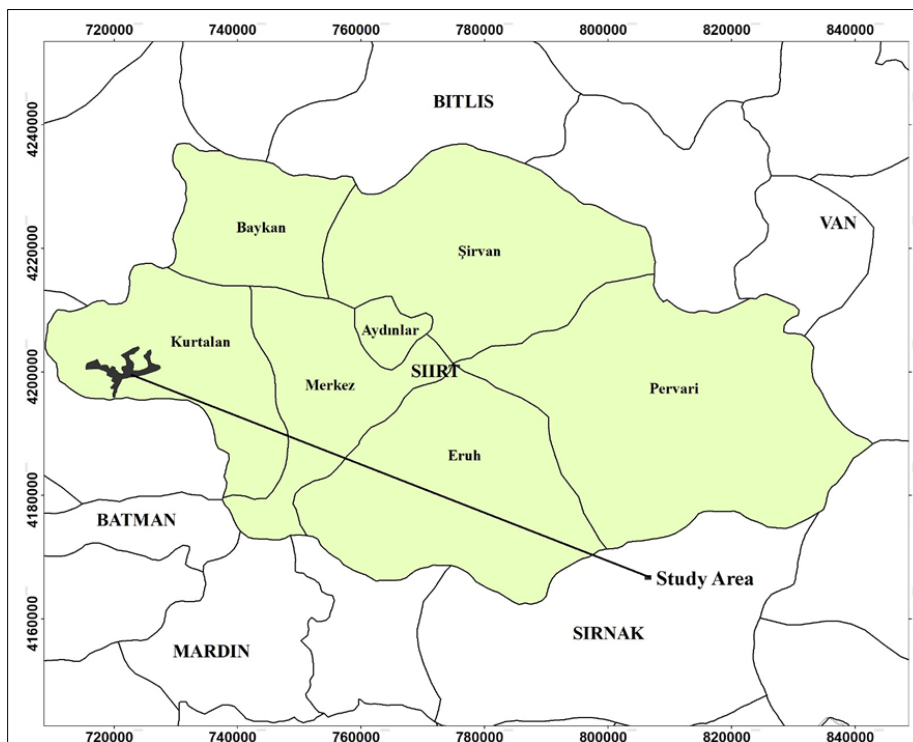


Figure 1. Location of study area

Soil Sampling

To assess the carbon storage potential of soils in pistachio orchards located in the Kurtalan

district of Siirt province, soil samples (disturbed and undisturbed) were collected from 72 orchards (Figure 2) at a depth of 0–20 cm using a

random sampling method. Undisturbed soil samples were collected using two 100 cm³ steel cylinders from each sampling point. In each orchard, disturbed soil samples were taken from four different points, combined in a container, thoroughly mixed, and approximately 1 kg of the

composite sample was brought to the laboratory for analysis. Immediately after collection, disturbed samples were placed in airtight polyethylene bags and undisturbed cores in steel cylinders were wrapped with parafilm to preserve structure and moisture.

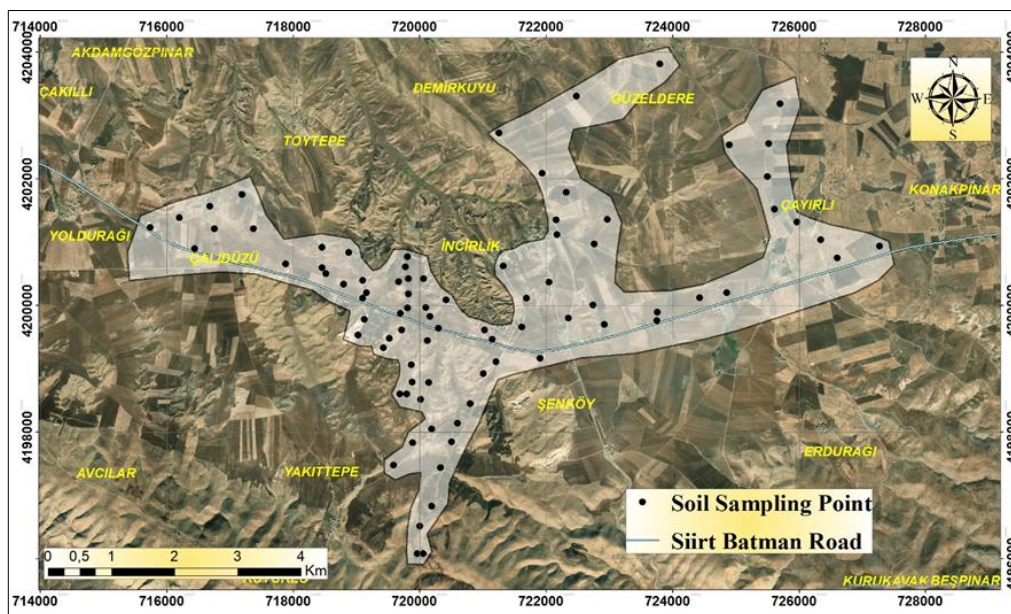


Figure 2. Soil sampling locations in the study area

Soil Analysis

Disturbed soil samples were air-dried at room temperature and sieved through a 2 mm mesh for laboratory analyses. The clay content was determined using the hydrometer method (Bouyocous, 1962), soil organic carbon (SOC) was determined using the modified Walkley-Black method (Nelson and Sommers, 1982), while bulk density (BD) was measured in undisturbed soil samples following the cylinder method (Blake & Hartge, 1986).

Following the determination of SOC content and BD in the study area, the soil organic carbon stock (SOCS) for each sampling point was calculated using Equation 1 (Zhang et al., 2013).

$$SOCS = BD \times D \times SOC \times A \dots\dots\dots 1$$

In this equation, BD represents the bulk density of the soil (Mg m⁻³), D denotes the depth at which the soil sample was collected (meters), SOC refers to the SOC content (g kg⁻¹) determined using the Walkley-Black method, and A

represents the total coverage of the study area (ha: 10⁴ m²).

The SOC:clay ratio was used to evaluate the relationship between SOC and clay content. Following the criteria proposed by Johannes et al. (2017), soils with an SOC:clay ratio above 1:8 were classified as "very well-structured," those between 1:10 and 1:8 as "well-structured," those between 1:13 and 1:10 as requiring "structural improvement," and those below 1:13 as "degraded." To assess these classifications, soil samples were analyzed in the laboratory, and the SOC:clay ratio was calculated for each sample. However, the SOC:Clay ratio may not be sensitive enough to detect changes in SOC due to management practices, particularly in high-clay soils where SOC is more stable. To address these limitations, Poeplau and Don (2023) proposed the SOC:SOC_{exp} ratio, which compares measured SOC to an expected value derived from a regression with clay content. This method accounts for the natural variation in SOC across soil types, providing a more context-sensitive

assessment of SOC dynamics. Unlike the SOC:Clay ratio, the SOC:SOCexp ratio is less prone to extreme biases and better aligns with soil structural properties such as porosity and bulk density.

Statistical Analysis and Spatial Distribution of Soil Samples

Descriptive statistics for the SOC, BD, SOCS, and SOC:clay content were calculated using the SPSS 21.0 software. Spatial distributions were modeled using the GS+ 7.0 software, and the most suitable model parameters (sill, nugget, range) were utilized to generate spatial distribution maps in ArcGIS 10.2. Selection of the most appropriate model was based on its ability to best describe spatial variability as a function of distance while constructing semivariograms for SOC, BD, and SOCS values. Coefficient of determination (R^2) and the residual sum of squares (RSS) indicating measurement errors, were considered as key criteria in this selection process. The optimal model was identified based on the highest possible R^2 value, approaching 1.0, and the lowest RSS value, approaching zero (Chang and Lin, 2000).

Results and Discussion

Descriptive Statistics of Soil Properties

The bulk density ranged between 1.16 and 1.74 g cm⁻³, with a mean value of 1.45 g cm⁻³ (Table 1). Bulk density can significantly vary depending on soil organic matter content, texture, aggregation, and pore structure of soils (Chaudhari et al., 2013). Although the coefficient of variability (CV) value of 8.35% indicates a relatively narrow range of variation, the minimum and maximum bulk density values suggest significant differences in soil compaction. Bulk density was low enough to pose no risk in some areas, while it was high enough to potentially hinder root development and plant growth in other areas. The variability in bulk density within the study area can be attributed to soil texture, organic matter content, and stoniness.

Additionally, differences in soil management practices play a significant role; while some orchards undergo soil tillage at least six to eight times per year, others experience little to no tillage, contributing to the observed variations. Clay content varies between 10% and 62.5% and sand content ranges from 17.1% to 75%. The high variability in clay (CV = 26.2%) and sand content (CV = 38%) (Budak et al., 2025) is a key factor contributing to the variation in bulk density.

The frequency of soil tillage operations, coupled with the diversity of implements used (such as plows, cultivators, and disc harrows), plays a crucial role in modulating soil bulk density. In this context, Fallahzade et al. (2020) demonstrated that traditional tillage methods markedly reduce surface soil bulk density in pistachio orchards in Iran. Complementing these findings, Salem et al. (2015) observed that conventional tillage practices lead to a significantly greater decline in bulk density compared to more conservative approaches, such as reduced or no-till systems. Moreover, Topa et al. (2021) revealed that the long-term impacts of tillage are depth-dependent; while the surface layer (0-10 cm) experiences a reduction in bulk density, deeper soil layers exhibit a significant increase.

The skewness and kurtosis values provide insight into the distributional characteristics of the soil parameters measured. For SOC and SOCS, the skewness values of 0.73 and 0.77, respectively, indicate a moderate positive skew. This suggests that both datasets have a slight asymmetry, with a tendency for a longer right tail, which may reflect the presence of higher value outliers or a clustering of lower values. In addition, the kurtosis values for SOC (1.07) and SOCS (1.05) imply that these distributions are moderately leptokurtic, meaning they are somewhat more peaked with heavier tails than a normal distribution. This characteristic can have implications for statistical analyses, as it suggests a higher probability of extreme values compared to a mesokurtic (normal) distribution (Hayes et al., 2022). In contrast, skewness (0.22) and

kurtosis (-0.03) of BD indicate a distribution that is nearly symmetrical and close to normal in terms of tail weight and peakedness.

Table 1. Descriptive statistics of soil organic carbon, bulk density, soc/clay and soil organic carbon stocks (0-20 cm)

		Minimum	Maximum	Mean	Standard Dev.	CV*	Kurtosis	Skewness
SOC	g kg ⁻¹	2.85	26.00	10.92	4.22	38.6	1.07	0.73
BD	g cm ⁻³	1.16	1.74	1.45	0.12	8.4	-0.03	0.22
SOC:Clay		0.07	0.86	0.27	0.14	50.59	4.35	1.70
SOCS	t ha ⁻¹	8.97	73.74	31.28	11.75	37.6	1.05	0.77

*Coefficient of variability (%)

The SOC is a critical component of the global carbon cycle, functioning both as a source and a sink of atmospheric CO₂. As a major reservoir in terrestrial ecosystems, SOC plays a pivotal role in regulating carbon fluxes between the soil and the atmosphere. The mineralization of plant and animal residues represents a fundamental process in the carbon cycle, directly influencing the SOC pool. This decomposition process is driven by microbial activity, which breaks down organic matter into simpler compounds, releasing CO₂ into the atmosphere (Xu et al., 2023). In intensively managed agricultural systems as in pistachio orchards, soil disturbances such as plowing contribute to the breakdown of macroaggregates, which are crucial for stabilizing organic carbon. The disruption of these aggregates exposes previously protected SOC to microbial degradation, leading to its conversion into CO₂ and subsequent release into the atmosphere. Consequently, the loss of SOC is accelerated, reducing soil fertility and diminishing the soil's capacity to act as a carbon sink (Wang et al., 2019). The extent of SOC depletion depends on the intensity and frequency of soil disturbances, indicating the importance of sustainable land management practices in mitigating carbon losses.

Relationship between orchard age and carbon storage potential of soils

A one-way analysis of variance (ANOVA) was performed to determine whether SOC, SOCS, and OC:Clay ratio exhibited statistically significant variations across pistachio orchards of different age groups (Table 2). The results indicated that the mean SOC and SOCS values did not differ

significantly among orchard age groups ($p > 0.05$). However, the OC:Clay ratio showed a statistically significant variation across age groups ($p < 0.05$). Although no statistically significant change was observed according to orchard age, the average SOC (%20.37) and SOCS (%22.76) values in the soils of 5-year-old and 20-year-old orchards significantly decreased with increasing orchard age. This difference was particularly pronounced between young orchards (≤ 5 years old) and mature orchards (≥ 20 years old), suggesting that soil organic matter stabilization mechanisms may vary with orchard development. The observed variation could be attributed to differences in root system maturity, microbial activity, organic matter decomposition rates, and soil structural evolution over time. These findings highlight the necessity of age-specific soil management strategies to optimize carbon sequestration and maintain long-term soil health and productivity in pistachio orchards

Orchards, as long-term perennial agricultural systems, play a significant role in SOC sequestration. Unlike annual cropping systems, which involve frequent soil disturbances, orchards are typically managed with minimal soil disruption, allowing for greater SOC accumulation and stabilization. The presence of deep-rooted perennial trees contributes to enhanced carbon inputs through continuous leaf litter deposition, root exudates, and organic matter incorporation into the soil profile (Montanaro et al., 2017). In traditional pistachio orchards, where intensive soil tillage is commonly practiced, SOC content varies between 2.85 g kg⁻¹ and 26 g kg⁻¹, with an average value of 10.92 g kg⁻¹ (Table 1). When classified based on orchard age, a clear trend of

declining SOC content with increasing orchard age is observed (Table 2). Specifically, in young orchards aged 1–5 years, the average SOC content is 11.83 g kg^{-1} , whereas in older orchards exceeding 20 years, this value declines to 9.42 g kg^{-1} . This pattern suggests that intensive soil disturbance in pistachio orchards accelerates SOC depletion over time, counteracting the expected accumulation of organic carbon in perennial systems. Soil aggregates play a crucial role in restricting the access of microorganisms, enzymes, and oxygen to organic compounds, thereby slowing down the mineralization of organic carbon. This physical protection mechanism helps maintain higher SOC levels over time (Topa et al., 2021). Unlike well-managed perennial orchards, where undisturbed soils promote stable macroaggregate formation and protect SOC from oxidation (Cao et al., 2021), frequent tillage in pistachio orchards disrupts soil structure, exposing SOC to microbial decomposition and increasing its loss as CO_2 . The stability of carbon-associated aggregates differs between macroaggregates and microaggregates, affecting their ability to preserve SOC. As macroaggregates gradually break down into smaller microaggregate-sized particles, the rate of mineralization intensifies, leading to greater carbon losses (Jat et al., 2019). Research indicates that soils subjected to minimal tillage or no-till practices tend to develop macroaggregates with a higher capacity for organic carbon storage compared to those undergoing conventional tillage (Zheng et al., 2018). In pistachio orchards, where soil disturbance becomes more frequent as orchard age increases, a significant portion of macroaggregates is broken down. This continuous disruption likely contributes to the decline in SOC, further accelerating carbon depletion in these intensively managed systems.

In the study area, SOCS values range from 8.97 t ha^{-1} to 73.74 t ha^{-1} , with an average of 31.28 t ha^{-1} (Table 1). Similar to SOC content (38.6% CV), SOCS values also exhibit high variability, with a CV of 37.6%, likely due to differences in orchard age and management practices. Research conducted

in the Dicle Basin, which has a similar climate, reported that among various land-use types, including croplands, orchards, vegetable fields, grasslands, and forests, the lowest average SOCS values were found in croplands (28.91 t ha^{-1}), followed by fruit orchards (31.97 t ha^{-1}) (Budak and Günel, 2018). These findings show the relatively lower SOC storage capacity of orchard soils compared to natural ecosystems, emphasizing the impact of land management on carbon sequestration potential. The significantly lower SOCS values recorded in pistachio orchards in the current study, compared to the findings of Budak and Günel (2018), can be primarily attributed to the lack of fertilization (both organic and inorganic) and the frequent soil tillage, which is performed 6 to 8 times per year. These intensive management practices have likely contributed to the depletion of soil organic carbon over time. Additionally, the conversion of lands previously used for intensive wheat and barley cultivation into pistachio orchards has further exacerbated SOC losses. The prolonged history of intensive agricultural production in these areas has led to a substantial reduction in organic carbon levels, ultimately resulting in lower SOCS values. Furthermore, the reduction in SOC accelerates the depletion of plant nutrients and the decline in carbon stocks, thereby adversely affecting soil fertility and potentially destabilizing ecosystem balance. Research has demonstrated that prolonged intensive tillage can reduce SOCS by 20 to 50% (Conant et al., 2007). This phenomenon represents a significant risk factor not only for sustainable agricultural practices but also for the global carbon balance. Öztürk (2025) reported that in pistachio cultivation areas in Şanlıurfa, which has a similar climate, SOC values ranged from 8.12 to 42.69 g kg^{-1} , while SOCS varied between 10.5 and 45.3 Mg ha^{-1} , with these variations generally attributed to differences in land use. In addition, in a study conducted by Yakupoğlu et al. (2017) on the erodibility of soils in areas converted from native shrubland to pistachio orchards, it was reported that the average SOC content in the natural area

was 12.96%, while in the pistachio orchards it was 7.30%. The change in SOC values in the same region can be associated with soil tillage and management systems.

The SOC to clay ratio (SOC:Clay) is a widely recognized indicator of soil structural quality, influencing key attributes such as porosity, aggregate stability, and resistance to erosion. This ratio serves as a reliable metric for assessing soil organic matter (SOM) sufficiency, with established threshold values delineating soil structural conditions ranging from very good to degraded (Prout et al., 2021). In this study, the SOC:Clay ratio exhibited a mean of 0.27, with values ranging from 0.07 to 0.86 and a standard deviation of 0.14. The CV was high at 50.59%, indicating substantial variability across the sampled soils. The average SOC:Clay ratio of 0.27 surpasses the threshold of 0.125 (1:8) considered indicative of very good structural quality (Johannes et al., 2017; Prout et al., 2021). This suggests that, on average, the soils in this study exhibit excellent structural conditions, supporting findings that a SOC:Clay ratio above 1:10 (0.10) generally corresponds to stable soil aggregation and improved soil functions (Dexter et al., 2008; Prout et al., 2021). However, despite the high average SOC:Clay ratio, the range of values indicates that some soil samples fall below the critical 0.077 (1:13) threshold, suggesting potential structural degradation (Prout et al., 2021). This is particularly relevant as arable soils and those under lower precipitation conditions with frequent tillage have been found to be more susceptible to SOC depletion, often falling below the 1:13 threshold (Verheijen et al., 2005; Prout et al., 2021). The positive skewness (1.70) and high kurtosis (4.35) observed in this dataset further indicate that while many soils have high SOC:Clay ratios, there is a tail of soils with significantly lower values that may require management interventions to enhance their structural integrity.

stock (SOCS), and SOC:Clay ratio across different age groups of Siirt pistachio orchards

Orchard Age	SOC	SOCS	SOC:Clay
≤5	11.83 ^a	35.45 ^a	0.32 ^a
6-10	10.9 ^a	30.48 ^a	0.26 ^{ba}
11-20	10.07 ^a	27.77 ^a	0.21 ^{ba}
≥20	9.42 ^a	27.38 ^a	0.20 ^b
ANOVA	0.131	0.134	0.045

Studies have demonstrated that the SOC:Clay ratio is a robust predictor of soil structural conditions across various land uses and soil types. In England and Wales, for example, SOC:Clay ratios have been used as a guideline to classify soil conditions, revealing that arable lands frequently exhibit degraded structural quality due to lower SOC retention relative to clay content (Prout et al., 2021). Given this, maintaining SOC:Clay ratios above 1:10 should be an essential soil management goal to prevent degradation and improve soil functionality (Johannes et al., 2017; Prout et al., 2021).

When the SOC:Clay ratio is equal to or greater than 1/8 (0.125), clay minerals effectively protect SOC from decomposition (Sauzet et al., 2024). However, intensive and repeated soil tillage disrupts soil aggregates, leading to their fragmentation into smaller particles. As aggregates break down, organic carbon becomes more exposed to microbial activity, accelerating its decomposition and subsequent loss. This process ultimately results in a decline in the SOC:Clay ratio (Prout et al., 2022). Analysis of SOC:Clay values in relation to orchard age revealed a clear decline in the ratio as orchards matured. In pistachio orchards aged five years or younger, the SOC:Clay ratio averaged 0.32. However, with increasing orchard age, a substantial reduction was observed, aligning with the decreasing trends in SOC and SOCS values and ultimately reaching 0.20. The differences in the OC:Clay ratio according to orchard age were found to be statistically significant ($p = 0.045$) (Table 2). The approximately 40% decline in the SOC:Clay ratio in orchards aged 20 years and older, compared to their younger counterparts, indicates that the practices in pistachio orchards may contribute to aggregate disruption and

Table 2. Soil organic carbon (SOC), soil organic carbon

accelerate organic carbon mineralization. While the SOC:Clay ratio has been widely used as an indicator of soil structural stability and organic carbon stabilization potential, recent studies have raised concerns about its applicability and reliability. Poeplau and Don (2023) argue that the SOC:Clay ratio is strongly biased towards clay content, leading to an exaggerated classification of fine-textured soils as degraded, while falsely indicating that coarse-textured soils are in good condition. In their analysis of over 2,900 topsoil samples from the German Agricultural Soil Inventory, they found that 94% of Chernozems, soils known for their high fertility and SOC content, were classified as degraded or moderate based on the SOC:Clay ratio. This suggests that the metric does not adequately reflect the true carbon sequestration potential or soil health status.

Spatial Distribution of Soil Organic Carbon in Pistachio Orchards

The spatial distribution maps of SOCS, SOC, and bulk density are presented in Figure 3. The southern and northern sections of the study area contain pistachio orchards with the highest SOCS values, whereas the central regions consist of orchards with the lowest SOCS values (Figure 3). The land between İncirlik Village and Şenköy is primarily composed of 15-year-old and older pistachio orchards, while other areas represent lands that have been converted into pistachio orchards within the last decade. Since SOCS values are calculated using bulk density and SOC content as key factors, their spatial distribution closely mirrors that of SOC (Figure 3). The loss of organic matter critically impairs soil structure, water retention, and nutrient cycling, initiating a cascade of detrimental effects on both soil health and overall ecosystem functionality. Organic matter is essential for maintaining soil aggregation and moisture conservation, and its depletion can exacerbate erosion processes while reducing the soil's capacity to sequester carbon (Lal, 2004). In this context, pistachio orchards with older trees are found to be particularly

susceptible to erosion. This increased vulnerability can be attributed to several factors: older trees often exhibit diminished canopy cover and less vigorous root systems, which weakens the soil's protective structure and thereby enhances the likelihood of surface erosion. Furthermore, intensive land management practices that accelerate organic matter loss further compromise soil structural integrity, exacerbating erosion risks and undermining the sustainability of these agroecosystems.

Specifically, the SOC values within the study area exhibit a distinct spatial distribution, with the southern and northern sectors recording the highest values, while the central zone has the lowest. The spatial variation map created for bulk density reveals that the highest values are concentrated along a north–south trending belt between the villages of Şenköy, İncirlik, Demirkuyu, and Güzeldere. In the pistachio orchards located in these areas, the elevated bulk density relative to other orchards can be primarily attributed to the soils' high sand content. Indeed, the spatial variation map for sand content prepared by Budak et al. (2025) clearly indicates that the highest sand content (exceeding 50%) is found within these particular zones. This pronounced sandy nature of the soils is indicative of a historical active fluvial system along this alignment. The observed distribution of SOC may be influenced by several interrelated factors, including variations in vegetation cover, land management practices, and inherent soil properties (Six et al., 2002; Lal, 2004). Soils with high sand content typically exhibit increased bulk density and reduced water retention capacity (Brady and Weil, 2008), conditions that can hinder the stabilization and accumulation of organic matter. Furthermore, the hydrological processes associated with past stream activity likely contributed to the depositional patterns observed in the current soil texture, thereby affecting both SOC distribution and bulk density (Blake and Hartge, 1986). Shangguan et al. (2014) also emphasizes that spatial variability in soil physical properties is often closely linked to both natural processes and anthropogenic impacts, underscoring the complexity of soil dynamics in agricultural landscapes.

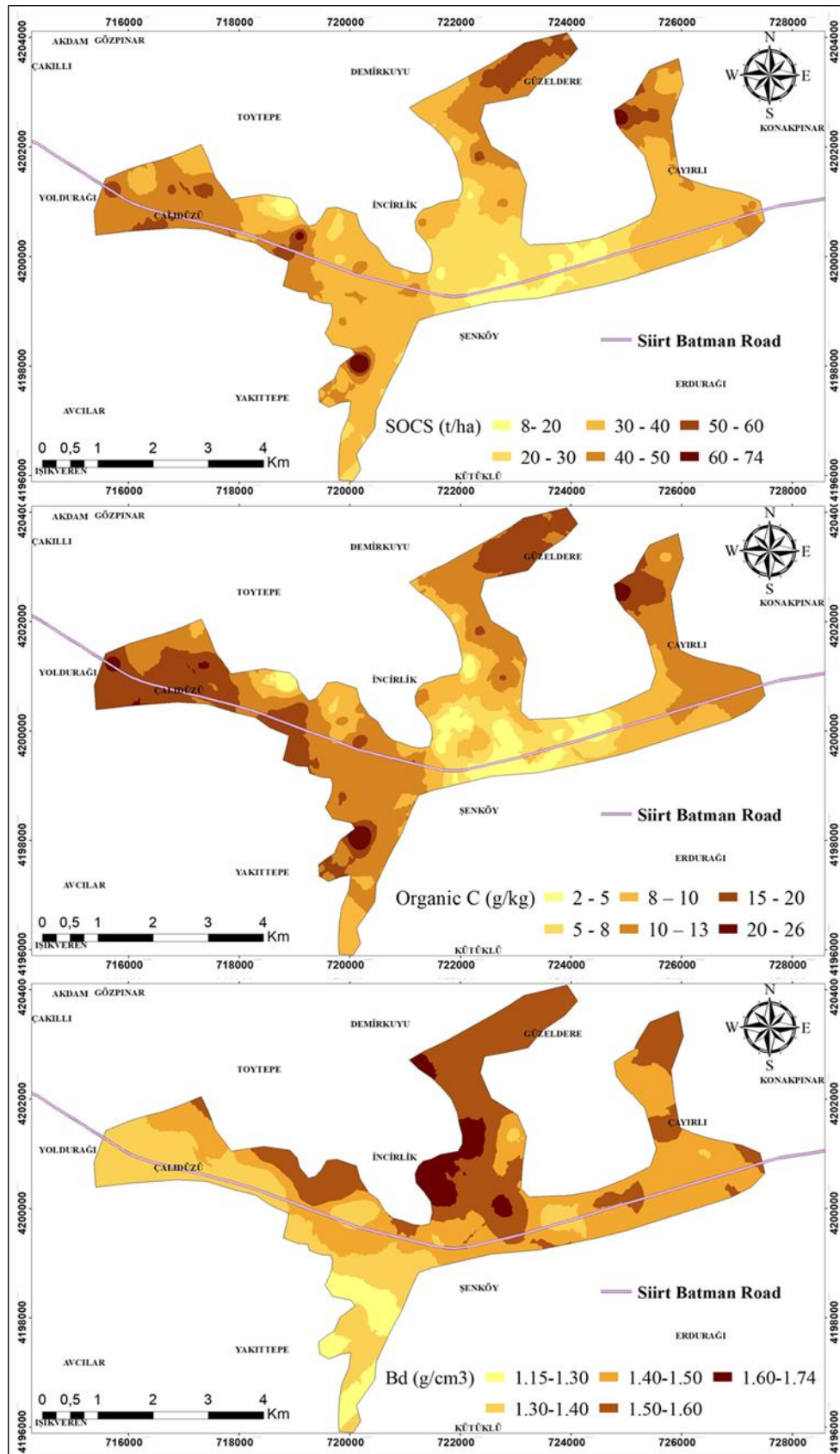


Figure 3. Spatial distribution maps of SOCS, SOC and bulk density values in the study area

Conclusion

The results indicate that the soil management practices applied in the pistachio orchards have led to a significant decrease in both organic carbon and total organic carbon stocks. Intensive

tillage, in particular, has accelerated the loss of organic matter, thereby disrupting soil carbon dynamics. Additionally, the sloped nature of the study area has heightened the risk of water erosion, resulting in the loss of nutrient-rich topsoil and a consequent decline in soil fertility. This erosion-driven loss of organic carbon disrupts

the intricate relationships between soil, water, and vegetation, which can ultimately reduce agricultural productivity over the long term.

A key indicator of soil structural stability, the SOC:Clay ratio, further reveals the impact of these management practices. The observed decline in SOC:Clay ratios, particularly in older orchards, suggests a progressive reduction in SOC:Clay ratio, which can impair aggregate stability and increase susceptibility to degradation. Since an SOC:Clay ratio below the critical threshold of 1:10 (0.10) is often associated with poor structural quality and reduced carbon sequestration potential, the significant decrease in this ratio across older orchards shows the urgency of adopting sustainable management interventions.

In response to these challenges, it is imperative that sustainable soil management practices be adopted in pistachio orchards established on sloped terrains. Strategies such as reduced tillage, the addition of organic amendments, and the maintenance of vegetative cover are critical for preserving the soil's carbon storage capacity and preventing further erosion in pistachio orchards. Moreover, the spatial variation maps developed in this study provide a valuable framework for monitoring changes in SOCS of pistachio orchards over time, thereby supporting the timely implementation of measures to mitigate soil degradation and ensure the long-term sustainability of pistachio production.

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Conflict of Interest

The authors declare that there is no conflict of

interest.

Author Contributions:

Data processing and manuscript preparation were carried out by Mesut Budak, Elif Günel, and Miraç Kılıç. Fieldwork, laboratory analyses, and statistical evaluations were conducted by Kübra Polat, Mesut Sırrı, and Reşat Yolbaş.

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Modeling and evaluation of Siverek (Şanlıurfa) groundwater potential by GIS and TOPSIS method

Siverek (Şanlıurfa) yeraltı suyu potansiyelinin CBS ve TOPSIS yöntemiyle modellenmesi ve değerlendirilmesi

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ABSTRACT

In addition to being an important part of the hydrological and hydrogeological systems in its area, it is an important strategy in terms of protecting and developing the gradually depleted groundwater within the resources. It is inevitable to evaluate the groundwater potential and quality of the Siverek basin for gains such as determination of water supply and distribution. Therefore, the mechanism of groundwater should be determined by sensitive hydrological and hydrogeological modeling studies. While current groundwater studies can explain groundwater hydraulics and hydrology, there is little literature on groundwater behavior under variable parameters and different scenarios involving surface water and groundwater interactions. In this study, the TOPSIS method based on the GIS program modeling the groundwater flow was used and the evaluation parameter weights were calculated using MCDM methods. By comparing the parameters with each other, the weights of the criteria were determined by the TOPSIS method. A total score was determined according to the evaluation form for 8 different suggestions entered into the system, such as rainfall, aquifer, slope, drainage density, soil type, geology, geomorphology. The highest scoring parameters were ranked in both methods. In the last stage, the parameters were evaluated and an order of superiority was determined for the ideal solution. The study was concluded by comparing the methods among each other.

Key Words: GIS software program, Hydrological and Hydrogeological system, Siverek basin, TOPSIS method

Öz

Bölgesindeki hidrolojik ve hidrojeolojik sistemlerin önemli bir parçası olmasının yanı sıra kaynaklar içerisinde giderek tükenen yeraltı sularının korunması ve geliştirilmesi açısından da önemli bir stratejidir. Siverek havzasının yeraltı suyu potansiyeli ve kalitesinin, su temini ve dağıtımının belirlenmesi gibi kazanımlar için değerlendirilmesi kaçınılmazdır. Bu nedenle yeraltı suyunun mekanizmasının hassas hidrolojik ve hidrojeolojik modelleme çalışmalarıyla belirlenmesi gerekmektedir. Mevcut yeraltı suyu çalışmaları yeraltı suyu hidrolojisini ve hidrojeolojisini açıklayabilse de değişken parametreler ve yüzey suyu ile yeraltı suyu etkileşimlerini içeren farklı senaryolar altında yeraltı suyu davranışı hakkında çok az literatür bulunmaktadır. Bu çalışmada yeraltı suyu akışını modelleyen GIS programına dayalı TOPSIS yöntemi kullanılmış ve değerlendirme parametre ağırlıkları MCDM yöntemleri kullanılarak hesaplanmıştır. Parametreler birbirleriyle karşılaştırılarak kriterlerin ağırlıkları TOPSIS yöntemi ile belirlenmiştir. Yağış,



akifer, eğim, drenaj yoğunluğu, toprak tipi, jeoloji, jeomorfoloji gibi sisteme girilen 8 farklı öneri için değerlendirme için değerlendirme formuna göre toplam puan belirlenmiştir. Her iki yöntemde de en yüksek puan alan parametreler sıralanmıştır. Son aşamada parametreler değerlendirildi ve ideal çözüm için üstünlük sırası belirlendi. Çalışma, yöntemlerin birbirleriyle karşılaştırılmasıyla sonuçlandırıldı.

Anahtar Kelimeler: CBS yazılım programı, Hidrolojik ve Hidrojeolojik sistem, Siverek havzası, TOPSIS yöntemi

Introduction

Groundwater is an important factor that directly affects the hydrological behavior within the basin and plays an important role in water supply and quality. Groundwater contributes to water supply with wells and various water structures, and affects the quality and quantity of surface waters through interactions with streams, lakes, rivers and swamps. Therefore, groundwater reserve, quality and control are of great importance. In order for "Ground water" water resources to be used efficiently, they must be modeled hydrologically correctly. Many characteristics of groundwater must be taken into account in precision groundwater models. One of the most important of these characteristic features is surface water-groundwater interactions that can vary regionally. Surface water-groundwater interactions affect the hydrological behavior and pollution transport between different water sources such as rivers, lakes, streams and aquifers (Sarkar and Mandal, 2024).

In dry and semi-arid areas, groundwater is viewed as a potential alternative to traditional water supply methods. Different kinds of minerals are carried in solution by groundwater, and the kinds and concentrations of these minerals are primarily influenced by things like rock chemistry, interactions between surface water and groundwater, the geological environment (which offers a preferred pathway for groundwater flow), and other potential sources (Sevik and Cetin, 2015; Fehdi et al., 2016). The final result of the interaction between water and rocks from various geological eras is represented by the chemistry of groundwater. This explains why dissolved chemical components of various sorts and

amounts are present in groundwater (Akbari et al., 2020).

The availability of groundwater is influenced by factors such as the geology, geomorphology, slope, soil type, drainage density, lineament concentration, rainfall, and land use of a region. The geological and geomorphological nature of an area determines how groundwater forms, moves, and is stored there. Geomorphological and geological elements have an impact on the hydrogeological settlement of the area, either directly or indirectly. In contrast, physiographic elements including relief, slope, and infiltration and seepage rates all affect flow rate. (Das and Pardeshi, 2018; Adiat et al., 2024).

Groundwater quality evaluation, a crucial part of groundwater environmental assessment, has recently attracted a lot of national and worldwide interest. Groundwater quality and quantity are now equally important in the assessment of groundwater resources, rather than only the quantity of groundwater. In several basins, groundwater monitoring network optimization projects are also in progress. These initiatives benefit the monitoring and defense of groundwater quality. Numerous water quality evaluation methods have arisen as a result of the growing demand for water quality assessment and preservation. According to data, there are already at least 60 techniques for determining the quality of water. Each technique has benefits and drawbacks, making it challenging to choose the optimal one. The Water Quality Index method is the one that is most frequently utilized abroad (Ejaz et al., 2024; Karakus, 2023). This study introduces and applies the ranking preference method based on how closely it resembles the ideal answer to groundwater quality assessment, which will serve as the foundation for the choice of groundwater quality assessment methods (Yildirim, 2021)

It is important to reveal the trends of the factors that threaten the potential and quality of groundwater over the years, in terms of carrying out quality status assessments. It will be possible to define the measures to be taken and to establish a regular monitoring system for a groundwater body whose situation has been assessed and the effects on it have been determined. In this context, In order to evaluate and manage the natural resources of the planet, remote sensing, GIS, and MCDM techniques will be extremely important. These methods are now highly efficient in terms of cost and time when attempting to determine a region's potential for groundwater. The definition of groundwater potential regions utilizing various geo environmental special layers has been successfully accomplished by numerous prior studies. Therefore, in this study, a study was conducted to model and evaluate the different groundwater potential and quality of the Siverek basin with the TOPSIS method by using the

techniques of positively affecting the groundwater in the GIS environment.

Material and Method

Siverek located in the north of Sanliurfa province, Kahta District of Adiyaman in the west, Ataturk Dam Lake extending from west to north, Adiyaman's Gerger district, Diyarbakir's Cermik and Cungus districts, and Mardin province with a small border in the southeast are neighbors, as are the counties of Viransehir and Hilvan. Siverek town center is located at the intersection of 37.45 north 39.19 east longitudes. The altitude of Siverek district center from sea level varies between 801 and 840 meters. Its total surface area is 4367 km.

The typical continental climate of Southeast Anatolia prevails in the region. The summers are hot and dry, the winters are cold and rainy. The long-term average of precipitation is 572 mm

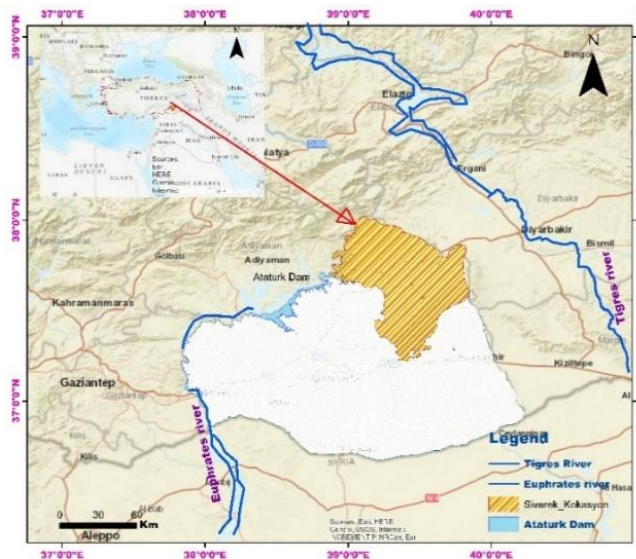


Figure 1. Study site location map

Eocene aged limestone; Miocene clay, sandy clay; Detritic materials consisting of Plioquaternary clay, silt, sand and gravel; Plioquaternary basalt and Quaternary alluvium were detected. There are no notable water streams in Siverek. However, there are streams that reach the Euphrates in the northeast. Since they are fed by these seasonal rains, they tend to dry towards the summer (Ozyilmaz et al., 2018).

Geological Structure of the Study Site

Eocene aged limestone and Miocene aged sandy clay, which are among the units in the study area, show bedding. In both units, the layer slopes are horizontal and close to the horizontal and are around 10°. Fracture systems, which are less common in sections dominated by clayey and chalky limestone levels, are highly developed in

basalts and crystallized limestone. Basalts are massive in structure and flow structures and cooling cracks are dominant. Irregular and excessive cooling cracks in basalts cause difficult monitoring of structural and

TOPSIS Method

The TOPSIS approach, one of the Multi-Criteria Decision Making (MCDM) strategies, was created by Hwang and Yoon in 1981 and is frequently used for ranking purposes (Ada and Cakir, 2022). In this method, the aim is to determine the alternative that is the closest to the ideal solution and the farthest to the non-ideal (anti-ideal; negative ideal) solution among many alternatives (Karaatli et al., 2015). Six different stages are followed in the ranking process of the alternatives made according to certain

criteria with the TOPSIS method. These are explained below, respectively (Dehghan Rahimabadi et al., 2024; Aslan and Sepetcioglu, 2022).

Step 1: Making the Decision Matrix: The decision maker creates a $m \times n$ matrix for the decision. The columns display the criteria, while the rows provide the options. Matrix elements x_{ij} show the value of the i -th alternative according to the j -th criterion, and the general structure of the decision matrix is in the form.

$$D=[X_{ij}], \quad i=1,2,3,\dots,n \quad (1)$$

Here, row A_i is the success values of the i th alternative according to all criteria, X_j column is the success values of all alternatives according to the j th criterion (Equation 1).

Step 2: Achieving the Normalized Matrix: The normalized matrix is obtained by descaling to evaluate criteria with different scales, and the following equation is used for this;

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{p=1}^m (x_{pj})^2}} \quad i = 1, 2, \dots, m; \quad j=1,2,\dots,n \quad (2)$$

Depending on the values calculated with this equation, $R= [r_{ij}]_{m \times n}$ normalized decision matrix is obtained.

So in other words; each x_{ij} value is normalized by dividing by the square root of the sum of the values in the column it is in, and r_{ij} values are obtained (Equation 2)

Step 3: Achieving the Weighted Normalized Matrix: At this stage, the criteria weights determined by the decision maker are used and the sum of the weights should be equal to 1. $V=[v_{ij}]_{m \times n}$ weighted normalized decision matrix.

$$V_{ij}= w_j r_{ij}, \quad i = 1, 2, \dots, m; \quad j=1, 2, 3, \dots, n \quad (3)$$

obtained by the formula. Here W_j is the weight value of j criterion. It is calculated by multiplying the values in each column of the R_{ij} matrix by the weight of the relevant criteria (Equation 3).

Step 4: Finding Values for Ideal and Anti-Ideal Solutions: At this stage, while determining the ideal solutions, if the goal is benefit, etc. The anti-ideal solution has the least value if the ideal solution has the biggest value. If the goal is cost etc. The ideal solution is the smallest value, and the anti-ideal solution is the largest value. Accordingly, (ideal solution) and (anti-ideal solution) are defined as follows:

$$A^* = \left\{ (\max_i v_{ij} | j \in J), (\min_i v_{ij} | j \in J') \right\} = \{V_1^*, V_2^*, V_3^*, \dots, V_j^*, \dots, V_n^*\} \quad (4)$$

$$A^- = \left\{ (\min_i v_{ij} | j \in J), (\max_i v_{ij} | j \in J') \right\} = \{V_1^-, V_2^-, V_3^-, \dots, V_j^-, \dots, V_n^-\} \quad (5)$$

Here, the maximum value for each column is A^+ the minimum value for each column. If the goal is minimization, the situation is the opposite. A^* is the minimum value for each column, the maximum value for each column (Equation 4, 5).

Step 5: Obtaining the Distance Values from the Ideal and Anti-Ideal Solution: Euclidean metric is used for the distance calculation and the formula is given below (Equation 6, 7):

$$S_i^* = \sqrt{\sum_{j=1}^n (V_{ij} - V_j^*)^2}, \quad i = 1, 2, 3, \dots, m \quad (6)$$

$$S_i^- = \sqrt{\sum_{j=1}^n (V_{ij} - V_j^-)^2}, \quad i = 1, 2, 3, \dots, m \quad (7)$$

Step 6: Making the order of preference: Finally, S_i^* is used to calculate how close each decision point is to the ideal answer (Equation 6).

$$C_i^* = \frac{S_i^-}{S_i^* + S_i^-}, \quad 0 < C_i^* < 1, \quad i = 1, 2, 3, \dots, m \quad (8)$$

Alternatives are sorted by ordering the C_i^* values from greatest to least (Equation 8).

Where; V^+ is nearest to good and farthest to poor, V^- is Farthest to good, closest to poor.

Results

When previous studies were examined, it was seen that many parameters (5 - 8 parameters) were used comprehensively for the groundwater potential index (GWP_Z) evaluation research. On the other hand, a few studies used more parameters for GWP_Z determination. It was determined that land use (LU), soil type, drainage density, lineament density and geology were standard parameters and basic when GWP_Z studies were examined. Çelik et al. (2024) evaluated the precipitation and drainage density parameters related to groundwater potential. It was stated that the annual average precipitation in Mersin is 613.2 mm and therefore precipitation is an excellent parameter in the determination of GWP_Z in his study.

However, more is needed to determine the groundwater potential. Because a region with high rainfall does not always indicate the presence of groundwater. Soil diversity and texture in a region, land use, geological texture, erosive power of water, water holding capacity, infiltration amount and inflow directly affect the groundwater potential. For these reasons, the specified rainfall parameter and other parameters should be considered together for an optimum GWPZ analysis (Yilmaz et al., 2021).

Thematic maps created with the data-based model and GIS technique for the groundwater

exploration criteria selected for the Siverek groundwater potential assessment study (precipitation, aquifer, slope, soil texture, land use, geology, geomorphology and drainage density) are presented in Figures 2 and 3, respectively. As can be seen from these maps, the thematic layers created in the GIS tools on each map are listed according to their effects on groundwater. Individual criteria have been reclassified according to their importance in determining groundwater potential zones. Detailed information on individual criteria is summarized below.

Rainfall

The main source of recharge of groundwater is precipitation. A permeable soil receiving high amounts of rainfall can contribute to the recharge of high stress water. The volume of water stored in the aquifer is a function of the degree and variation of precipitation. Water is stored in the aquifer only if it can be conveyed to the compartment and geological units. The rechargeability of groundwater depends on the empty spaces in the soil, geological structures and topographic conditions in the region. There is a high probability that the rains will continue for a long time and its functioning will reoccur. In potential and permeable aquifers, large amounts of water are stored when rain reaches the water table. The results of the distribution of reclassified precipitation maps on the GIS platform are presented in Figure 3a. The representations of thematic maps revealed almost the same categories in both approaches (Ahmad et al., 2020) (Figure 2a).

As can be seen in the regional precipitation map in Figure 2a, the part of the basin between 347-364 mm/year constitutes 40.25% of the annual precipitation average, while the part between 365-374 mm/year has 31.23% of the basin. The part between 375-382 mm/year has 10.23% of the basin and the part between 383-390 mm/year has approximately 9.30% of the precipitation amount. The precipitation amount between 391-400 mm/year covers only 9% of the

basin (Celik, 2019).

Aquifer

Provides vital fluid for humans and other living things. They are geological formations consisting of porous rock, sand and gravel, containing significant amounts of water. The importance of these natural water sources stems from the fact that they contain almost all of the liquid fresh water on the planet.

Slope

Slope is a significant factor in identifying groundwater recharge zones within a basin. Its importance primarily lies in its influence on water retention time - flatter slopes allow more time for water to infiltrate the ground, thereby enhancing recharge potential. The speed of surface runoff, retention time and infiltration capacity of geological units are affected by slope. Flat areas in the basin are good indicators of groundwater potential zones due to their high retention times and infiltration rates. The majority of the lower parts of the basin are classified under steep slopes, and the groundwater potential zone varies from very low to low. Due to the steepness of the topography, the surface flow rate is very high and the retention time is very low. When the slope is flat enough, surface runoff has more time to percolate underground (Tamiru et al., 2022).

SRTM was used to create slope map (in degrees) from DEM in ArcGIS environment. The slope function defines the slope or maximum rate of change in the z value in each cell of the DEM, from 0° to 90°. The slope tool was used to calculate the maximum rate of change of a given cell to neighboring cells. The maximum change in elevation across the distance between the cell and its eight neighboring cells defined the steepest downhill descent from the cell (Figure 2c).

In Figure 2c, in approximately 54% of the basin, the slope is over 10%, in 20% of the basin, the slope is between 10-18%, and the desired slope for groundwater potential is 7%, that is, 13% of the area.

Soil texture

Groundwater is stored in the spaces between soil particles. The volume of water stored in specific hydrological units depends on the texture and size of soil particles. A reclassification was made according to the permeability and infiltration capacity of the soil. However, according to the information obtained from productive wells, the effect of the soil is relatively low if the wells are deep. Therefore, other factors such as rainfall, land use and drainage intensity have played an important role in recharge and contributing to groundwater. One of the interesting features of rain is that it filters through the soil and reaches the water. It is obvious that a large amount of water is stored in the aquifer if there is a possibility of leakage through the soil. Although there is no well-documented information about the aquifer of the sub-basin, it has been determined that there is a high level of infiltration, as can be understood from geophysical studies (Figure 2d).

The soil map of the basin shows that approximately 2% is alluvial soil, 58% is brown forest soil, 39% is brown soil and 2% is reddish-brown soil.

Land Use

Groundwater recharge is the main process by which rainfall infiltrates the aquifer. This process is a function of land use. Compared to areas that were not urbanized in the past, urbanization is increasing in the basin and the rechargeability of groundwater is increasing (Hamed et al., 2018).

The difference between current and past land use typically reflects changes resulting from human activities. These changes can be outlined as follows:

- **Urban Expansion and Increase in Settlements:** Areas that were previously used for agriculture, forests, or natural landscapes have been converted into residential, industrial, or commercial zones in the new land use.
- **Reduction of Agricultural Land:** Agricultural areas have been rezoned and

repurposed for other uses such as housing or industrial development.

- **Degradation of Natural Areas:** Natural landscapes such as forests, pastures, and wetlands have been disturbed or destroyed due to construction, mining, or other anthropogenic activities in the new land use.

- **Change in Land Use Intensity:** While some areas are now used more intensively (e.g., high-rise buildings, roads), certain natural areas have been lost altogether.

- **Environmental Impacts:** New land use practices often lead to increased environmental effects, such as erosion, water pollution, and habitat loss.

Reclassified land use thematic maps created with GIS technique are presented in Figures 2e and 3e. There are five dominant land uses in the study area (Figure 2e). The water body and irrigated areas in the discharge area of the basin are quite small and there are generally unirrigated agricultural lands (31%), pastures (17%) and other areas with vegetation.

Geology

One of the most important factors determining the formation of groundwater is geology. Groundwater can only be stored and circulated in the cracks and cavities of the rock. This means that it is the porosity and permeability of materials that ultimately limits groundwater potential. In practice, the challenge often consists of identifying formations that are more likely to hold water. These include, but are not limited to, unconsolidated deposits, weathering products, and fractured and soluble sedimentary rocks (Figure 2f).

The presence of groundwater in an aquifer depends on the nature of the geology and the permeability of the geological environment. When a geological formation captures and transmits percolating water, the water is stored in an aquifer. The amount and volume of water stored in geology is a function of the hydrogeological environment of the region. Water can only be stored if there is sufficient

space between geological units. The aquifer is assumed to be potential when there is sufficient space in the hydrogeological environment. Geological units with uniform grain size generally have sufficient porosity and high permeability. Groundwater recharge occurs from surface runoff, precipitation seeping into the soil from lakes and streams (Berhanu et al., 2020).

Geomorphology

There are five dominant landforms in the study area: flats, plains, plateaus, hills and mountains. GIS tools were used to reclassify landforms according to the importance of their contribution to groundwater. In this study, a thematic layer was created and the dominant landforms were reclassified into five groups. The landforms of a geomorphological unit differ in terms of their characteristics and spatial distribution. The formation and movement of groundwater depends on the geomorphological characteristics of the region. Low mountainous and flat plains are indicative of the presence of groundwater if hydrogeological environments conduct water. Landform classification based on slope was applied for a geomorphological unit and a quantitative classification was made.

As seen in Figure 2g, the basin consists of topographic maps showing parts such as flats, plains, plateaus, hills and mountainous areas. While plains and plain parts contain high groundwater potential, hilly and mountainous regions are the parts with the lowest potential. The basin has an area of mountainous areas (16%), hilly areas (17%), plain areas (18%), plains (43%) and plateaus (6%).

Drainage density

The permeability and infiltration rate of rainfall in certain regions is controlled by drainage density. Stream networks and corresponding land surface discharge along the length of the channel may indicate potential groundwater zones. If the drainage density in the basin contributes to the recharge of groundwater, the aquifer is potential. The hydrogeological conditions and moisture

retention capacity of the soil initiate drainage density, which in turn supports the rechargeability of groundwater. Drainage density is affected by topographic conditions and the permeability of geological units. If the slope is flat, the permeability is very high and the drainage density is very low.

In the basin, where the drainage density is mostly low, the coefficient of performance is higher and the flow is slower, so infiltration occurs at a higher rate. First of all, raster thematic maps were created in the light of the explanation of the GIS method.

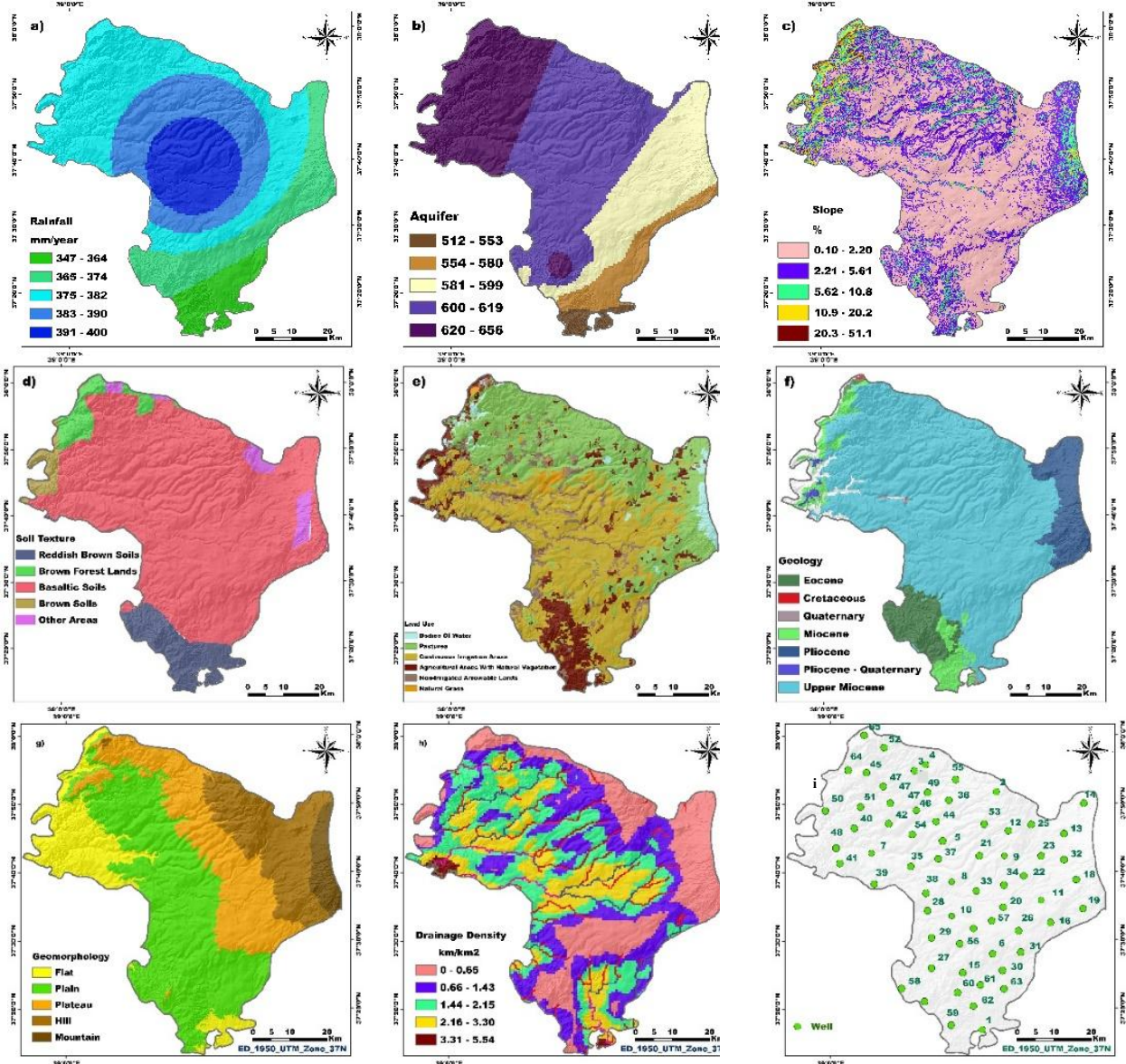


Figure 2. Raster thematic maps created in ArcGIS, ArcMap 10.5 software environment (a) Precipitation, b) Groundwater level, c) Slope, d) Soil texture, e) Land use, f) Geology, g) Geomorphology, h) Drainage density and i) Drilling data)

Table 1. Partial well data drilled by institutions, organizations and private sectors

Rank	District	Neighborhood-Village	SWL	DWL	Water Flow 1/sn	Kot No	depth	Coordinate	
								X (East)	Y (North)
1	Siverek	Soydan	80	110	2	1150	210	564498	4180509
2	Siverek	Soydan	50	65	10	1148	220	"_"	"_"
3	Siverek	Soydan	80	110	44	1155	225	563078	4180563
4	Siverek	Soydan	53	102	26	1147	216	559598	4181201
5	Siverek	Soydan	55	67	8	1149	205	"_"	"_"
6	Siverek	Soydan	75	102	1	1151	240	563045	4178724
7	Siverek	Soydan	110	120	11	1152	245	559042	4181050
8	Siverek	Soydan	80	110	1	1153	246	563142	4179136
9	Siverek	Soydan	110	120	10	1151	236	560072	4180198K
10	Siverek	Soydan	110	120	15	1155	253	560042	4180148
11	Siverek	Gedik	60	73	20	1154	270	"_"	"_"
12	Siverek	Karacadag	30	66	15	1115	216	563866	4176509
13	Siverek	Karakoyun	110	120	15	702	120	518656	4171841
14	Siverek	Karakoyun	110	120	10	700	126	420384	4170052
15	Siverek	Karakoyun	110	120	15	701	135	520992	4171541
16	Siverek	Karakoyun	80	90	23	703	140	526975	4172722
17	Siverek	Karakoyun	110	120	15	702	152	520611	4171942
18	Siverek	Karakoyun	7	120	5	705	160	521536	4170092
19	Siverek	Karakoyun	110	120	15	701	150	519915	4172301
20	Siverek	Karakoyun	110	120	5	704	145	519759	4169821
21	Siverek	Karakoyun	110	120	10	703	135	521055	4171510
22	Siverek	Darlıcalı	110	120	5	702	130	521096	4171693
23	Siverek	Darlıcalı	18	30	15	692	170	523026	4165087
24	Siverek	Darlıcalı	40	50	15	691	175	523080	4165123
25	Siverek	Darlıcalı	30	65	45	693	143	516200	4166400
26	Siverek	Darlıcalı	20	45	15	069	162	522585	4164603
27	Siverek	Darlıcalı	20	45	20	695	175	521643	4163806
28	Siverek	Darlıcalı	55	75	20	693	180	517113	4177799
29	Siverek	Darlıcalı	60	65	10	690	182	522268	4167968
30	Siverek	Darlıcalı	40	78	10	692	144	517186	4178696
31	Siverek	Darlıcalı	110	120	10	691	145	522183	4177869
32	Siverek	Darlıcalı	60	65	20	695	155	517445	4166306
33	Siverek	Darlıcalı	55	75	10	693	156	"_"	"_"
34	Siverek	Darlıcalı	110	120	10	694	148	"_"	"_"
35	Siverek	Darlıcalı	30	65	45	692	139	518191	4164812
36	Siverek	Darlıcalı	35	40	10	695	146	522234	4164558
37	Siverek	Darlıcalı	110	120	39	696	129	523209	4165192
38	Siverek	Darlıcalı	110	120	30	697	138	523806	4164510
39	Siverek	Darlıcalı	90	110	13	692	149	524109	4164123
40	Siverek	Darlıcalı/Kadıoğlu	30	65	45	691	160	522454	4164883
41	Siverek	Darlıcalı/Kadıoğlu	20	55	25	695	154	"_"	"_"
42	Siverek	Darlıcalı/Kadıoğlu	20	48	20	699	157	"_"	"_"
43	Siverek	Darlıcalı/Kadıoğlu	20	65	40	698	138	"_"	"_"
44	Siverek	Darlıcalı/Kadıoğlu	20	45	10	694	156	"_"	"_"
45	Siverek	Darlıcalı	110	120	1	692	150	517220	4168001
46	Siverek	Karacadag	15	42	1.5		100	"_"	"_"

Well Number	Location	Well Name	License owner	License Status	Elevation	Coordinate		Flow Rate
1	Ataturk Camlik	Exterior 1	Siverek Belediyesi	Existing	720.04	526700.939,	4178597.093	25lt/sn
2	Ataturk Camlik	Bahce 1	Siverek Belediyesi	Existing	718.12	526624.876,	4178597.093	281t/sn
3	Ataturk Camlik	Bahce 2	Siverek Belediyesi	Existing	716.30	526473.007,	4178403.903	201t/sn
4	Ataturk Camlik	Well No. 3	Siverek Belediyesi	Existing	715.77	526473.007	4178299.907	251t/sn
5	Ataturk Camlik	Well No. 4	Siverek Belediyesi	Existing	714.09	526427.256	4178241.163	251t/sn
6	Ataturk Camlik	Well No. 5	Siverek Belediyesi	Existing	42.00	526373.740	4178127.751	251t/sn
7	Sanayi	Ciraklik Okulu	Siverek Belediyesi	Existing	694.96	525635.845	4176938.506	281t/sn
8	Sanayi Park	Well No. 6	Siverek Belediyesi	Existing	696.60	525676.103	4177020.640	281t/sn
9	Camlik Karsisi	Astroturf Field	Siverek Belediyesi	Existing	721.91	526745.018	4178350,818	201t/sn
Spring Waters								
1	Gedik Koyu Karacadag	Gedik Well No. 1	Siverek Belediyesi	Existing	1142.73	561421.424	4180389.075	201t/sn
2	Gedik Koyu Karacadag	Gedik Well No. 2	Siverek Belediyesi	Existing	1143.07	561442.890	4180359.331	201t/sn
3	Ileri Koyu Karacadag	Ileri Well No. 1	Siverek Belediyesi	Existing	1248.19	564733.544	4175252.577	151t/sn
4	Ileri Koyu Karacadag	Ileri Well No. 1	Siverek Belediyesi	Existing	1246.72	564645.333	4175230.252	151t/sn

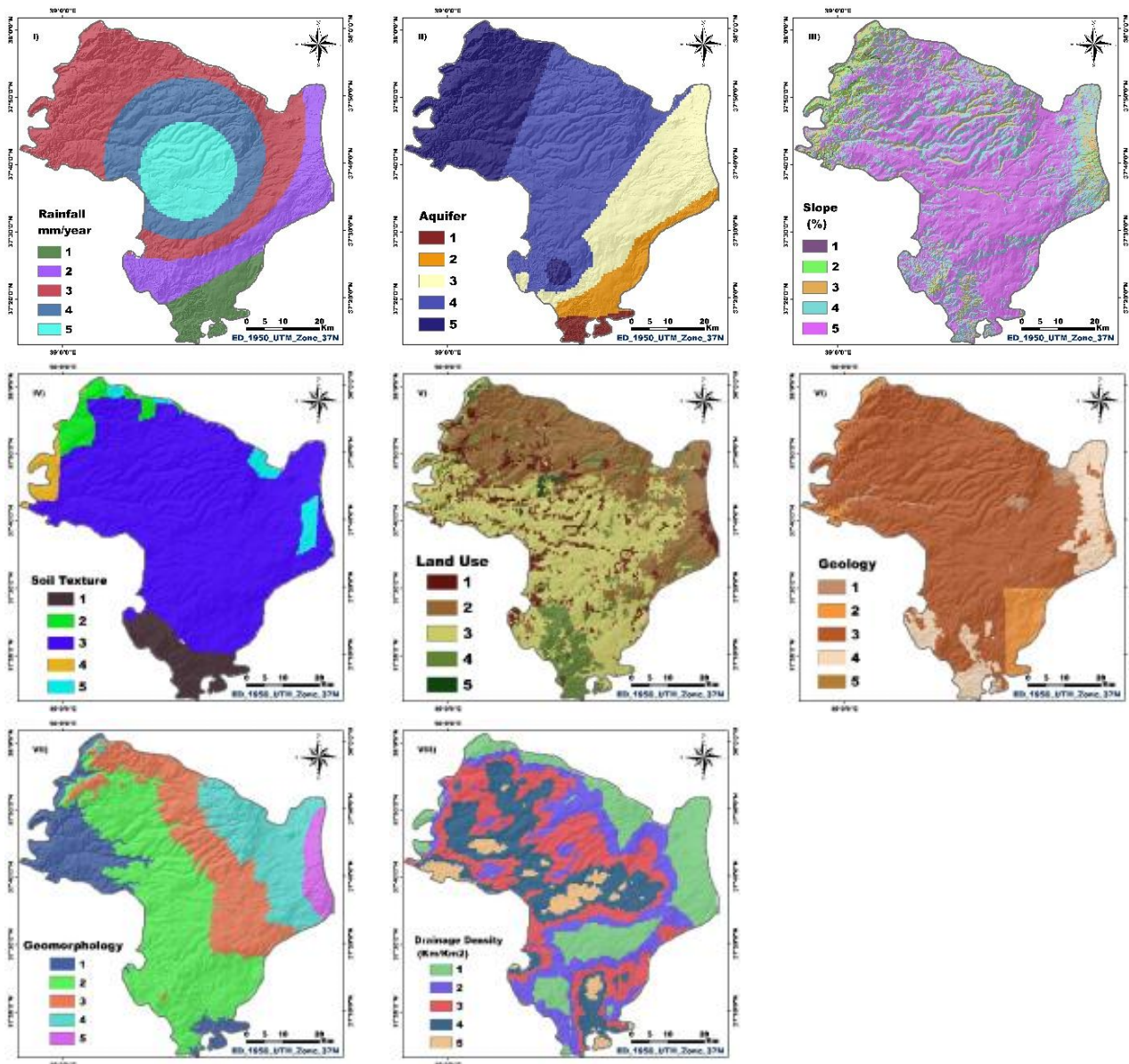


Figure 3. Generated maps of 8 reclassified parameters with ArcMap 10.5 program ((I). Rainfall, II). Aquifer, III). Slope, IV). Soil texture, V). Land use, VI). Geology, VII). Geomorphology, VIII). Drainage density)

Here;

SWL: Static Water Level

DWL; Dynamic Water Level

The reason for creating Table 1 for groundwater potential is;

Providing real data; coordinated drilling data includes real site-specific measurements such as groundwater depth, flow rate, water level and lithology. These data increase accuracy in modeling and analysis.

Validation; the accuracy of the hydrogeological models made on the map is tested by comparing them with existing drilling data. Thus, the reliability of the map is increased.

Understanding groundwater distribution; Measurements at drilling points help to show in which areas water is more abundant and in which

areas it is limited.

Determining areas: Drilling data is one of the basic inputs in the classification of areas with high, medium and low groundwater potential.

Increasing map sensitivity; Especially in areas with very wide or heterogeneous geological structures, when drilling data is geographically placed, the potential map is prepared more sensitively and in accordance with local conditions.

A total of 82 data were obtained from wells opened by relevant institutions, organizations and private companies regarding the groundwater potential of the Siverek basin. About 17 of these wells, It was removed because it was out of bounds and because its coordinates were missing. The reason for using the obtained wells in the

basin is to create observation wells for the distribution of groundwater potential and quality analysis.

In order to create the infrastructure of the TOPSIS method and the groundwater potential distribution map, the 8 parameters used in the

study were reclassified.

Application of TOPSIS Method

The TOPSIS method application was made in the light of the explanations in the methodology section (Table 2).

Table 2. Pairwise comparison matrix

	Beneficial Performance	Non-Beneficial Cost	Beneficial	Beneficial Sustainability
Weight Values	0.4	0.2	0.3	0.1
Rainfall	60	55	65	75
Aquifer	50	55	50	45
Slope	40	45	40	35
Soil Texture	30	35	50	30
Land Use	25	30	45	45
Geology	20	25	40	40
Geomorphology	15	20	35	35
Drainage Density	10	15	30	30

By dividing the sum of the squares of the relevant column's values by the square root, the normalized decision matrix is created, with each

value in the columns being reduced to a single denominator (Table 3).

Table 3. Obtaining the normalized matrix

	Performance	Cost	Beneficial	Sustainability
Weight Values	0.4	0.2	0.3	0.1
Rainfall	0.601505649	0.513996245	0.504878164	0.601929265
Aquifer	0.627455805	0.599208182	0.449921271	0.452267017
Slope	0.644628082	0.612357456	0.403028963	0.394396246
Soil Texture	0.632349375	0.602408457	0.550466625	0.367867925
Land Use	0.680118897	0.646846494	0.593410447	0.593405150
Geology	0.741977933	0.706722112	0.655304374	0.655310093
Geomorphology	0.829459431	0.798863527	0.759028943	0.759040553
Drainage Density	0.98732505	0.994973736	0.999054489	0.99909052

By multiplying the weights by the values in the standard decision matrix, a weighted standard

decision matrix is produced Table 4)

Table 4. Obtaining a weighted normalized matrix

	Performance	Cost	Beneficial	Sustainability
Rainfall	0.240602259	0.102799249	0.151463449	0.060192927
Aquifer	0.250982322	0.119841636	0.134976381	0.045226702
Slope	0.257851233	0.122471491	0.120908689	0.039439625
Soil Texture	0.252939750	0.120481691	0.165139988	0.036786792
Land Use	0.272047559	0.129369299	0.178023134	0.059340515
Geology	0.296791173	0.141344422	0.196591312	0.065531009
Geomorphology	0.331783772	0.159772705	0.227708683	0.075904055
Drainage Density	0.394930020	0.198994747	0.299716347	0.099909052

In order to obtain the ideal solution from each column in the weighted decision matrix, the ideal values for the positive ideal and negative ideal solutions are selected and the solution sets are determined. In the explanation below, the first line shows the positive ideal solution and the second line shows the negative ideal solution set.

Obtaining Ideal and Anti-Ideal Solution Values

Worst and best (closest to good and farthest from bad)

$$V^+ 0.240602259 \ 0.198994747 \ 0.299716347 \ 0.099909052$$

$$V^- 0.394930020 \ 0.102799249 \ 0.120908689 \ 0.036786792$$

Table 5. Ordering the choice (C_i^*) and calculating the distances between the ideal and anti-ideal solutions (S_i^* ve S_i^-)

	S_i^*	S_i^-	C_i^*	Rank
Rainfall	0.047510749	0.308255572	0.866455181	1
Aquifer	0.048809111	0.312439149	0.864887623	2
Slope	0.04883046	0.312507471	0.864862071	3
Soil Texture	0.053559419	0.327290145	0.859368568	4
Land Use	0.062979911	0.354908189	0.849290010	5
Geology	0.075502852	0.388594523	0.837312478	6
Geomorphology	0.096610229	0.439568492	0.819817114	7
Drainage Density	0.147690169	0.543489041	0.786321454	8

The distance values to the positive ideal and negative ideal solution were calculated by subtracting the positive ideal and negative ideal values from the values in the column of each parameter (Table 5).

According to the application in the TOPSIS method, the ideal solution is respectively; rainfall, aquifer, slope, soil texture, land use, Geology, Geomorphology, drainage density.

In TOPSIS implementation, indices for groundwater potential and quality assessment are often artificially chosen based on subjective experience, which greatly reduces the relevance, accuracy and reliability of assessment results.

Verification of Siverek Basin Groundwater Potantial Index (GWPI) Results with Field Well Data

Normally, it is very costly to obtain up-to-date data from wells in the field. Therefore, it is shorter and more economical to determine the groundwater potential and quality of a site with multi-criteria decision making methods. Therefore, it is necessary to determine the parameters and weights that affect the groundwater correctly. GWPI area distribution map was produced with the method mentioned above in ArcGIS ArcMap 10.5 environment by using the data of 11 of the wells drilled before, by checking the determined method and the results obtained in the field.

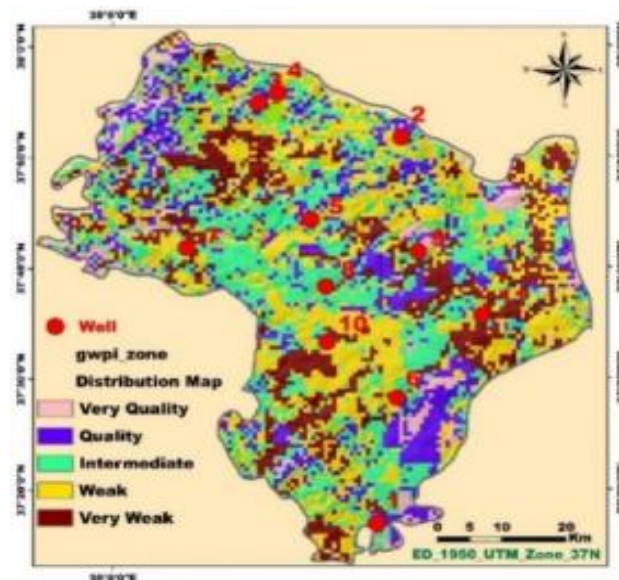


Figure 4. Groundwater potential distribution map of the Siverek basin survey site

Table 6. Definition of Siverek basin according to GWPI values

GWPI Value	Definition	Rate (%)	Covered area (km ²)
204 - 257	Very Well	8,00	356,08
258 - 293	Well	22,00	987,17
294 - 311	Moderate	35,07	1515,37
312 - 361	Poor	23,03	1002,22
362 - 395	Very Poor	11,93	508,12

The eight parameters used for the groundwater potential index distribution map were first reclassified, and then a precise map of the groundwater potential zone (GWP_Z) was produced in ArcToolbox, Spatial Analyst Tools, Overlay, Weighted Overlay (Figure 4).

Groundwater potential and quality distribution values were obtained between approximately 204 and 361, and as explained in Table 6, it can be said that the groundwater potential and quality are partially at a good level in the scattered regions of the basin (Table 6).

Groundwater potential index distribution (GWPI) is a map showing the groundwater potential of the study area. This map shows the possibility of obtaining groundwater resources for the study area. GWPI for the study area changes according to the variability of the characteristics that constitute the groundwater potential. This GWPI map produced for the study shows groundwater discharge zones for very poor: very low, poor: low, medium, quality:

medium low, quality: high rate and very high rate: very high quality.

Accordingly, when the description of the evaluation of the basin's GWPI map in Table 6 is examined; very poor (508.12 km²; 11.93%), poor (1002.22 km²; 23.03%), medium (1515.37.22 km²; 35.07%), good (987.17 km²; 22%) and very good (356.08 km²; 8%). When the basin's GWPI map is examined, it is seen that the average potential is around 35%.

Validity (Verification)

Groundwater potential value data from 11 observation wells in the basin shown in Figure 4 represent water well locations. Almost all of the irrigation pump wells were evaluated in terms of groundwater potential area with very good, good, medium, weak, very weak categories. In the evaluation, 11 of these wells were found to be compatible

Table 7. Comparison of well data

Number Sequence	Town	X	Y	Z	Depth	SWL	DWL	Yield	GWPI	Evaluation	Coherence
1	Siverek	520611	4171942	512	216	30	66	3	201	Poor	Conformable
2	Siverek	521536	4170092	681	120	110	120	2	214	Moderate	Conformable
3	Siverek	519915	4172301	583	126	110	120	1	226	Good	Conformable
4	Siverek	519759	4169821	735	135	110	120	5	247	Poor	Conformable
5	Siverek	521055	4171510	577	140	80	90	1	279	Good	Conformable
6	Siverek	521096	4171693	735	152	110	120	3	312	Moderate	Conformable
7	Siverek	523026	4165087	666	160	7	120	1	351	Good	Conformable
8	Siverek	523080	4165123	713	150	110	120	1,5	384	Weak	Conformable
9	Siverek	516200	4166400	664	145	110	120	4	397	Good	Conformable
10	Siverek	522585	4164603	712	135	110	120	1	405	Weak	Conformable
11	Siverek	521643	4163806	856	130	110	120	0,1	414	Moderate	Conformable

Trying to validate the groundwater model using the terrain-based groundwater potential map of the study area, this puts forward the

assumption that water is the main factor affecting the groundwater potential quality and generally the amount of water available for runoff

Table 8. Classification of parameters affecting the groundwater potential zones

Sequence No.	Parameters	Rank	Sub - Parameters	Land Coverage Area	Area Covered (%)	Groundwater View	Degree
1	Rainfall	9	347 - 364	347	0.19	Very Weak	3
			365 - 374	354	0.18	Weak	4
			375 - 382	384	0.20	Moderate	5
			383 - 390	391	0.21	Quality	7
			391 - 400	399	0.22	Very Quality	8
2	Aquifer (Hydrogeology)	8	512 - 553	655	0.23	Very Weak	4
			554 - 580	648	0.22	Poor	5
			581 - 599	629	0.20	Moderate	6
			600 - 619	637	0.21	Quality	7
			620 - 656	511	0.16	Very Quality	8
3	Slope	7	0,10 - 2,20	70315	0.29	Very Good	9
			2.21 - 5.61	68706	0.27	Very Good	8
			5.62 - 10.8	40455	0.17	Good	7
			10.9 - 20.2	25572	0.11	Moderate	5
			20.3 - 51.1	37852	0.15	Poor	3
4	Soil Texture	6	Reddish Brown Soils	2488	0.30	Very Good	8
			Brown Soils	681	0.08	Good	6
			Brown Forest Lands	1183	0.14	Moderate	5
			Other Areas	874	0.11	Poor	4
			Basaltic Soils	2994	0.36	Very Weak	3
5	Land Use	5	Vineyards	3272	0.14	Very Good	7
			Irrigated Arable Lands	8151	0.34	Good	6
			Orchards	3587	0.15	Moderate	5
			Meadows and Pastures	5032	0.21	Poor	4
			Natural Grassland	3778	0.16	Very Poor	3
6	Geology	6	Eocene	2543	0.15	Very Good	6
			Miocene	2284	0.13	Good	5
			Pliocene – Quaternary	159	0.009	Moderate	4
			Pliocene	3244	0.18	Poor	3
			Upper Miocene	8957	0.52	Very Poor	2
7	Geomorphology	5	413 – 667	154223	0.06	Very Good	7
			668 – 835	374528	0.19	Good	6
			835 – 1030	555164	0.29	Moderate	5
			1031 – 1311	572161	0.30	Poor	4
			1312 – 1793	278014	0.14	Very Poor	3
8	Drainage Density	7	0,10 - 0,65	17	0.001	Very Poor	3
			0,66 – 1,43	374	0.012	Poor	4
			1.44 – 2,15	542	0.017	Moderate	5
			2,16 – 3,30	1347	0.043	Good	6
			3,31 – 5,54	28779	0.93	Very Good	7

As a result of the classification of the parameters affecting the groundwater potential regions in the ArcMap environment, both classification and weighting of these parameters according to their effects on groundwater were provided (Table 8).

Conclusion and Recommendations

In the determination of the underground water potential and change of the Siverek basin with the geographical information system, the well data obtained firstly was arranged in accordance with the purpose of the study. In the study, 8 raster maps were created using Spatial Analyst Tools, Interpolation, IDW methods and other techniques in GIS environment in order to

determine the formation of groundwater in the basin, where and how much groundwater is found and its usage possibilities. After that these thematic maps were reclassified and the ground was prepared for the potential status of groundwater. These parameters, which were obtained as a result of the application of the TOPSIS method, were matched with the percent values in their ideal order in the ArcMap program. With this map, which was created as a result of overlapping (Figure 4), a database was provided in order to be able to control the groundwater level in the future and to make hydrogeological evaluations.

In the study, the operating performance of Siverek groundwater potential was evaluated with the GIS supported TOPSIS method and the

success status of each parameter was listed. According to the effects of the eight parameters used in the study on the groundwater, the method was applied in the rate analysis in the process. With the applied method, the examination rates were converted into a single concrete value mathematically and the results were expressed numerically. In this context, it was observed that the most successful parameter of the method application was precipitation (approximately 8.7%), while the parameter with the lowest success rate was drainage density (approximately 7.9%). (Table 5).

By using multi-criteria decision making methods, it can be ensured that the decisions are effective in the groundwater potential of the institutions and organizations, and the working performance can be increased by choosing the right alternatives. Evaluating the business with other performance criteria and other businesses and comparing it with the method may be more meaningful for decision makers on behalf of the business. Studies for decision makers and researchers at all levels can contribute to the measurement and evaluation of business success.

Considering the usable water potential per capita, Siverek is a district experiencing water pressure. It is pointed out that there will be water shortages at medium and high levels in many parts of the basin. This situation shows that, contrary to popular belief, it is a candidate basin to face serious water problems in the near future. What to do in the short and long term; In order to determine the groundwater reserves correctly and healthily, the hydrogeological studies of the district-wide groundwater basins should be completed quickly, the groundwater potential of the basins should be determined, and the groundwater allocations should be followed by establishing a monitoring system. In addition,

Preventing excessive and uncontrolled water withdrawal; preventing unlicensed and uncontrolled use of underground water wells,

Using Efficient Irrigation Techniques;

switching to drip or sprinkler irrigation methods instead of traditional wild irrigation.

Detection of illegal wells; carrying out strict inspections by water management institutions.

Informing the public; Providing them with training on water saving and groundwater protection.

Serious and deterrent measures should be taken, especially for the protection of groundwater, and the opening of uncontrolled and unlicensed groundwater wells, the number of which is increasing day by day, should be prevented. Groundwater aquifers research program should be initiated, the existence and characteristics of groundwater aquifers should be revealed.

Major problems such as desertification, disappearing wetlands and pastures, unplanned agricultural policies and unplanned hydroelectric power generation, and the effects of global climate change should also be taken into account.

Waste water should be made reusable and measures should be taken to prevent leakages in city and irrigation networks.

In industrial sectors with high water use, water savings should be achieved by expanding the use of advanced technology and waste water.

It causes salinization and sterility due to excessive irrigation in agricultural production areas. In order to prevent this, the use of the flood irrigation method should be changed rapidly, and the irrigation system of agricultural areas should be changed by using advanced technology methods or drip irrigation methods. In addition, water conservation should be ensured and the necessary support and training should be given to the farmer in this regard.

Necessary sensitivity should be shown in the protection of groundwater resources, wells should not be drilled in a way that affects each other, excessive drafts should be controlled to prevent wastage of water.

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Conflict of interest

The author declares no conflict of interest

Author's Contributions

Veysel ASLAN conceptualized the study, developed the methodology, validated the results, and wrote the original draft.

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Sürdürülebilir tarımda yeşil sentez ile üretilen ZnO-nanopartiküllerinin etkinliği: Yapraktan spreyleme uygulaması

Effectiveness of ZnO-Nanoparticles produced with green synthesis in sustainable agriculture: Leaf spraying applications

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Öz

Çinko oksit nanoparçacıklarının (ZnO-NP'ler) yeşil sentezi, bitki büyümesini artırma potansiyelleri nedeniyle önemli ölçüde dikkat çekmiştir. Bu çalışmada, kekik ekstresi kullanılarak sentezlenen ZnO-NP'lerin (Tv-ZnO-NP'ler) buğday bitkisi üzerindeki etkileri, 250 ila 5000 mg L⁻¹ arasında değişen yedi farklı konsantrasyon uygulanarak araştırılmıştır. Amaç, bu nanoparçacıkların bitki büyümesi üzerindeki etkilerini değerlendirmek ve büyüme performansını artırmadaki potansiyel rollerine ilişkin öngörüler sağlamaktır. Tv-ZnO-NP'ler, ortalama 19,72 nm parçacık boyutu ortaya koyan SEM, FTIR ve XRD analizleri ile karakterize edilmiştir. Buğday fidelerinin büyümesi üzerindeki etkileri değerlendirmek amacıyla sentezlenen çinko oksit nanoparçacıkları (Tv-ZnO-NP'ler) yapraktan püskürtme yöntemiyle uygulanmıştır. Uygulamanın ardından, tüm uygulama konsantrasyonlarında kontrol grubuna kıyasla fide boyunda anlamlı bir artış gözlemlenmiştir. Ayrıca, düşük konsantrasyonlarda (250 ve 500 mg L⁻¹) klorofil içeriğinde anlamlı bir artış meydana gelmiş; buna karşın, daha yüksek konsantrasyonlarda (≥4000 mg L⁻¹) istatistiksel olarak anlamlı bir değişim tespit edilmemiştir. Bununla birlikte, 250 ila 3000 mg L⁻¹ konsantrasyonları arasında Tv-ZnO-NP'lere maruz kalan bitkilerde kuru/yaş ağırlık oranında istatistiksel olarak anlamlı değişiklikler ve Zn²⁺ içeriğinde belirgin bir artış gözlemlenmiştir. Bu sonuçlar, Tv-ZnO-NP'lerin buğday fidelerinin büyüme ve gelişimini olumlu yönde etkilediğini ortaya koymaktadır. Ayrıca, nanoparçacık konsantrasyonu ile bitki tepkisi arasında açık bir doz-tepki ilişkisi saptanmıştır. Bu bulgu, bitki kaynaklı nanoparçacıkların tarımda kullanım potansiyelini göstermekte ve en yüksek tarımsal verimin sağlanabilmesi için uygun dozun dikkatle belirlenmesi gerektiğini vurgulamaktadır.

Anahtar Kelimeler: Buğday, Kekik, Klorofil, Sürdürülebilir tarım, Yaprak uygulaması

ABSTRACT

The green synthesis of zinc oxide nanoparticles (ZnO-NPs) has garnered significant attention due to their potential to enhance plant growth. In this study, the effects of ZnO-NPs synthesized using thyme extract (Tv-ZnO-NPs) on wheat plants were investigated by applying seven different concentrations ranging from 250 to 5000 mg L⁻¹. The aim was to evaluate the effects of these nanoparticles on plant growth and to provide insights into their potential roles in improving growth performance. Tv-ZnO-NPs were characterized by SEM, FTIR, and XRD analyses, revealing an average particle size of 19.72 nm. To assess their effects on the growth of wheat seedlings, the synthesized ZnO nanoparticles (Tv-ZnO-NPs) were applied via foliar spraying. Following the application, a significant increase in seedling height was observed at all treatment concentrations compared to the control group. Additionally, a significant increase in chlorophyll content was noted at low

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concentrations (250 and 500 mg L⁻¹), whereas no statistically significant change was detected at higher concentrations (≥4000 mg L⁻¹). However, in plants exposed to Tv-ZnO-NPs at concentrations between 250 and 3000 mg L⁻¹, statistically significant changes in dry/fresh weight ratio and a marked increase in Zn²⁺ content were observed. These findings indicate that Tv-ZnO-NPs positively affect the growth and development of wheat seedlings. Furthermore, a clear dose-response relationship between nanoparticle concentration and plant response was established. This shows the agricultural potential of plant-derived nanoparticles and emphasizes the need to optimize the appropriate dose for achieving maximum agricultural benefits.

Key Words: Chlorophyll, Foliar treatment, Sustainable agriculture, Thyme, Wheat

Giriş

Benzersiz fiziksel ve kimyasal özellikleri sayesinde nanobilim, son yıllarda en hızlı gelişen ve en fazla ilgi gören çok disiplinli araştırma alanlarından biri haline gelmiştir. Nanopartiküller (NP'ler), boyutları 1–100 nanometre arasında değişen ve nanoteknolojinin temel bileşenlerini oluşturan partiküller olarak tanımlanır. Bu yapılar hem doğada doğal olarak bulunabilir hem de kontrollü laboratuvar koşullarında sentetik olarak üretilebilir. Boyut ve morfolojik özelliklerinden kaynaklanan yüksek yüzey alanı, iletkenlik, mekanik dayanıklılık gibi özgün özellikleri sayesinde, nanopartiküller günümüzde sağlık, çevre, enerji ve tarım gibi birçok alanda yaygın olarak kullanılmaktadır. Özellikle çevre dostu özellikleriyle öne çıkan doğal ve biyojenik nanopartiküller, son dönemde sürdürülebilir teknolojiler kapsamında dikkat çeken bir araştırma konusu olmuştur. Bu nanopartiküllerin biyosentezine dayalı üretim yöntemleri, geleneksel kimyasal ve fiziksel sentez tekniklerine kıyasla daha az enerji ve kimyasal gereksinimi duymaları, çevresel toksisiteyi azaltmaları ve daha düşük maliyetli olmaları nedeniyle avantajlı bir alternatif sunmaktadır (Chimbekujwo ve ark., 2024).

Nanoteknoloji, birçok alanda potansiyel uygulamalara ve avantajlara sahip, laboratuvar alanından pratik alana geçen yeni bir teknoloji olarak günlük hayata dahil olmuştur (Sharma ve ark., 2023). Bitki özlerinin kullanıldığı yeşil sentez metodu, sürdürülebilir, uygun maliyetli ve çevre dostu alternatif bir metod olarak ortaya çıkmaktadır. Bol ve kolay erişilebilir olan bitkiler, nanopartikül üretiminde etkili kaynaklar olarak hizmet etmektedir. Bitki özlerinde bulunan

fitokimyasallar kütle formundaki metalleri kaplayıcı, stabilize edici ve indirgeyici ajanlar olarak kullanılmakta ve diğer kimyasal ajanlara olan ihtiyacı azaltmaktadır. Bitki özlerindeki fitokimyasallar, metal iyonunu indirgeme ve ortaya çıkan nanopartiküllerin stabilizasyonunda oldukça önemlidir (Ovais ve ark., 2018). Birçok çalışmada, bitki seçiminin nanoparçacık özellikleri üzerindeki etkisi vurgulanarak farklı bitki türlerinin kullanıldığı ZnO nanopartiküllerinin (ZnO-NP'ler) yeşil sentezi araştırılmıştır (Pakzad ve ark., 2019; Potbhare ve ark., 2019; Sone ve ark., 2020).

Özellikle iklim değişikliği ve çevresel sürdürülebilirlik ile ilgili sorunların üstesinden gelebilmek ve ekosistem dostu nanomalzemeler üretmek için uygulanabilir ve etkili çözümler bulmak gereklidir. İklim değişikliğinden en çok etkilenen sistemlerden biri tarım sistemleridir. Tarımsal üretimi arttırmak ve bitki büyümesini desteklemek için kullanılan kimyasal gübrelerle alternatif olarak geliştirilen nano-gübreler, son yıllarda yaygın olarak araştırılan bir konudur (Zhu ve ark., 2020). İklim değişikliğine bağlı öngörülemeyen mevsimsel değişikliklere bağlı olarak bitkilerin değişen yaşam döngüsü, ürünün beslenme kalitesini değiştirir (Sharma ve ark., 2023). Buna bağlı olarak kimyasal gübre ve pestisitlerin aşırı kullanımı toprak verimliliğini azaltmakta, gıda güvenliğini tehdit etmekte, yeraltı suyu ve hava kirliliği gibi doğal ekosistemleri bozmaktadır (Verma ve ark. 2022; Sharma ve ark., 2023). Tüm bu olumsuzluklarla başa çıkmak, tarımsal uygulamaları verimli hale getirmek ve doğal kaynakları korumak için sistematik çalışmalarla tarımsal ve çevresel sürdürülebilirlik sağlanabilir (Babu ve ark., 2022). Bu bağlamda, nanoteknolojik gelişmelerle ortaya çıkan nano-gübrelerin araştırma ve geliştirme

çalışmaları, özellikle besin alımının artırılmasında ve toprak-bitki sistemindeki besin eksikliklerini azaltma konusunda umut vericidir (Saurabh ve ark., 2024). Nano-gübre olarak kullanılan nanopartiküller, biyotik ve abiyotik stres koşulları altında savunma sistemlerini aktive ederek bitkilerin stresle başa çıkma yeteneğinde oldukça önemli bir rol oynarlar (Usman ve ark., 2020a; Usman ve ark., 2020b; Sharma ve ark., 2023). Nano-gübreler makro (azot-N, fosfor-P, potasyum-K, kalsiyum-Ca, magnezyum-Mg ve kükürt-S) ve/veya mikro (demir-Fe, çinko-Zn, bakır-Cu, bor-B, nikel-Ni vb.) besin elementleri içeren nanopartiküller olarak tanımlanabilir. Bu gübreler iklim değişikliği nedeniyle toprak-bitki sisteminde oluşabilecek besin elementi noksanlığının yol açacağı tarımsal zararları azaltmak için araştırmacılara ve çiftçilere yeni bir çözüm yolu sunmaktadır. Özellikle metal ve metal oksit nanopartiküller besin elementi alımını kolaylaştırabilme, tohum çimlenmesini arttırma ve patojenlere karşı bitki direncini iyileştirebilme gibi özelliklere sahiptirler (Dimpka ve ark., 2012). Çinko (Zn), bitkilerin enzim ve protein yapılarının biyosentezinde aktif bir rol oynayarak çimlenme, büyüme ve gelişim aşamalarını etkileyen en önemli mikro besin elementlerinden biridir (Sturikova ve ark., 2018; Saini ve ark., 2021; Azam ve ark., 2022). Bitkilerde Zn eksikliğinde, enzim aktiviteleri bozulur, büyüme ve gelişme azalır ve kloroz gibi semptomlar gözlenebilir (Chandra ve ark., 2015; Sturikova ve ark., 2018; Azam ve ark., 2022). Bu nedenle, bir metal oksit nanopartikülü olan ZnO nanopartiküllerinin (ZnO-NP), pestisitler ve diğer gübrelerden daha iyi performans sergilemesi, üretkenlik artışına katkısı ve toprak sağlığının korunması gibi birçok avantajından dolayı tarımsal sürdürülebilirlik alanında dikkat çekmektedir (Ruttikay-Nedecky ve ark., 2017). Öte yandan, yüksek konsantrasyonlardaki ZnO-NP uygulamalarının bitkilerde toksik etki gösterdiği çalışmalar da mevcuttur (Cao ve ark., 2021; Babu ve ark., 2022). Bu nedenle, uygulanan ZnO-NP'lerin etkili eşik konsantrasyonunun doğru bir şekilde araştırılması ve değerlendirilmesi gerekmektedir.

Biyojenik olarak üretilen ZnO-NP'lerin tarım sektöründe uygulanabilirliğinin araştırıldığı bu çalışmada, sürdürülebilir ve çevre dostu bir üretim yaklaşımı ile kekik bitki özütü kullanılarak çinko oksit nanopartiküller sentezlenmiştir. Çalışmada, kekik özütünü stabilize edici ve indirgeyici ajan olarak kullanarak ZnO-NP üretimi için basit ve sürdürülebilir bir metod uygulanmıştır. Ayrıca, kekik özütünden elde edilen ZnO-NP'lerin buğdayın (*Triticum aestivum* - Adana 99) büyüme parametreleri ve klorofil içeriği üzerine etkisi açıklanmaya çalışılmıştır. Bu nanopartiküllerin bitki yapraklarına spreyleme metodu ile uygulaması gerçekleştirilerek nanopartiküllerin bitki tarafından alımı belirlenmiştir. Bu çalışmada; I) bitki özütü kullanılarak elde edilen ZnO-NP'lerin yeşil sentezi hakkında değerli bilgiler sağlamak, II) ZnO-NP'lerin bitki büyümesi üzerindeki etkilerine ışık tutmak ve III) sürdürülebilir tarım uygulamaları kapsamında bitkisel üretimde karşılaşılan beslenme sorunlarına yeni çözümler üretmek amaçlanmıştır.

Materyal ve Yöntem

Materyal

ZnO-NP'lerin sentezinde kullanılan çinko asetat dihidrat ($Zn(CH_3COO)_2 \cdot 2H_2O$ > %98) ve sodyum hidroksit (NaOH) özel bir firmadan satın alınmıştır. Kekik (*Thymus vulgaris* L.) yaprakları ve buğday (*Triticum aestivum* - Adana 99) tohumları, Mersin ili yerel piyasasından temin edilmiştir.

Bitki özütü hazırlama ve ZnO-NP'lerin yeşil sentezi

Bitki özütünün hazırlanması yöntemi Abdullah ve ark. (2020) tarafından geliştirilen yöntemle uyarlanmıştır. Kekik (*Thymus vulgaris* L.) yaprak özütü elde etmek için yapraklar musluk suyu ve damıtılmış su ile yıkanarak tüm kirliliklerden arındırılmıştır. Daha sonra, 30 g kekik yaprağı tartılmış ve 450 mL distile su içeren bir behere bırakılarak hot-plate üzerinde (80°C) 30 dakika ısıtılmıştır. Süre sonunda hazırlanan çözelti oda sıcaklığına kadar soğutulmuş ve iki kez filtre edilmiştir. Elde edilen bitki özleri daha sonra kullanılmak üzere 4°C'de buzdolabında saklanmıştır.

ZnO-NP'lerin yeşil sentezi için ilk olarak 33.33 g/L çinko asetat dihidrat ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) stok çözeltisi hazırlanmıştır (Abdullah ve ark., 2020). Daha önce hazırlanan bitki özütü (90 mL) ve çinko asetat stok çözeltisi (150 mL) 10 dakika boyunca hızlı bir şekilde karıştırılmıştır. Süre sonunda, pH 12.0'a ayarlamak için karışıma 1 M NaOH damlatılmış ve ardından oda sıcaklığında 2 saat boyunca sürekli olarak çalkalanmıştır. Elde edilen çözelti içerisindeki ZnO-NP'lerin çökmesini sağlamak amacıyla bekletilmiş ve çökelti süzülerek deiyonize su ile 3 kez yıkanmıştır. Filtre kâğıdı üzerinde toplanan çökelti bir etüvde 60°C'de gece boyunca kurutulmuştur. Yeşil sentez metodu ile üretilen kekik özütü katkılı ZnO-NP'ler (Tv-ZnO-NP) ince bir toz haline gelene kadar öğütülmüş ve karakterizasyon için saklanmıştır.

Karakterizasyon çalışmaları

Üretilen Tv-ZnO-NP'lerin karakterizasyonu taramalı elektron mikroskobu (SEM, FEI Quanta FEG 250 Model), Fourier dönüşümlü kızılötesi spektroskopisi (FTIR, Bruker ALPHA Model) ve X-ışını kırınımı (XRD, Bruker D8 Advance Model) kullanılarak gerçekleştirilmiştir. Tv-ZnO-NP'lerin ortalama boyutları Debye-Scherrer denkleminde (Denklem 1) göre hesaplanmıştır.

$$D = 0.9\lambda / \beta \cos\theta \quad (1)$$

Burada; D: kristal tanelerin boyutunu, β (rad): XRD zirvesinin yarım-maksimumda tam genişliğini (FWHM), λ : X-ışınlarının dalga boyunu, θ : gözlemlenen açının pik değerini ve k: sabit değeri ifade etmektedir ($k = 0.9$) (Zakirov ve ark., 2018).

Saksı denemesi: Tv-ZnO-NP süspansiyonlarının yapraktan uygulaması

Ülkemiz toprakları Zn açısından fakir olup özellikle tahıl bitkilerinin Zn ihtiyacı fazladır (Ekiz ve ark. 1998; Çakmak ve Kutman, 2018). Bu nedenle özellikle bitki gelişiminin erken döneminde Zn içeren yaprak gübreleme uygulamasının bitkiler üzerinde olumlu etkilerinin olduğu bilinmektedir (Prasad ve ark., 2012; Davarpanah ve ark., 2016; Kah ve ark., 2018).

Saksı denemelerinde bitkiler torf (pH 6.0-7.0,

organik madde: %76.4, nem eşdeğeri: %107) ortamında, oda sıcaklığında laboratuvar koşullarında yetiştirilmiştir. Her saksıya 80 g torf doldurulmuş ve 5'er adet buğday tohumu ekilerek musluk suyu ile sulanmıştır. Çimlenmenin ardından bitkiler erken gelişim evresi olan 3 yapraklı döneme (Zadoks skalasına göre Z20: ana saptta dördüncü yaprağın gelişmeye başladığı dönem (Zadoks ve ark., 1974; Tiryakioğlu ve ark., 2014) kadar 28 gün büyütülmüştür. Süre sonunda her saksının torf yüzeyi folyo ile kaplanarak üretilen Tv-ZnO-NP'lerin toprakla teması kesilmiş ve yapraktan spreyleme işlemi gerçekleştirilmiştir. Adrees ve ark. (2021)'nin çalışmasına benzer şekilde, Tv-ZnO NP'lerin ilk yaprak uygulaması çimlenmeden sonraki ikinci haftada (çimlenmeden sonraki 14. gün) bir el püskürtücüsü ile yapılmıştır. Toplamda, tüm deneyde birer haftalık aralıklarla üç spreyleme gerçekleştirilmiştir. Bu işlemde bitkiler her spreylemede 5 mL olmak üzere 3 kez toplamda 15 mL farklı konsantrasyonlarda hazırlanmış Tv-ZnO-NP'ler (250, 500, 1000, 2000, 3000, 4000 ve 5000 mg L⁻¹) ile muamele edilmiştir.

Denemeler tam tesadüf deneme desenine göre 3 paralelli olarak, laboratuvar koşullarında gün ışığı altında gerçekleştirilmiştir. Spreylemenin yapıldığı son hafta (çimlenmeden sonraki 28. gün) bitkilerin boyları ve klorofil içerikleri (SPAD-502 Klorofil metre, Konica-Minolta, Japonya) ölçülmüştür. Tüm bitkiler toprak yüzeyinin 1 cm yukarisından hasat edildikten sonra olası kirleticilerden uzaklaştırmak için damıtılmış su ile yıkanmış, kurulanmış ve hassas terazi yardımı ile yaş ağırlıkları belirlenmiştir.

Kuru ağırlıkları, 48 saat 65°C'de bir etüvde tutulan bitki örnekleri tartılarak kuru ağırlıkları hesaplanmıştır. Bitkiler oda sıcaklığına gelene kadar soğuması için bir desikatörde bekletilmiş ve sabit tartıma gelen kuru bitkilerin ağırlıkları bir hassas terazi yardımı ile belirlenmiştir.

Bitkilerde Zn içeriği tayini

Kuru ağırlıkları belirlenen bitki örnekleri, bitki öğütme değirmeninde öğütülmüştür. Ardından yaklaşık 0.5 gram kuru bitki örneği, 5 mL %12 derişimli nitrik asit (HNO_3) içerisinde, 220 °C

sıcaklıktaki bir hot-plate üzerinde çözündürülmüştür (Doğaroğlu ve Köleli, 2017). Elde edilen çözelti, çinko (Zn) içeriğinin belirlenmesi amacıyla indüktif eşleşmiş plazma-kütle spektrometresi (ICP-MS, Agilent 7500ce modeli) ile analiz edilmiştir.

İstatistiksel analiz

Her bir analizin üç tekrarlı yapıldığı deneylerden elde edilen veriler ayrı ayrı varyans analizine (ANOVA) tabi tutulmuştur. ANOVA, SPSS sürüm 26.0 (IBM) kullanılarak gerçekleştirilmiştir. Normallik testi ve varyansların homojenliği testi yapılarak verilerin normal dağılıma sahip olduğu ve gruplar arasında homojenliğin olduğu tespit edilmiştir. En küçük önemli fark (LSD) testi post-hoc olarak kullanılarak istatistiksel analizler gerçekleştirilmiştir. Çalışma kapsamında $p < 0.05$ değeri istatistiksel olarak anlamlı kabul edilmiştir.

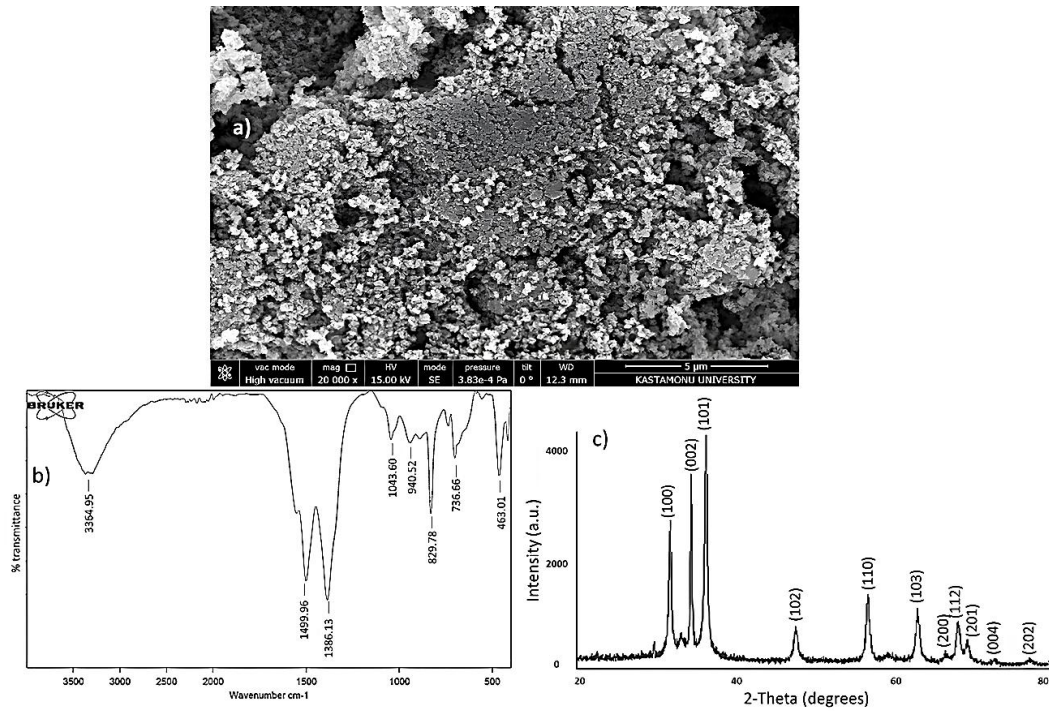
Araştırma Bulguları ve Tartışma

Üretilen Tv-ZnO-NP'lerin karakterizasyonu

Çalışma kapsamında kekik bitkisi özütü kullanılarak yeşil sentez yoluyla sentezlenen Tv-ZnO-NP'lere ait SEM görüntüsü, FTIR spektrumu ve XRD difraktogramı Şekil 1'de verilmektedir. Üretilen kekik özütü Tv-ZnO-NP kristallerinin amorf yapıda olduğu belirlenmiştir (Şekil 1a). Önceki çalışmalarda, geleneksel metodlarla sentezlenen ZnO-NP'ler genel bir eşit dağılım sergilediği belirlense de, bu çalışmada olduğu gibi özellikle yeşil yöntemiyle sentezlenen nanopartiküllerde yaygın bir karakteristik özellik olarak zaman zaman önemli bir agregasyona sahip oldukları görülmektedir. Bu olgu, biyojenik NP'lerin yükseltilmiş yüzey alanına ve kalıcı afinitesine atfedilir ve bu da kümelenme veya bir

araya gelme eğilimlerini arttırmaktadır (Vidya ve ark., 2013). Parçacık kümelenmesi, nanopartiküllerin polaritesinden ve elektrostatik çekimlerinden etkilenmektedir (Aminuzzaman ve ark., 2018).

Üretilen nanopartiküllerle ilişkili fonksiyonel grupları belirlemek için Tv-ZnO-NP'lerin FTIR analizi gerçekleştirilmiştir. Tv-ZnO-NP'lerin üretiminden sorumlu biyomoleküller, 4000 ile 400 cm^{-1} arasındaki FTIR çalışmaları kullanılarak tanımlanmıştır (Şekil 1b). Çinko asetattan gelen Zn^{2+} iyonları bitki özütünde bulunan polifenollerle bağlanarak ZnO-NP'lerin sentezi sırasında Zn^{2+} 'ya indirgenmesi nedeniyle reaksiyon çözeltisinde Zn^{+} -polifenollerin karmaşık bir üretimi olarak gözlenmektedir. Bu reaksiyonlarda rol alan fonksiyonel gruplar, bitki özütlerinden sentezlenen yeşil Tv-ZnO-NP'lerin FTIR spektrumlarındaki karşılaştırılabilir tepe noktalarıyla gösterilmektedir (Şekil 1b). Tv-ZnO-NP'lerin FTIR spektrumunda gözlenen 3357, 1572, 1496, 1403, 854, 676, 544, 468 ve 415 cm^{-1} 'deki pikler absorpsiyon bantlarını temsil etmektedir. 3357 cm^{-1} 'deki geniş ve çok güçlü bant hidroksil fonksiyonel gruplarını göstermektedir. 1572 cm^{-1} 'deki tepe noktası, polifenolik maddelerin aromatik halkalarındaki C=C gerilme titreşimleriyle ilişkilidir. Asitlerin simetrik COO gerilme modu, 1496 ve 1403 cm^{-1} 'deki tepe noktalarından sorumludur (Matinise ve ark., 2017). 1048 ve 1020 cm^{-1} 'deki spektrumlar ise bitki özündeki doymuş esterler, alkoller, fenoller, sikloalkanlar ve asit anhidritlerden kaynaklanan C-O, C-C ve C-O-C bağlarının gerilmesine bağlanabilir (Gabriela ve ark., 2017; Arumugam ve ark., 2021).



Şekil 1. Yeşil sentez metodu ile sentezlenen Tv-ZnO-NP'lerine ait a) SEM görüntüsü, b) FTIR spektrumu ve c) XRD difraktogramı

Figure 1. Tv-ZnO-NPs synthesized by green synthesis method a) SEM image, b) FTIR spectrum, and c) XRD Difraktogram

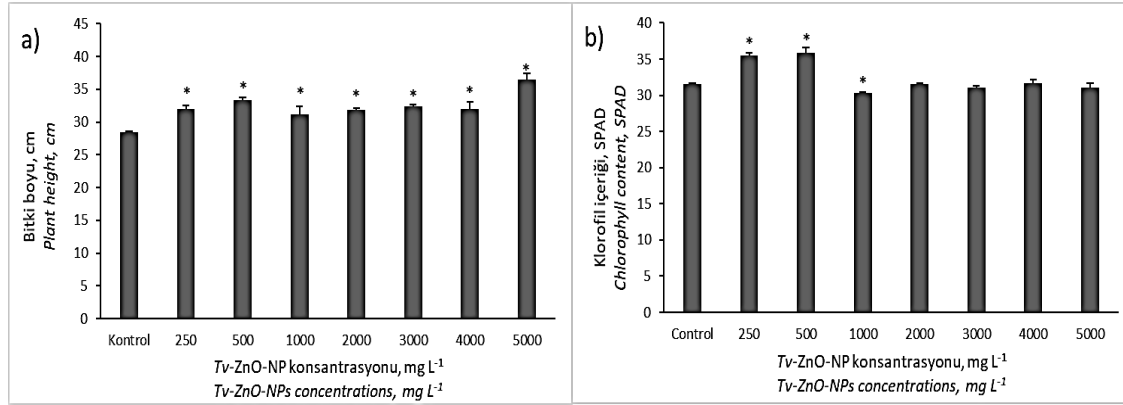
Tv-ZnO-NP'lerin XRD difraktogramlarında gözlenen pikler (JCPDS kart numarası: 36-1451), hekzagonal wurtzite yapısını tanımlamaktadır (Şekil 1c) (Kumar ve ark., 2019). Tv-ZnO-NP'lerin XRD desenleri $2\theta = 31.76^\circ, 34.40^\circ, 36.17^\circ, 47.52^\circ, 56.58^\circ, 62.83^\circ, 66.36^\circ, 67.93^\circ, 69.07^\circ, 72.52^\circ, 76.94^\circ$ ve 81.34° 'de belirgin tepe noktaları olduğunu ortaya koymuştur. Bu tepe noktaları, Tv-ZnO-NP'ler için düzlem Miller indisleriyle (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) ve (104) iyi uyuşmaktadır (Kaliraj ve ark., 2019). XRD tepelerinin gözlemlenebilir pik genişlemesi, malzemenin nanometre ölçeğindeki kristal boyutunu doğrulamaktadır. XRD verilerine dayanarak, ortalama parçacık boyutlarını hesaplamak için Debye-Scherrer denklemi kullanılmıştır ve sentezlenen Tv-ZnO-NP'lerin 19.72 nm boyutunda olduğu belirlenmiştir. Bu sayede hızlı, kolay ve çevresel açıdan güvenli yeşil sentez metodunun yüksek kristalli nanomalzemeler oluşturulmasında kullanılabilir. Bu nedenle, enerji verimliliği açısından, bu çalışma ZnO-NP'ler sentezlemek için geleneksel sentez metodlarına göre üstün performans ve uygulanabilir bir seçenek olarak ortaya çıkmaktadır.

Saksı denemeleri

Spreyleme işlemi sonunda Tv-ZnO-NP'lerin düşük konsantrasyonları da dahil tüm uygulamalarında, kontrol bitkilerine kıyasla buğday bitki boyunu önemli düzeyde ($p < 0.05$) artırmıştır (Şekil 2a). Tv-ZnO-NP'lerin artan konsantrasyonuna bağlı olarak bitki boylarında gözlenen artışın, bitki boyunun uzamasına sebep olan oksin hormonu sentezi ile Zn arasındaki ilişkiden kaynaklandığı bildirilmiştir (Rizwan ve ark., 2019; Pandaya ve ark., 2023). Literatür çalışmaları ZnO-NP'lerin buğday bitkisine spreyleme yolu ile verildiğinde bitki boyunun ZnO-NP konsantrasyonuna ve bitki yetiştirme koşullarına göre farklılık gösterdiğini bildirmektedir. Iqbal ve ark. (2019), ZnO-NP spreylemesinin buğdayın bitki boyunu kuraklık stresi altında önemli ölçüde etkilenmediğini ortaya koyarken, Raza ve ark. (2025), buğdayın erken dönem gelişiminde kuraklık koşullarında ZnO-NP uygulamasının bitki boyunu arttırdığını bildirmişlerdir. Bununla birlikte, Nazir ve ark. (2024), yeşil sentez metodu ile *Withania coagulans* bitki özütünden üretilen ZnO-NP'lerin buğday bitki boyunu kontrole kıyasla % 23 arttırdığını bildirmişlerdir.

Klorofil içeriği, bitki sağlığı için hayati bir parametredir ve sonuçlar, Tv-ZnO-NP'lerin süspansiyonunun yapraktan uygulamasının, düşük konsantrasyonlarda (250 ve 500 mg L⁻¹, p <0.05) kontrole kıyasla buğday klorofil içeriğini desteklediğini ve daha yüksek konsantrasyonlarda anlamlı bir değişiklik olmadığını göstermektedir

(Şekil 2b). Bu sonuç Dimpka ve ark. (2012) ve Das ve ark. (2023)'ün, ZnO-NP'lerin yapraktan spreyleme uygulaması ile çinko-kitosan-salisilik asit nanopartikülleri ile sorgum ve buğday bitkilerinde artan fotosentetik pigment içeriğini gösteren çalışmalarıyla uyumluluk göstermektedir.



Şekil 2. Tv-ZnO-NP'lerin yapraktan spreyleme uygulamasının buğday bitkisi a) bitki boyuna ve b) klorofil içeriğine etkisi (* istatistiksel olarak anlamlı, p <0.05)

Figure 2. Effect of foliar spraying of Tv-ZnO-NPs on a) plant height and b) chlorophyll content of wheat plants (* statistically significant, p <0.05)

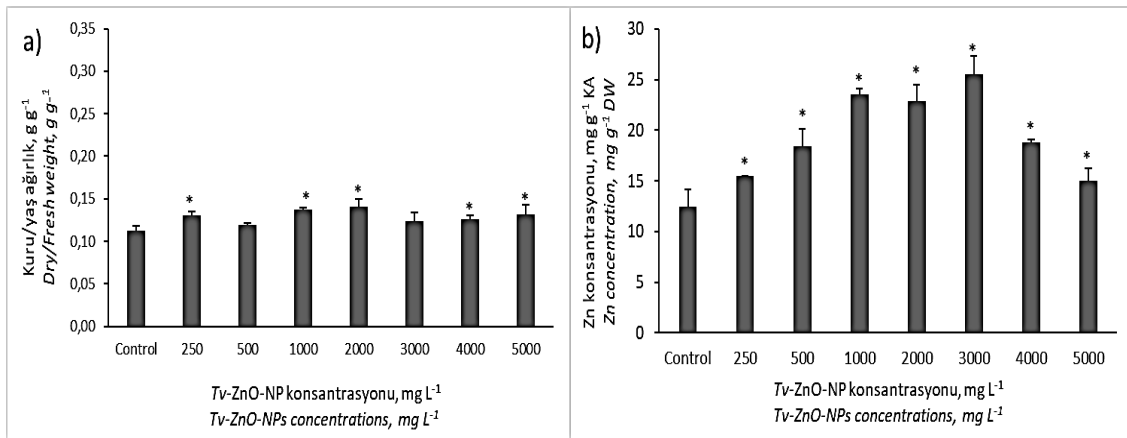
Yapraktan spreyleme uygulaması, özellikle düşük konsantrasyonlarda, en yüksek SPAD değeri ile belirtildiği gibi klorofil içeriğinin teşvik edilmesinde etkili bir yöntem olarak ortaya çıkmaktadır. Maksimum klorofil içeriği, yapraktan spreyleme uygulamasında 35.9 SPAD değeri (500 mg L⁻¹) olarak belirlenmiştir. Hu ve Xianyu (2021), sağlıklı bitki büyümesini teşvik etmek için çalışmalarda yaygın olarak kullanılan biyokimyasal ajanların çoğunluğunun amaçlanan hedef bölgeye sınırlı bir penetrasyon sergilediğini vurgulamıştır. Bu sınırlamayı ele almak için nanoteknolojinin kullanılması yaygınlık kazanmaktadır. Araştırmalar, nanopartiküllerin bitkilere biyokimyasalların iletilmesini kolaylaştırmada önemli rolünü göstermiştir. Örneğin, CeO₂, SiO₂ ve karbon noktaları spreyleme yolu ile bitkilere verildiğinde koruma hücrelerine % 100, hücre dışı boşluğa % 90.3 ve mısır ve pamuk yapraklarındaki kloroplastlara ise % 55.8'lik dağılım yüzdeleri sergiledikleri tespit edilmiştir (Adhikari ve ark., 2016; Hu ve ark., 2020). Ayrıca, Adhikari ve ark. (2016), ZnO-NP'lerin uygulanmasının hem bitki büyüme performansını hem de klorofil içeriğini artırabileceğini belirtmişlerdir.

Bu çalışmada, yapraktan spreyleme yolu ile Tv-ZnO-NP'lere maruz kalan buğday bitkilerindeki önemli bir büyüme parametresi olan kuru/yaş ağırlık oranı da değerlendirilmiştir. Yapraktan spreyleme yolu ile yedi farklı konsantrasyonda (250-5000 mg L⁻¹) Tv-ZnO-NP'lere maruz kalan buğday bitkilerinin kuru/yaş bitki ağırlık oranında istatistiksel olarak anlamlı değişiklikler gözlenmiştir (p <0.05) (Şekil 3a). Bazı çalışmalar genellikle yaş ve kuru ağırlıkları ayrı ayrı rapor ederken, yaprak kuru ağırlığının yaş ağırlığa oranına odaklanmak büyüme dinamikleri hakkında daha kapsamlı bir bilgi sağlamaktadır (Huang ve ark., 2019; Rani ve ark., 2020).

Çinko, bitkilerde enzimatik ve hormonal aktiviteyi teşvik eder (Asmat-Compos ve ark., 2022). Bunu destekleyen Sahoo ve ark. (2021), kuru maddenin tahıl bitkisi verimiyle ilgisini vurgularken, Rai-Kalal ve Jajoo (2021), ZnO-NP'lerle muamele edilen buğday fidelerinde yaş ve kuru kütlelerin arttığı bildirmiştir. Yapraklar arasındaki farklı kuru/yaş ağırlık oranının değişkenliğine rağmen, birçok araştırmacı yaprak biyokütlesi ve yaprak yüzey alanı arasındaki ölçeklendirme ilişkisini araştırırken, yaprak kuru

ağırlığı biyokütlenin temsili bir ölçüsü olarak kabul edilir. Bu ölçeklendirmenin, kuru biyokütlenin artışı etkileyen fotosentetik aktivite (ışık yakalama yüzey alanı) ile doğrudan ilişkili olduğu bilinmektedir. Ancak çoğu bitki türü için yaprak kuru ağırlığının yaprak yaş ağırlığı ile orantılı bir ilişki göstermediği de yapılan çalışmalarda belirtilmiştir (Huang ve ark., 2019). Yapılan bir çalışmada domates bitkilerine 50 mg L⁻¹ ZnO + CaO'in kombine uygulamasının sürgün uzunluğu, sürgün sayısı, kök sayısı, bitki başına verim, meyve

ağırlığı ve yaprak alanı üzerinde olumlu etkiler gösterdiği bildirilmiştir. Tersine, kök uzunluğu, bitki ağırlığı ve meyve çapının 200 mg L⁻¹ ZnO + CaO konsantrasyonunda daha yüksek değerler sergilediği vurgulanmıştır. Ayrıca, Zn nanopartiküllerinin spreyleme işleminin geleneksel çinko tuzu ile muamele edilmiş domates bitkilerine kıyasla bitki yaş ağırlığında % 7.7'lik bir artışa neden olduğu bildirilmiştir (Farooq ve ark., 2023).



Şekil 3. Tv-ZnO-NP'lerin yapraktan spreyleme uygulamasının buğday fidelerinin a) kuru/yaş ağırlık ve b) çinko konsantrasyonuna etkisi (* istatistiksel olarak anlamlı, p < 0.05)

Figure 3. Effect of foliar spraying of Tv-ZnO-NPs on a) dry/fresh weight and b) zinc content of wheat seedlings (* statistically significant, p < 0.05)

Çinko, protein sentezi, gen ekspresyonu, metabolik fonksiyonlar, su alımı ve taşıma ve enzim aktivitesi dahil olmak üzere bitki yaşamının birçok farklı yönünü etkileyen önemli bir besin elementidir (Hafeez ve ark., 2013). Çinko nanopartiküllerinin bitkilere uygulanmasında yaprak ve toprak uygulamaları olmak üzere iki temel yöntem bulunmaktadır. Yaprak uygulaması, toprak uygulamasına kıyasla besin maddesini bitkiler tarafından alınmasında daha etkili bir yoldur (Zhu ve ark., 2020). Bu çalışmada, spreyleme metodu ile buğday bitkisine artan dozlarda Tv-ZnO-NP uygulaması gerçekleştirilmiş ve sonuçlar, bitki büyümesinin bu uygulamadan olumlu etkilendiğini göstermiştir. Yapraktan spreyleme ile Tv-ZnO-NP uygulanan buğday fidelerinde 250-3000 mg L⁻¹ dozları arasında Zn²⁺ konsantrasyonunda önemli bir artış gözlenmiştir. 4000 ve 5000 mg Tv-ZnO-NP L⁻¹ uygulama dozlarında ise, Zn²⁺ alımı düşük dozlara göre azalış

gösterse de kontrol bitkilerine göre artış olduğu belirlenmiştir (Şekil 3b, p < 0.05). Yapraktan spreyleme uygulamasında, maksimum Zn²⁺ konsantrasyonu 3000 mg Tv-ZnO-NP L⁻¹ dozunda 25.59 mg g⁻¹ olarak belirlenirken, kontrol bitkilerinde 12.41 mg g⁻¹ olarak belirlenmiştir. Literatürde farklı bitkiler üzerine ZnO-NP'lerin yapraktan uygulanmasının bitki büyüme ve gelişimine olumlu etkisini rapor eden birçok çalışma bulunmaktadır. Wang ve ark. (2023), pirinçteki Zn konsantrasyonunun, ZnO-NP uygulaması ile kontrole kıyasla % 82.4 artış gösterdiği, Lv ve ark. (2023) ise, ZnO NP'lerin yaprak uygulamasının, buğdayın Zn konsantrasyonunu (40.34 mg kg⁻¹) önemli ölçüde arttırdığı bildirilmiştir. Kadmiyum (Cd) ile kirlenmiş toprakta yetiştirilen buğday bitkilerine yapraktan uygulanan ZnO-NP'lerin ise farklı kuraklık koşullarında bile Zn alımını önemli ölçüde artırırken Cd alımını azalttığı rapor edilmiştir

(Adrees ve ark., 2021). Yapılan çalışmalarda spreyleme ile buğdaya uygulanan ZnO-NP'lerin ilk olarak stomalar aracılığı ile bitkiye alındığını ve zamanla apoplast boyunca hareket ettiğini, ZnO-NP'lerin bir kısmının apoplastta çözünerek mezofil hücreleri tarafından emildiğini ve çözülmemiş ZnO-NP'lerin ise kısmen hücrelere aktarıldığını göstermiştir (Zhu ve ark., 2020).

Sonuçlar

Bu çalışmada, kekik bitkisi ekstraktı kullanılarak yeşil sentez yöntemiyle çinko oksit (ZnO) metal oksit nanoparçacıkları (Tv-ZnO-NP'ler) üretilmiş ve yapraktan uygulama yöntemiyle buğday bitkisinin klorofil içeriği, büyüme performansı ve Zn alımı üzerindeki etkileri incelenmiştir. Yapraktan spreyleme uygulamasının, düşük konsantrasyonlarda dahi bitki büyümesini ve klorofil içeriğini artırma potansiyeline sahip etkili bir yöntem olduğu belirlenmiştir.

Elde edilen bulgular, Tv-ZnO-NP'lerin buğday bitkisinin gelişimi üzerinde anlamlı ve olumlu etkiler yarattığını ortaya koymaktadır. Bu çalışma, geleneksel toprak uygulamalarına alternatif olarak yapraktan nanogübre uygulamasını ele almakta ve bu yöntemin buğdayın büyüme parametreleri üzerindeki etkisini kapsamlı şekilde değerlendirmektedir. Ayrıca, kekik ekstraktı ile çevre dostu bir şekilde sentezlenen Tv-ZnO-NP'lerin sürdürülebilir tarım uygulamaları açısından taşıdığı potansiyeli vurgulamakta ve bu konuda değerli bilgiler sunmaktadır.

Bununla birlikte, çalışmada yüksek konsantrasyonlarda uygulanan Tv-ZnO-NP'lerin olumsuz etkiler gösterebildiği de belirlenmiştir. Bu durum, tarımsal uygulamalarda kullanılacak ZnO nanoparçacıklarının optimum konsantrasyonlarının belirlenmesine yönelik doz-temelli araştırmalara ihtiyaç duyulduğunu göstermektedir. Sonuç olarak, bu bulgular dikkatli doz ayarlamalarının gerekliliğine ve potansiyel çevresel etkilerin değerlendirilmesinin önemine işaret ederek, gelecekte yapılacak çalışmalar için yol gösterici niteliktedir.

Çıkar çatışması beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması yoktur.

Yazar katkısı

Araştırma konseptinin oluşturulması, verilerin işlenmesi ve düzenlenmesi, istatistik analizler ve makalenin yazımı Zeynep Görkem DOĞAROĞLU ve Yağmur UYSAL tarafından eşit oranda katkı sağlanarak gerçekleştirilmiştir.

Etik Kurulu Kararı

Bu çalışmada etik kurul onayına ihtiyaç yoktur.

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Seed germination characteristics of *Xanthium spinosum* populations from different climatic regions of Türkiye: Implications for weed management

Türkiye'nin farklı iklim bölgelerinden toplanan *Xanthium spinosum* popülasyonlarının tohum çimlenme özellikleri: Yabancı ot mücadelesi açısından ipuçları

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ABSTRACT

Xanthium spinosum is a troublesome invasive weed widely distributed through many agroecological zones in Türkiye, adversely affecting crop production and local biodiversity. Understanding its seed germination biology is essential for formulating efficient, region-specific management measurements. This study assessed the influence of photoperiod, temperature, pH, salinity, osmotic potential, and seed burial depth on the germination characteristics of *X. spinosum* populations collected from four geographical regions (Southeastern Anatolia, Mediterranean, Black Sea, and Central Anatolia) in Türkiye. Peak germination was observed under a 12-hour light/dark photoperiod, with Southeastern Anatolia and the Mediterranean populations exhibiting higher responsiveness to light. The highest germination occurred at ~30 °C for Southeastern Anatolia and Mediterranean populations, whereas Black Sea and Central Anatolia populations preferred relatively lower temperatures (25–27 °C) for peak germination. Similarly, neutral pH (~7.0–7.6) resulted in the highest germination of all populations, whereas Southeastern Anatolia had better pH tolerance. Salinity and osmotic stress tests revealed significant demographic differences, with the Southeastern Anatolia population exhibiting higher tolerance to salinity and drought stresses. Seedling emergence peaked at lower burial depths (~2–4 cm) and had a sharp decline at increased depths. These results highlight significant population-specific adaptation patterns associated with local climatic and edaphic conditions. Deep tillage and management strategies based on environmental tolerance determined in the current study may restrict the further spread of *X. spinosum*.

Keywords: Invasive weed management, Photoperiod sensitivity, Population variability, Seed germination

ÖZ

Xanthium spinosum, Türkiye'nin birçok agroekolojik bölgesine yayılmış, tarımsal üretimi ve yerel biyoçeşitliliği olumsuz etkileyen sorunlu bir istilacı yabancı ottur. Bu türün tohum çimlenme biyolojisinin anlaşılması, etkili ve bölgeye özgü yönetim önlemlerinin oluşturulması açısından büyük önem taşımaktadır. Bu çalışmada, Türkiye'nin dört farklı coğrafik bölgesinden (Güneydoğu Anadolu, Akdeniz, Karadeniz ve İç Anadolu) toplanan *X. spinosum* popülasyonlarının çimlenme davranışı üzerinde fotoperiyot, sıcaklık, pH, tuzluluk, ozmotik potansiyel ve tohum gömülme derinliğinin etkileri değerlendirilmiştir. En yüksek çimlenme, 12 saat ışık/12 saat karanlık fotoperiyotta gözlenmiş, Güneydoğu Anadolu ve Akdeniz popülasyonları daha yüksek ışık duyarlılığı sergilemiştir. Sıcaklık açısından en yüksek çimlenmenin Güneydoğu Anadolu ve Akdeniz popülasyonlarında yaklaşık 30 °C'de gözlemlenirken, Karadeniz ve İç Anadolu popülasyonlarında ise daha ılıman sıcaklıklarda (25–27 °C) gerçekleştiği ortaya konulmuştur. Benzer şekilde, tüm popülasyonlarda nötr pH (~7.0–7.6) koşullarında en yüksek çimlenme kaydedilmiş, Güneydoğu Anadolu popülasyonu daha geniş bir pH toleransı göstermiştir. Tuzluluk ve ozmotik stres testleri, Güneydoğu Anadolu popülasyonunun tuz ve kuraklık streslerine

karşı daha yüksek tolerans sergilediğini ortaya koymuştur. Fide çıkışı, düşük gömülme derinliklerinde (~2–4 cm) zirveye ulaşmış, derinlik arttıkça keskin bir düşüş göstermiştir. Bu sonuçlar, yerel iklimsel ve edafik koşullarla ilişkili önemli popülasyon-özü adaptasyon kalıplarını vurgulamaktadır. Bu çalışmada belirlenen çevresel toleranslara dayalı derin sürüm teknikleri ve yönetim stratejileri, *X. spinosum*'un gelişimini sınırlayabilir. Bu çalışmada ortaya konulan popülasyon düzeyindeki değişkenlik, bölgeye özü etkili yabancı ot yönetim stratejileri için kritik öneme sahiptir.

Anahtar Kelimeler: İstilacı yabancı ot yönetimi, Fotoperiyot duyarlılığı, Popülasyon varyasyonu, Tohum çimlenmesi

Introduction

Seed germination is a critical factor in the ecology and management of weed species as it influences timing and pattern of seedling emergence (Batlla & Benech-Arnold, 2014). Soil seed bank is the principal source of future weed infestations as it harbours millions of viable seeds (Schwartz-Lazaro & Copes, 2019). Weed infestation is caused by the seeds that can enter dormancy and endure adverse conditions until germination (Batlla & Luis Benech-Arnold, 2007; Benech-Arnold et al., 2000; Qasem, 2020). Consequently, understanding seed germination biology is essential for predicting weed emergence patterns and developing effective management strategies (Chauhan & Johnson, 2010; Marschner et al., 2024). Weed management methods, such as stale or false seedbeds are designed to use germination cues, facilitating germination of weed seeds (Travlos et al., 2020). Therefore, understanding the environmental factors that facilitate or inhibit weed seed germination is essential for the implementation of available management strategies or designing alternative methods (Batlla & Benech-Arnold, 2014).

Seed germination and dormancy of weed species is altered by several environmental factors. Temperature is the most critical indicator of germination, and each species has an optimal temperature range and often exhibits specific diurnal requirements (Dürr et al., 2015). Numerous summer-annual weeds germinate under temperatures ranging from 25 to 35°C, while others need alternating day/night temperatures (Travlos et al., 2020). Fluctuating temperatures may promote weed species to sprout near the soil surface, where daily thermal

variations are more pronounced (Steinmaus et al., 2000).

Numerous weed seeds exhibit positive photoblasticism, indicating that they need light exposure to terminate dormancy (Chauhan et al., 2006a). Seeds buried deeper in darkness remain dormant, while those closer to the surface germinate. Cocklebur (*Xanthium* spp.) seeds germinate well in both darkness and light (Weaver & Lechowicz, 1983). Osmotic potential and soil moisture are the other important factors regulating seed germination in weeds. Seeds may remain dormant if the soil moisture tension is elevated (Hegarty, 1978). Certain weed species may germinate under extreme water stress (i.e., osmotic potentials < –0.5 MPa), whereas others need abundant moisture (Ahmadi et al., 2024; Farooq et al., 2019, 2021; Malka et al., 2023; Önen et al., 2018). Osmotic stress or ion toxicity resulting from elevated salinity may impede germination (Hakim et al., 2011). Local adaptation is crucial for successful germination in saline soils as salt-adapted seeds exhibit superior germination under salinity (DiTommaso, 2004). Soil pH is another major factor influencing seed germination of weeds (Alberto Ortiz et al., 2019). While excessive acidity or alkalinity may restrict germination, most weed seeds thrive throughout a broad pH spectrum (Chauhan et al., 2006b, 2006c). Agricultural weeds may thrive in several soil types owing to their wide pH germination range.

Seed burial depth significantly influences germination and seedling emergence by altering light, temperature, and oxygen exposure (Tian et al., 2024; Yuan et al., 2025). Seeds on the soil surface often fail to germinate or survive owing to light-induced dormancy or desiccation; however, shallow burying may provide moisture and enable

light penetration (Kigel, 2017; Nautiyal et al., 2023). Small-seeded weeds are unable to emerge from deep soil layers due to restricted energy resources (Benvenuti & Mazzoncini, 2019), while larger seeds will remain dormant if buried beyond their optimal emergence depth (Grundy et al., 2003). The burs (fruits) of *Xanthium spinosum* buried at depths of 1–4 cm exhibit optimal emergence, whereas surface seeds do not germinate, and emergence rates decline markedly at depths above 5–10 cm (Auld, 1993; Hocking & Liddle, 1986; Tao et al., 2022).

Xanthium spinosum L. is a globally significant annual broadleaf weed. It is native to South America and now distributed across all continents except Antarctica, and regarded as a significant invasive weed in Europe, Asia, Africa, and Australia (CABI, 2022). Its invasive nature and impact make it one of the most noxious weeds (CABI, 2022; Holm et al., 1978). *Xanthium spinosum* infests agricultural fields, pastures, roadsides, and riparian zones, flourishing in warm climates and disturbed habitats (Assefa & Tilaye, 2023). It spreads by adhering to animal fur, sheep wool, vehicles, and agricultural machinery, so contaminating harvested crops and seed lots with its spiny burs (CABI, 2022). It may establish dense monospecific stands, outcompeting crops, forage grasses, and native vegetation (Kelečević et al., 2024). Severe infestations in row crops such as maize, soybean, cotton, and sunflower may decrease yields, threatening agriculture. Hence it is a high-priority target for management in several countries, where it is regarded as a noxious weed (CABI, 2022).

Xanthium spinosum is distributed in many habitats of various regions in Türkiye. It has been reported from ~30 provinces in Mediterranean, Aegean, and southeastern Anatolian regions (Bükün, 2005; Kadioğlu & Kekeç, 2020; Ozturk et al., 2017). The ability of *X. spinosum* to inhabit cultivated and ruderal areas is because of its resistance to many environments. Different populations of *X. spinosum* in Türkiye may face diverse selection pressures, ranging from the humid Black Sea coast to the arid southeastern

Anatolia. Hence, it is essential to understand germination biology of these populations to ascertain the differences in tolerance limits (e.g., temperature or moisture) that might influence management strategies. Understanding germination characteristics will allow to predict peak emergence in Turkish agroecological zones and schedule actions such as soil cultivation or herbicide use to target emerging cohorts of *X. spinosum*.

Although *X. spinosum* is significant threat to agriculture; few studies have investigated its seed germination ecology in various regions of the world (Ahmadi et al., 2024; Kelečević et al., 2024; Tao et al., 2022). It exhibits similar germination to that of other *Xanthium* species (CABI, 2022). It produces twin-seeded burs with one larger and one smaller arranged at bottom and top, respectively (Esashi & Leopold, 1968). The bigger seed is often non-dormant and germinates in the first favorable season, whereas the smaller seed has an intrinsic dormancy and frequently remains viable (CABI, 2022). This dual-seed strategy helps in longer persistence. Seeds of *X. spinosum* have the potential to germinate in the absence of light (Auld, 1993; Weaver & Lechowicz, 1983). Elevated soil temperatures facilitate the germination of *X. spinosum* throughout late spring and summer. Kelečević et al. (2024) discovered that *X. spinosum* seeds germinate between 15 to 40°C, exhibiting reduced rates at lower temperatures. The optimal temperature range is 30–35°C. Optimal germination occurs at pH levels ranging from 5 to 10 and tolerance to high salinity, with a 50% germination threshold at 200-250 mM NaCl (Kelečević et al., 2024).

Another significant component of weed germination biology is variation across different populations of the same species. Weeds often colonize extensive geographic areas and many ecosystems, and they may develop population-specific germination responses adapted to local conditions (Farooq et al., 2019). Several weed species in Türkiye exhibited significant differences among seed germination requirements (Farooq et al., 2019, 2021; Önen et al., 2018; Ozaslan et al.,

2017). Therefore, determining the seed germination biology of different populations can help to develop region-specific management strategies. Unfortunately, no such data exist for *X. spinosum* populations distributed across different regions in Türkiye.

This study investigated the seed germination characteristics of *X. spinosum* populations stemming from several regions in Türkiye. The objectives were to (1) determine the effects of photoperiod, temperature, pH, osmotic potential, and seed burial depth on germination and seedling emergence of *X. spinosum*, and (2) evaluate germination responses across various climatic and geographic locations. The results will help to develop region-specific management strategies in the future.

Materials and Methods

Seed collection

Seeds (burs) were obtained from four independent populations distributed across various ecosystems in different geographic

regions of Türkiye. Seeds were harvested from 50 mature mother plants, transported to the laboratory, desiccated in the shade, and kept at 25°C until used in the studies. The details of the geographic locations of seed collection sites and habitat are given in Table 1.

Seeds were collected from four different places in Türkiye, i.e., Şanlıurfa (Southeastern Anatolia), Adana (Mediterranean), Samsun (Black Sea), and Kayseri (Central Anatolia). The climatic conditions differed, with Şanlıurfa exhibiting a Hot Semi-Arid Climate (BSh), Adana having Hot-Summer Mediterranean Climate (Csa), Samsun with Warm-Summer Humid Continental Climate (Dfb), and Kayseri with Cold Semi-Arid Climate (BSk). Seed collection habitats ranged from agricultural areas (Şanlıurfa and Adana) to non-agricultural areas (Samsun) and roadside environments (Kayseri). The population size all sites was 100 to 200 plants.

Table 1. Background information on the seed collection sites, their geographic locations, prevailing habitat and climatic conditions

Population	Region	Climatic region/Köppen classification	Geographic location	Habitat	Population size
Şanlıurfa	Southeastern Anatolia	Hot Semi-Arid Climate (BSh)	37.325450 °N 39.656890 °E	Agriculture	> 100 plants
Adana	Mediterranean	Hot-Summer Mediterranean Climate (Csa)	36.875550 °N 35.567060 °E	Agriculture	> 200 plants
Samsun	Black Sea	Warm-Summer Humid Continental (Dfb)	40.986110 °N 35.641260 °E	Non-Agricultural	> 150 plants
Kayseri	Central Anatolia	Cold Semi-Arid Climate (BSk)	38.681333 °N 35.495861 °E	Roadside	> 100 plants

Three soil samples were collected from all seed collection sites and mixed to prepare a composite sample. The soil was analyzed according to standard procedures described in the literature. Soil characteristics of seed collection sites indicated significant variations that may influence seed germination. Soils in Adana had the highest calcium carbonate (CaCO₃) concentration (29.58%), indicating more calcareous, alkaline conditions, while Şanlıurfa exhibited a comparatively low CaCO₃ content (6.86%),

suggesting less calcareous soil. Organic matter (OM) content was typically low across all sites, indicating degraded or intensively cultivated soils, with Samsun exhibiting the highest OM content (2.46%), potentially improving soil moisture retention and nutrient availability. Soil pH indicated a little alkalinity across all locations, ranging from 7.55 to 8.23, with Kayseri and Samsun exhibiting the highest levels of alkalinity. Electrical conductivity (EC) (an indicator of soil salinity) was significantly higher in Şanlıurfa (839

mS/m) and a bit high in Adana (512.89 mS/m), suggesting saline soils that may impede germination. Kayseri had the lowest EC (205.15 mS/m), indicating mild salinity. Soil texture exhibited minor variations. All locations were mostly clayey; however, Kayseri had a comparatively lower clay content (49.5%) than

Şanlıurfa, Adana, and Samsun (>60%), possibly facilitating improved drainage. Available phosphorus (P) concentration was highest in Samsun (67.87 ppm), indicating possibly enhanced soil fertility, while Kayseri exhibited a very low level (10.03 ppm) (Table 2).

Table 2. Soil properties of the seed collection sites of *Xanthium spinosum* populations included in the study

Population	CaCO ₃ (%)	OM (%)	pH	EC (mS/m)	Clay (%)	Sand (%)	Silt (%)	P (ppm)
Şanlıurfa	6.86	1.69	7.62	839.00	61.10	23.90	15.00	28.10
Adana	29.58	1.93	7.55	512.89	63.22	32.20	25.14	41.20
Samsun	13.24	2.46	8.03	476.00	61.8	25.7	12.5	67.87
Kayseri	10.63	1.17	8.23	205.15	49.50	31.75	18.75	10.03

Experimental protocol

The burs of *X. spinosum* were used for all experiments. Due to the quick release of seed dormancy after seed dispersal, no seed dormancy release treatments were required. Each bur generally carries two seeds, one of which germinates in the current growing season while the other germinates in the subsequent season (CABI, 2022). Therefore, the burs were used in the experiments, i.e., seeds were not removed from the burs.

Germination tests were conducted in 90 × 15 mm Petri dishes infused with two layers of filter paper. The paper was saturated with either 4 mL of deionized water or the appropriate treatment solutions (salinity, pH, or osmotic potential experiments). Petri dishes were sealed with paraffin film to reduce moisture loss and incubated under conditions specific to each experiment. Each treatment had five replications, with 20 seeds placed in each dish. Two dishes constituted a single replication, resulting in a total of 10 dishes (200 seeds) per treatment. Incubation was conducted at 30 °C under a 12-hour photoperiod, unless specified otherwise. Germination was evaluated after 21 days and experiments were terminated.

A factorial experimental design was used for the experiments where populations were the main factor and experimental treatments were sub-factors. Each experiment was repeated over time to validate the findings.

Effect of photoperiod on seed germination

Seeds were exposed to three light/dark regimes, i.e., complete dark (0 h), a 12-hour light/12-hour dark cycle, and continuous light (24 h) to evaluate photoperiod sensitivity. Incubators were illuminated with cool, white, fluorescent lights with an intensity of 350 $\mu\text{Em}^{-2} \text{s}^{-1}$. Dishes in the complete dark treatment were wrapped in four layers of aluminum foil to prevent any light exposure.

Effect of constant temperatures on seed germination

Seeds were exposed to ten constant temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 °C). Each treatment was conducted in independently controlled incubators to ensure accurate temperature control.

Effect of different pH levels on seed germination

The effect of pH on seed germination was investigated across a wide range of pH levels, i.e., acidic (3.0, 4.0, 5.0, and 6.0), neutral (7.0), and alkaline (8.0, 9.0, 10.0, 11.0, and 12.0). All solutions were prepared using distilled water as the base. The desired pH values were adjusted using either 1N hydrochloric acid (HCl) to lower the pH or 1N sodium hydroxide (NaOH) to raise it, following the protocol described by Chauhan et al. (2006a). The pH of each solution was verified using a calibrated digital pH meter. Fresh solutions were prepared as needed, and any

stored solutions were rechecked for pH stability prior to use.

Effect of different salinity levels on seed germination

To evaluate salinity tolerance, seeds were exposed to NaCl solutions at concentrations of 50, 100, 150, 200, 300, 400, 500, and 600 mM. A control treatment with deionized water was included for the comparison. All saline solutions were prepared by dissolving analytical-grade NaCl in deionized water to get the desired concentrations.

Effect of different osmotic potentials on seed germination

The response of seeds to water stress was evaluated using polyethylene glycol (PEG 8000) solutions prepared to deliver osmotic potentials of 0 (control), -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, -1.4, and -1.6 MPa. Solutions were freshly prepared prior to each use for ensuring consistency and accuracy. The required concentrations of PEG 8000 for each osmotic potential were determined and prepared according to the method described by Michel (1983).

Effect of seed burial depth on seedling emergence

The impact of burial depth on seedling emergence was assessed in plastic pots by planting seeds at depths of 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 15 cm. Twenty seeds were allocated for each depth, with five replications per treatment. Experiments were performed in a greenhouse regulated at 30 °C with a 12-hour light/dark cycle.

Statistical analysis

Final germination percentages were evaluated using third-degree (cubic) polynomial regression and Gaussian model, depending upon the treatments. Germination percentages across varying salinity and osmotic potential levels were modelled using third-degree (cubic) polynomial regression to accurately describe the nonlinear

germination response curves. A third-degree polynomial function has been given in equation 1 (Obam, 2006).

$$Y = ax^3 + bx^2 + cx + d \quad (\text{Eq. 1})$$

where y is the predicted germination percentage x is either salinity (mM) or osmotic potential (-MPa), and a, b, c, and d are the polynomial coefficients estimated by least squares fitting.

For temperature, pH, and seed burial depth experiments, data were modeled using a three-parameter Gaussian model presented in equation 2 (Mesgaran et al., 2013).

$$G = a \times e^{-0.5 - \{(x - b)/c\}^2} \quad (\text{Eq. 2})$$

where a represents the peak germination or emergence percentage, b is the treatment level corresponding to peak response, and c describes the spread of the distribution.

The results from the photoperiod experiment were analyzed using a two-way analysis of variance (ANOVA). Before ANOVA, the assumptions of normality and homogeneity of variance were tested. The means were grouped using the Least Significant Difference (LSD) test at 95% probability level where ANOVA denoted significant differences.

All ANOVA analyses were executed in SPSS version 21.0, and curve fitting was carried out using SigmaPlot version 13.0.

Results and Discussion

Effect of photoperiod on seed germination

Analysis of variance revealed that seed germination was strongly influenced by population, photoperiod, and their interaction ($p < 0.0001$ for all; Table 3). The photoperiod had the most significant effect ($F = 272.93$), followed by population ($F = 10.17$) and the population $\times$ photoperiod interaction ($F = 11.53$).

Table 3. Analysis of variance for different photoperiods on seed germination of *Xanthium spinosum* populations stemming from different climatic zones in Türkiye

Source of variation	DF	Sum of squares	Mean squares	F value	P value
Populations (P)	3	229.22	76.41	10.17	< 0.0001
Photoperiod (PH)	2	4097.79	2048.89	272.93	< 0.0001
P × PH	6	519.70	86.61	11.53	< 0.0001

DF = degrees of freedom, the bold values in P value column denote significant effect of the respective individual or interactive effect on seed germination ($p < 0.05$)

Significant variations were observed across studied populations and photoperiods (Figure 1). The highest germination was recorded under a 12-hour light/12-hour dark photoperiod, indicating that alternating light exposure was optimum for seed germination. The Southeastern Anatolia population had the highest germination percentage under 12-hour light/12-hour dark photoperiod, followed by the Mediterranean population. Conversely, the populations from Central Anatolia and the Black Sea had significantly lower germination rates across all

photoperiods. Continuous dark caused the lowest germination rates across all populations, while seeds from Central Anatolia exhibited slightly better germination under these conditions relative to other regions. Populations from warmer, sunnier regions (Southeastern Anatolia and Mediterranean) exhibited more receptivity to light, whereas those from colder or variable-light availability (Black Sea and Central Anatolia) had lesser light dependency.

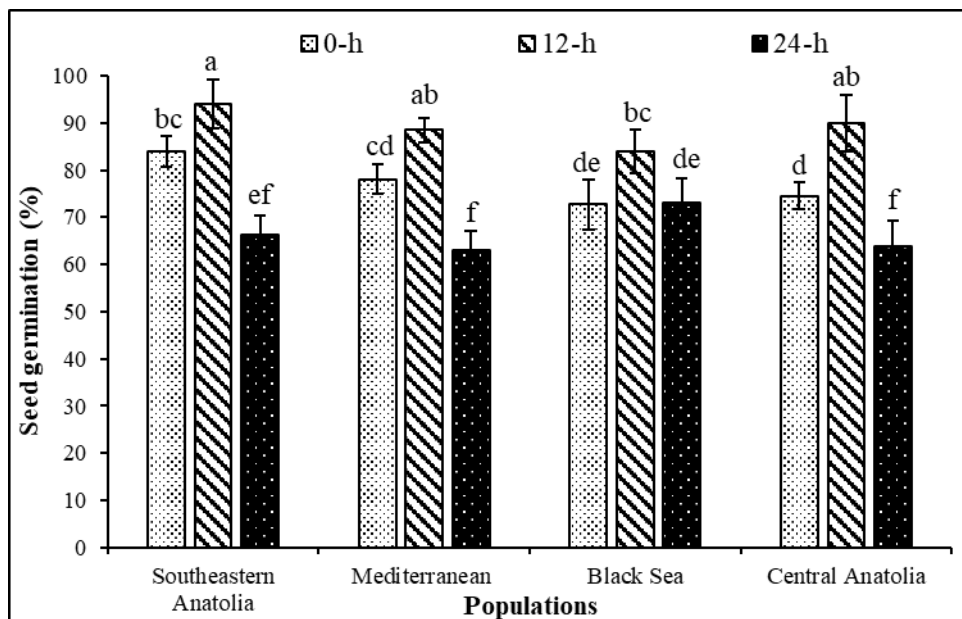


Figure 1. The influence of different photoperiods on seed germination of *Xanthium spinosum* populations stemming from different climatic zones in Türkiye. The data presented are means $\pm$ standard errors ($n = 5$). The means with different letters are statistically different from each other ($p < 0.05$).

The strong influence of individual and interactive effects of photoperiods and populations on seed germination demonstrates the importance of environmental cues and genetic variation in regulating the germination characteristics of *X. spinosum*. Higher germination of Southeastern Anatolia and the Mediterranean

populations under 12-hour light/12-hour dark conditions indicate adaptability to environments with higher solar radiation and uniform photoperiods. Fluctuating light conditions probably act as a dependable environmental cue in these regions, possibly facilitating germination when circumstances are optimal for seedling

establishment (Dürr et al., 2015; Kelečević et al., 2024). The significant relationship between population and photoperiod highlights local ecotypic variation within *X. spinosum*, affecting its invasive potential. Populations requiring excessive sunlight may be controlled by canopy cover methods that limit light penetration; thus, preventing germination. In contrast, populations exhibiting increased tolerance to low-light environments may need comprehensive strategies addressing both seed bank and newly germinated seedlings. The results stress the need to modify weed control strategies to the ecological traits and adaptive behaviors of various *X. spinosum* populations.

Effect of constant temperatures on seed germination

Temperature significantly influenced the germination of *X. spinosum* populations, showing a unique Gaussian response across all populations (Figure 2). Southeastern Anatolia and Mediterranean populations had the greatest germination ability, reaching maximum theoretical germination rates of 101.4% and 89.9%, respectively, with optimum germination temperatures estimated at 30.1 °C (Table 4). Conversely, the Black Sea and Central Anatolia

populations had lower optimum temperatures (27.0 °C and 25.5 °C, respectively), with corresponding maximum germination rates of 92.7% and 90.9%, respectively.

Germination was limited at lower temperature (≤ 15 °C), with all populations exhibiting <35% germination. Germination gradually increased with increasing temperature, reaching its peak at 25–30 °C, followed by a steep decline at temperatures besides 35 °C. Southeastern Anatolia population exhibited comparatively higher germination percentages at supra-optimal temperatures (35–40 °C) relative to other populations, indicating a greater thermal tolerance. The calculated spread (σ) values, between 8.1 and 9.2 °C, indicate significant thermal adaptability among populations. Southeastern Anatolia and Mediterranean populations exhibited adaptation to elevated temperatures, but Black Sea and Central Anatolia populations were better adapted to comparatively colder environments. These variations probably indicate ecological or evolutionary diversity across populations, perhaps influenced by the climatic circumstances of their native environments (Farooq et al., 2019, 2021; Önen et al., 2018).

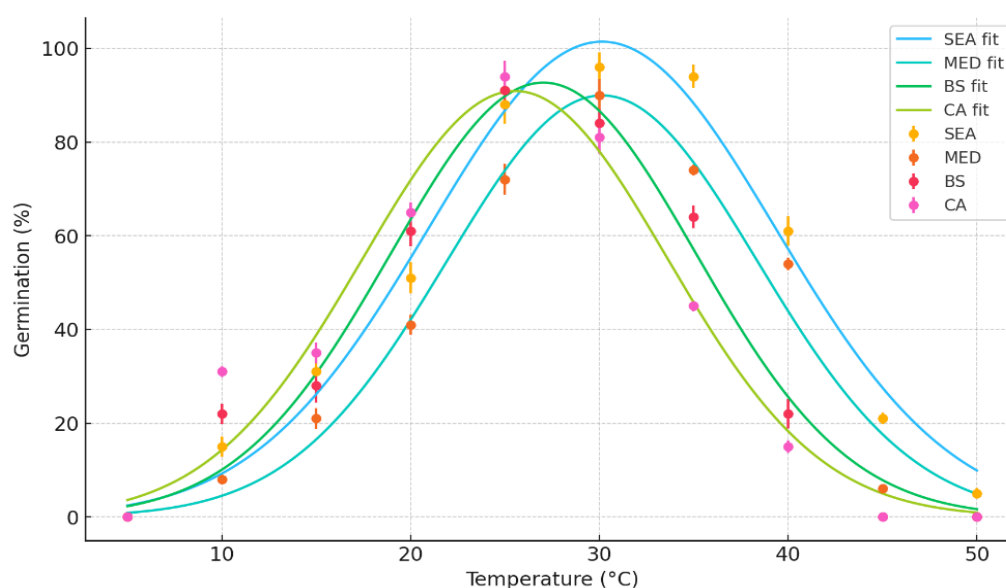


Figure 2. The influence of different constant temperatures on seed germination of *Xanthium spinosum* populations stemming from different climatic zones in Türkiye. The data presented are means $\pm$ standard errors ($n = 5$). The curves are three-parameter Gaussian model fitted on the final germination data.

Table 4. Summary of the fitted temperature response parameters for *Xanthium spinosum* seed germination across four regional populations

Populations	Maximum Germination (%)	Optimum Temperature (°C)	Spread (σ)
Southeastern Anatolia	101.42	30.15	9.20
Mediterranean	89.95	30.15	8.23
Black Sea	92.68	27.04	8.08
Central Anatolia	90.89	25.54	8.08

Maximum germination (%) denotes the estimated highest germination rate determined by Gaussian model fitting. The optimum temperature (°C) denotes the temperature at which highest germination is predicted to occur. The spread (σ , °C) is the standard deviation of the fitted Gaussian curve, reflecting the range of temperature tolerance around the optimum. High σ values imply increased temperature tolerance, whilst lower σ values indicate limited thermal requirements for germination.

The observed variations in optimum temperatures and germination responses among studied populations indicate significant ecotypic heterogeneity possibly influenced by local environmental variables (Cochrane et al., 2015; Walter et al., 2020). Southeastern Anatolia and Mediterranean populations demonstrated higher optimum temperatures (~30 °C) because of warmer and drier conditions in the seed collection sites, which aligns with evidence that *X. spinosum* flourishes in disturbed, open habitats characterized by significant thermal variations (Liu et al., 2025; Xiao et al., 2024). The lower optimal temperatures observed for Black Sea and Central Anatolia populations (~25–27 °C) correspond with adaptations to colder or more temperate habitats, since temperature conditions during early germination stages are crucial for weed establishment and invasion success (Dürr et al., 2015). Temperature breadth (σ values ranging from 8–9 °C) recorded in all populations suggests a level of physiological adaptability, supporting the concept that *X. spinosum* may thrive in a diverse array of thermal niches, a characteristic trait among invasive and cosmopolitan weeds (Fenner & Thompson, 2005). The reduction in germination at temperatures beyond 35 °C highlights thermal thresholds of *X. spinosum*, which may become more significant considering future global warming scenarios (Liu et al., 2025). These findings emphasize the need

to consider population-level diversity in germination characteristics when predicting the possible spread and control strategies of *X. spinosum* under changing conditions.

Effect of different pH levels on seed germination

The germination response to pH exhibited a Gaussian pattern, showing significant variations across populations for optimum pH values (Figure 3). The Central Anatolia population had the highest predicted germination rate (91.6%) at an optimum pH of 7.37, indicating strong adaptation to neutral soil conditions. The Southeastern Anatolia population exhibited better performance, reaching a maximum of 90.1% at lower optimum pH of 6.95, indicating a tolerance for moderately acidic soils. The Black Sea population exhibited an optimum pH of 7.59 and a highest germination rate of 89.6%, while the Mediterranean population reached a peak germination rate of 86.3% at an optimum pH of 7.07. These populations exhibited slightly reduced maximum germination but had a distinct preference for neutral to slightly alkaline environments.

Southeastern Anatolia population had the widest pH tolerance ($\sigma = 2.01$), indicating greater adaptability in both acidic and alkaline environments. The Mediterranean population had the least variability ($\sigma = 1.76$), indicating greater susceptibility to pH variations (Table 5).

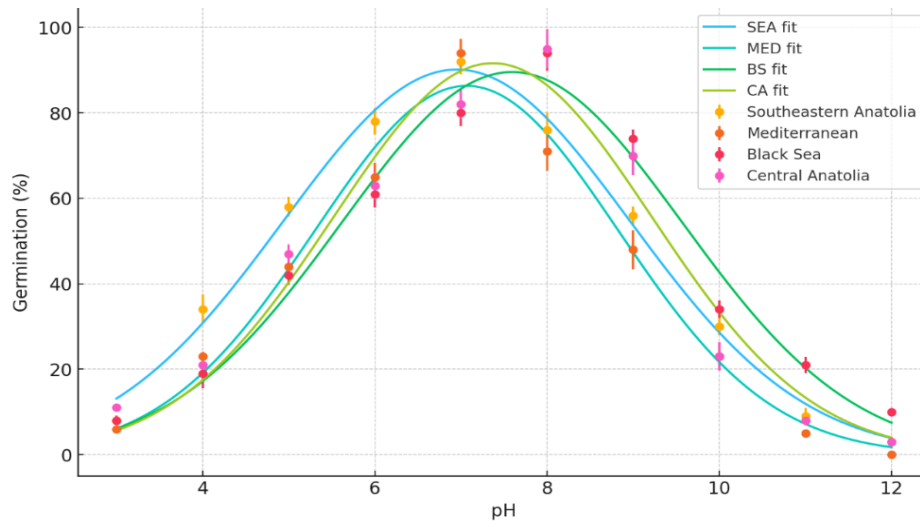


Figure 3. Germination response curves of *Xanthium spinosum* populations stemming from four geographic regions of Türkiye under different pH levels. Points indicate calculated mean germination percentages ($\pm$ standard error), whereas lines represent fitted Gaussian curves. Each population had a bell-shaped response, with peak germination occurring at neutral pH levels (7.0–7.6). Differences in peak values and spread (σ) indicate population-specific tolerance and adaptation to variations in solution pH.

Table 5. Summary of fitted Gaussian parameters describing the response of *Xanthium spinosum* seed germination to pH across four regional populations

Populations	Maximum Germination (%)	Optimum pH	Spread (σ)
Southeastern Anatolia	90.15	6.95	2.01
Mediterranean	86.33	7.07	1.76
Black Sea	89.57	7.59	1.98
Central Anatolia	91.63	7.37	1.85

Maximum germination (%) denotes the theoretical maximum germination derived from curve fitting analysis. The optimum pH is the pH level at which highest germination is expected to occur. Spread (σ , pH units) indicates the standard deviation of the Gaussian distribution and indicates the range of pH tolerance for each population, where higher values represent a wider tolerance.

The pH-dependent germination responses of studied populations were highly correlated with the soil properties of their native ranges. Populations from Southeastern Anatolia and the Mediterranean region had optimum germination at pH 7.0–7.1, indicating adaptability to moderately alkaline soils (pH 7.55–7.62). Conversely, populations from the Black Sea and Central Anatolia originated in more alkaline soils (pH 8.03–8.23), had somewhat elevated optimum pH values (7.4–7.6). The results align with earlier research indicating that seed germination characteristics of various populations of the same species often correlate with the soil pH conditions of their origin (Baskin & Baskin, 2014; Farooq et al., 2019, 2021; Önen et al., 2018; Ozaslan et al., 2017).

Extensive pH tolerance observed in the

Southeastern Anatolia population may be attributed to its origin in extremely saline, clay-dominant soils (EC = 839 mS/m; 61.1% clay), where dynamic soil chemistry encourages a more flexible germination behavior (Benvenuti, 2003). In contrast, the limited tolerance of the Mediterranean population correlates with its calcareous soil environment (29.58% CaCO_3), recognized for maintaining more stable pH values. These findings indicate that soil chemistry significantly impacts the germination niche width of *X. spinosum*, hence influencing its potential invasiveness in various habitats.

Effect of different salinity levels on seed germination

The highest germination under non-saline treatment exhibited slight variation across

populations, with 91.76% germination in the Anatolia population (Table 6). Black Sea population to 96.65% in the Central

Table 6. Maximum germination percentages at zero salinity, salinity levels required to inhibit 50% germination (EC_{50}), and coefficient of determination (R^2) values for third-degree polynomial fits describing the germination response of four regional populations under varying salinity levels

Population	Maximum Germination (%)	EC_{50} (ppm)	R^2 of Fit
Southeastern Anatolia	94.10	306.30	0.996
Mediterranean	93.48	227.41	0.987
Black Sea	91.76	198.87	0.993
Central Anatolia	96.65	155.76	0.993

EC_{50} = salinity levels required to inhibit 50% of maximum germination

Despite similar baseline germination, populations exhibited varied responses to increasing salinity levels. The Southeastern Anatolia population had the highest salt resistance, requiring a salinity level of 306.30 mM to decrease germination by 50% (EC_{50}). In

contrast, the Central Anatolia population had the highest sensitivity, with a 50% drop at just 155.76 ppm. Mediterranean and Black Sea populations exhibited intermediate tolerances, with EC_{50} values of 227.41 ppm and 198.87 ppm, respectively (Figure 4).

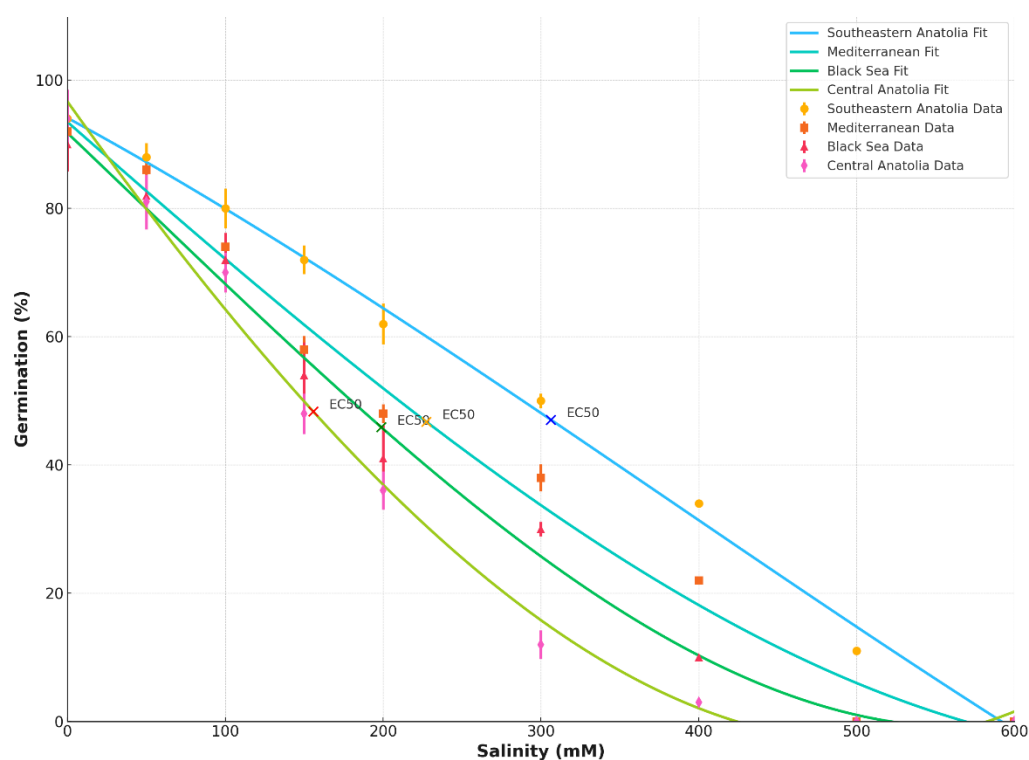


Figure 4. Germination response curves of four *Xanthium spinosum* populations under increasing salinity stress, fitted using third-degree polynomial models. Data points represent observed mean germination percentages $\pm$ standard error of the mean at each salinity level ($n = 5$). The lines indicate fitted curves. EC_{50} points (salinity concentrations causing a 50% reduction in maximum germination) are marked on each curve.

Tested populations had high and constant germination (91.76% to 96.65%) in non-saline conditions (0 mM), whereas tolerance to salt varied among populations. The EC_{50} values significantly varied across populations. Southeastern Anatolia population demonstrated

the highest salt resistance, with an EC_{50} of 306.30 mM, demonstrating strong physiological mechanisms for germination under salinity stress. These findings align with the soil properties of the site of origin, which had high electrical conductivity (839.00 mS/m) and a semi-arid

climate. Plant populations naturally exposed to highly saline and dry environments evolve seed-level tolerance traits such osmotic adjustments, antioxidant activity, and delayed germination (Gul et al., 2013; Qu & Huang, 2005). Central Anatolia population had the lowest salt tolerance, with an EC_{50} of 155.76 mM. This population stemmed from a cold semi-arid climate with low soil salinity (205.15 mS/m). Other continental steppe-origin plants have higher salinity sensitivity during germination owing to lower seed development selection pressure for salt tolerance (Song et al., 2005). Lower organic matter and phosphorus levels (1.17% OM and 10.03 ppm P) in soils may affect early-stage stress mitigation during germination.

The Mediterranean and Black Sea populations exhibited moderate salt tolerance, with EC_{50} values of 227.41 mM and 198.87 mM, respectively. Hot-summer Mediterranean climate and moderate salinity (512.89 mS/m) of

Mediterranean soils may have selected for salt tolerance. These results confirm the ecological concept that local adaptation to soil salinity and moisture regimes considerably affect seed germination (Baskin & Baskin, 2014). High soil salinity favors seed germination delay, and structural resistance, whereas mesic or temperate populations prioritize rapid germination under favorable conditions without costly stress adaptations.

Effect of different osmotic potentials on seed germination

The third-degree polynomial models correctly predicted germination responses under osmotic stress, with good coefficients of determination (R^2 values between 0.990 and 0.997). Peak germination under non-stressed environments varied from 88.84% in the Mediterranean to 94.78% in Southeastern Anatolia populations (Table 7).

Table 7. Maximum germination percentages at zero osmotic stress, osmotic potential values causing 50% reduction in maximum germination (EC_{50}), and coefficients of determination (R^2) for third-degree polynomial fits describing the germination responses of four *Xanthium spinosum* populations under increasing osmotic stress (-MPa).

Population	Maximum Germination (%)	EC_{50} (-MPa)	R^2 of Fit
Southeastern Anatolia	94.78	0.96	0.9974
Mediterranean	88.84	0.85	0.9900
Black Sea	92.08	0.70	0.9970
Central Anatolia	93.54	0.61	0.9955

EC_{50} = Osmotic potential levels required to inhibit 50% of the maximum germination

The osmotic potential required to inhibit maximum germination by 50% (EC_{50}) varied among population. Southeastern Anatolia had the highest tolerance at -0.96 MPa, followed by the Mediterranean at -0.85 MPa. The Black Sea and

Central Anatolia populations were more susceptible, with EC_{50} values of -0.70 and -0.61 MPa. Southeastern Anatolia appeared most resistant to osmotic stress (Figure 5).

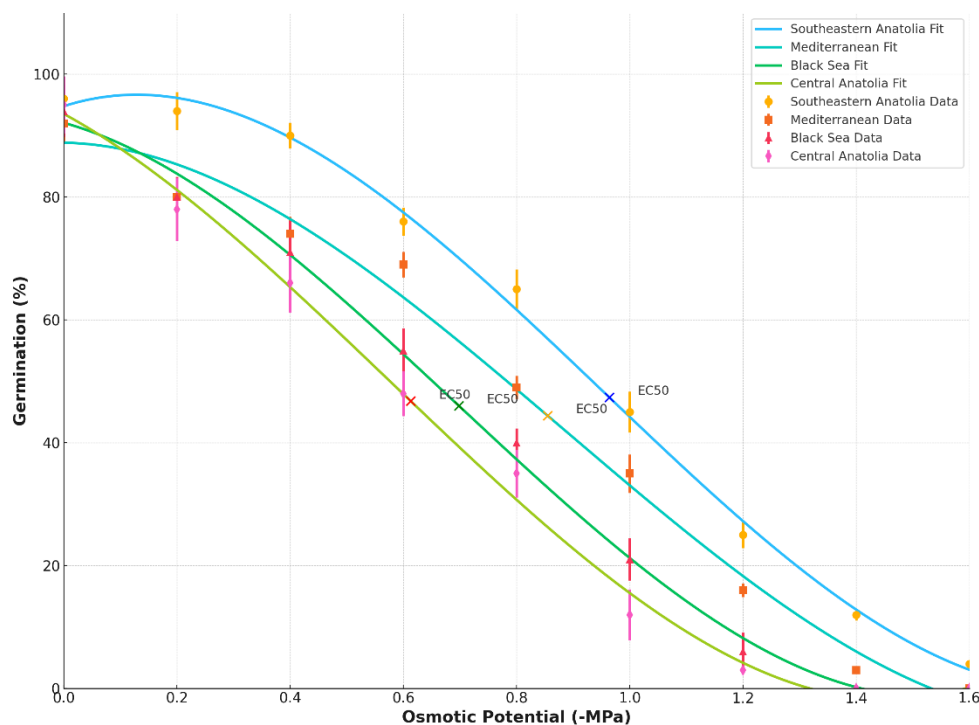


Figure 5. Germination response curves of four *Xanthium spinosum* populations under increasing osmotic stress, fitted using third-degree polynomial models. Data points represent observed mean germination percentages $\pm$ standard error of the mean at each osmotic potential level. Solid lines indicate fitted curves. EC₅₀ points are marked on each curve.

The results indicated that studied populations exhibit significant variation in germination responses to osmotic stress, strongly associated with their native environments. Southeastern Anatolia population had the highest resistance, with an EC₅₀ of -0.96 MPa, while the Mediterranean population followed with an EC₅₀ of -0.85 MPa. Conversely, Black Sea and Central Anatolia populations exhibited more susceptibility to water stress, shown by lower EC₅₀ values of -0.70 MPa and -0.61 MPa, respectively. These patterns indicate robust ecological adaptability (Sun et al., 2023) to the climatic and soil conditions of the seed collection sites. Southeastern Anatolia had a hot semi-arid climate, very high soil salinity (839.00 mS/m), and frequent drought, which likely help in the production of seeds with improved osmotic stress resistance. This correlates with earlier studies

(Farooq et al., 2019, 2021; Önen et al., 2018), which indicated that seeds from dry and saline habitats often have enhanced osmotic adjustment mechanisms, such as osmolyte accumulation and membrane stability, to facilitate germination under water-limited environments.

Effect of seed burial depth on seedling emergence

The seedling emergence from various burial depths was successfully modeled using three-parameter Gaussian functions. Seedling emergence demonstrated a typical bell-shaped curve, characterized by low emergence at surface sowing (0 cm), a rapid rise peaking at shallow to moderate burial depths (~2–4 cm), and a subsequent gradual drop at deeper depths (Table 8).

Table 8. Summary of fitted Gaussian model parameters describing the effect of burial depth on seedling emergence of *Xanthium spinosum* populations collected from four different climatic regions of Türkiye.

Population	Maximum Emergence (%)	Optimum Burial Depth (cm)	Spread (σ , cm)
Southeastern Anatolia	85.61	4.33	3.08
Mediterranean	76.48	4.35	3.11
Black Sea	81.80	4.17	2.66
Central Anatolia	75.90	4.06	2.59

Maximum emergence (%) represents the theoretical peak seedling emergence predicted by the fitted model. Optimum burial depth (cm) indicates the depth at which maximum seedling emergence is expected. Spread (σ , cm) denotes the standard deviation of the fitted Gaussian curve and reflects the breadth of burial depth tolerance; larger σ values indicate broader germination ability across depth variations.

Southeastern Anatolia population had the highest peak emergence, with maximum emergence recorded from 2 cm burial depth. Mediterranean and Black Sea populations had slightly reduced maximum emergence rates while demonstrating comparable optimum burial depths to those in Southeastern Anatolia. Central Anatolia population, although achieving a relatively high peak emergence, had a more significant drop beyond a burial depth of 4 cm, indicating greater vulnerability to deeper burial (Figure 6).

The burial depth responses directly impact integrated weed management strategies.

Significant emergence observed at shallow to intermediate burial depths (~2–4 cm) across all populations indicates that *X. spinosum* is particularly sensitive to seed placement within the soil profile. The significant reduction in emergence beyond 6 cm, especially for the Central Anatolia population, suggests that deep burial may function as an efficient cultural control strategy. Techniques like deep tillage, which displace seeds to depths more than 6–8 cm, may significantly diminish seedling recruitment, hence constraining the weed's soil seedbank over time (Chauhan et al., 2006a, 2006c).

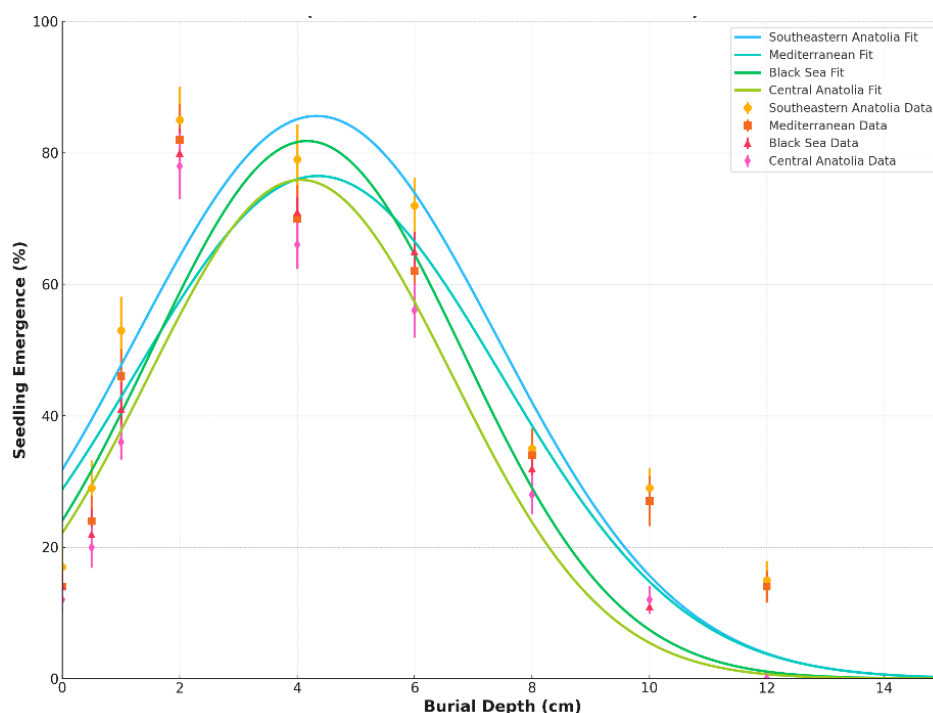


Figure 6. Seedling emergence response curves of four *Xanthium spinosum* in relation to burial depth, modeled using three-parameter Gaussian functions. Data points represent observed mean germination percentages $\pm$ standard error of the mean (SEM). Solid lines indicate fitted Gaussian curves. Burial depths are expressed in centimeters (cm).

Conclusion

The study revealed that germination characteristics of *Xanthium spinosum* seeds are significantly affected by environmental cues with considerable population-specific variability associated with climatic and edaphic conditions. The highest germination was recorded under moderate temperature (~30 °C), neutral to slightly alkaline pH, moderate osmotic stress, and shallow burial depths (~2–4 cm). Populations from warmer and more saline areas (Southeastern Anatolia) exhibited enhanced tolerance to thermal, saline, and osmotic stresses, along with a wider range of pH and burial depth tolerance. Conversely, populations stemming from temperate and semi-arid regions had smaller ecological tolerance. The results have significant implications for weed control, i.e., extensive tillage that buries seeds below 6 cm, along with moisture management and residue retention, might significantly reduce the establishment of *X. spinosum*. Moreover, incorporating population-specific responses to light, temperature, salinity, and burial depth into management strategies might enhance the accuracy and robustness of management measures. Predictive emergence models that include these variations in biology will enhance the timing of herbicide application, mechanical control strategies, and soil management techniques. Designing weed management strategies to the ecological adaptations of *X. spinosum* populations is essential for limiting the spread and attaining sustainable suppression across different agroecosystems in Türkiye.

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Not applicable

Ethics approval and consent to participate

Not applicable as the study is not a clinical trial and did not include any patients.

Competing interests

The author has declared no conflicts of interest.

Authors' contributions

Conceptualization, S.F.; methodology, S.F.; software, S.F.; validation, S.F.; formal analysis, S.F.; investigation, S.F.; resources, S.F.; data curation, S.F.; writing—original draft preparation, S.F.; writing—review and editing, S.F.; visualization, S.F. All authors have read and agreed to the published version of the manuscript.

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Effects of boiling and peeling treatments on the physicochemical properties of Durian (*Durio zibethinus*) seed flour

Durian (Durio zibethinus) Tohum Ununun Fizikokimyasal Özellikleri Üzerine Kaynatma ve Soyma İşlemlerinin Etkileri

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ABSTRACT

Durian (*Durio zibethinus*) seed flour represents an underutilized byproduct with significant potential for food applications. This study systematically evaluated how boiling and peeling pre-treatments affect the physicochemical properties of durian seed flour using a factorial completely randomized design with four treatments: boiled-peeled (BP), boiled-unpeeled (BUP), unboiled-peeled (UBP), and unboiled-unpeeled (UBUP). Results revealed that boiling significantly increased cohesiveness (Carr Index: 16.67% in BP vs 6.67% in UBUP) and thermal stability (peak temperature: 144.67°C in BP vs 26.87°C in UBUP) through starch gelatinization, while reducing flowability. Skin removal improved lightness ($L^* = 87.11$ in UBUP vs 60.02 in BUP) and flow properties. Unpeeled flours exhibited superior functional properties, with UBUP showing the highest water solubility (0.16%) and foam stability (94.44%), attributed to preserved seed coat polysaccharides and amphiphilic compounds. Thermal analysis confirmed boiling-induced structural stabilization, whereas unboiled-unpeeled flour displayed anomalous low transitions, suggesting enzymatic degradation. Color analysis aligned with consumer preferences, as peeled flours (UBP, BP) achieved higher lightness ($L^* > 83$) compared to unpeeled counterparts ($L^* < 71$). The study demonstrates that processing methods dictate flour functionality: peeled flours are optimal for visual quality and flowability, while unpeeled variants excel in foaming and solubility. These findings provide critical insights for tailoring durian seed flour production to specific applications, bridging the gap between agricultural waste valorization and functional ingredient development. Future research should explore protein isolation techniques and industrial-scale processing to fully exploit this sustainable resource.

Key Words: Boiling, Durian seed flour, Peeling

Öz

Durian (*Durio zibethinus*) tohumu unu, önemli potansiyele sahip ancak yeterince değerlendirilmemiş bir yan ürün olarak gıda uygulamaları açısından dikkate değerdir. Bu çalışma, haşlama ve kabuk soyma ön işlemlerinin durian tohumu ununun fizikokimyasal özellikleri üzerindeki etkilerini sistematik olarak değerlendirmiştir. Çalışmada, tamamen rastgeleleştirilmiş faktöriyel bir tasarım kullanılarak dört farklı işlem grubu incelenmiştir: haşlanmış-soyulmuş (BP), haşlanmış-soyulmamış (BUP), haşlanmamış-soyulmuş (UBP) ve haşlanmamış-soyulmamış (UBUP). Elde edilen sonuçlar, haşlama işleminin nişasta jelatinizasyonu yoluyla kohezyonu (Carr İndeksi: BP'de %16,67; UBUP'da %6,67) ve termal stabiliteyi (tepe sıcaklığı: BP'de 144,67°C; UBUP'da 26,87°C) önemli ölçüde artırırken akışkanlığı azalttığını ortaya koymuştur. Kabuk soyulması ise unun açıklığını ($L = UBUP'de 87,11; BUP'da 60,02$) ve akış özelliklerini iyileştirmiştir. Soyulmamış unlar fonksiyonel özellikler açısından daha üstün bulunmuş, özellikle UBUP grubu en yüksek su çözünürlüğü (%0,16) ve köpük stabilitesi (%94,44) değerlerine ulaşmıştır. Bu durum,

tohum kabuğunda korunan polisakkaritler ve amfipatik bileşiklere atfedilmektedir. Termal analiz, haşlamaya bağlı yapısal stabilizasyonu doğrularken, haşlanmamış-soyulmamış un düşük ve anormal geçiş sıcaklıkları göstermiş, bu durum enzimatik bozulmayı düşündürmektedir. Renk analizleri, tüketici tercihleriyle uyumlu olup, soyulmuş unların (UBP, BP) daha yüksek açıklık değerlerine ($L > 83$) ulaştığını, soyulmamış unların ise daha düşük değerlere ($L < 71$) sahip olduğunu göstermiştir. Bu çalışma, işleme yöntemlerinin unun fonksiyonelliğini belirlediğini ortaya koymaktadır: soyulmuş unlar görsel kalite ve akışkanlık açısından uygunken, soyulmamış unlar köpüklenme ve çözünürlük açısından daha üstündür. Elde edilen bulgular, durian tohumu ununun belirli uygulamalara uygun şekilde işlenmesine dair önemli bilgiler sunmakta ve tarımsal atıkların değerlendirilmesi ile fonksiyonel bileşen geliştirilmesi arasındaki boşluğu doldurmaya katkı sağlamaktadır. Gelecek çalışmaların, protein izolasyon teknikleri ve endüstriyel ölçekli işleme yöntemlerine odaklanarak bu sürdürülebilir kaynağın potansiyelini tam anlamıyla ortaya çıkarması önerilmektedir.

Anahtar Kelimeler: Kaynatma, Durian Tohumu Unu, Soyma

Introduction

Durian (*Durio zibethinus*), widely regarded as "The King of Fruits," holds significant agricultural and economic importance in Indonesia. Despite its popularity, the fruit's edible portion constitutes only 20–35% of its total weight, with the remaining 65–80% consisting of rind (60–75%) and seeds (5–15%) (Lestari et al., 2024). These byproducts are predominantly discarded as organic waste, representing both an environmental concern and a lost opportunity for resource utilization. Notably, durian seeds contain substantial nutritional value, with compositions of 54.90% water, 3.40% protein, 1.58% ash, 1.32% fat, and 18.92% starch (Srianta et al., 2012). While minimal efforts have been made to repurpose seeds as animal feed, their potential as a functional food ingredient remains underexplored. Converting durian seeds into flour presents a viable strategy to mitigate waste while enhancing their commercial value.

The production of durian seed flour involves sequential processing steps—washing, peeling, boiling, drying, and grinding—each of which may influence the final product's quality. Previous studies have demonstrated the successful incorporation of durian seed flour into various food products, including biscuits (Verawati and Yanto, 2019), bread (Humaira and Faisal, 2023), and noodles (Wibawani et al., 2023), suggesting its potential for broader food and beverage applications. However, the physicochemical properties of the flour are highly dependent on pretreatment methods, particularly boiling and peeling.

Boiling, for example, has been demonstrated to modify essential characteristics of flour. Cahyani and Hakim (2017) reported higher flour yields from unboiled seeds, whereas Amon et al. (2014) observed that boiling significantly modified moisture content, reducing sugars, crude fat, and phenolic compounds in taro flour. The boiling pretreatment was selected due to its role in starch gelatinization, protein denaturation, and antinutrient reduction, which simultaneously enhance the flour's functional properties (Ma et al., 2011). Similarly, peeling reduces seed mass, directly affecting flour yield. Despite these findings, no comprehensive study has evaluated the combined effects of boiling and peeling on durian seed flour's physicochemical properties, leaving a critical gap in optimizing processing techniques.

This study addresses this gap by systematically examining how different combinations of boiling (with/without) and peeling (with/without) influence durian seed flour's yield, nutritional profile, and functional quality. By elucidating the interactions between these pretreatment variables, the research aims to establish optimal processing conditions that maximize flour quality and economic viability. The findings will contribute to sustainable waste optimization strategies while expanding the utilization of durian by products in the food industry.

Material and Methods

The main ingredient used in making flour is durian seeds from ripe and fresh durian (Figure 1). Durian was obtained from farmer or community plantations in Desa Batee Meucanang, Labuhan Haji, South Aceh.



Figure 1. Durian and the seed

This study applied a Factorial Completely Randomized Design (CRD) with two main factors and two treatment levels, each of which was tested in three replications. The two main factors in this study were peeling of durian seed skin (with/without) and boiling of durian seed (with/without). Therefore, there were four samples in every replication, they were BP (boiled, peeled), BUP (boiled, unpeeled), UBP (unboiled, peeled), and UBUP (unboiled, unpeeled).

Preparation of Durian Seed Flour

Preparation of flour is shown by Figure 2. The initial stage in durian seed flour production involved manual sorting, wherein seeds were selected based on specific quality criteria: dense and fresh seed flesh, absence of pest damage, and free from rot. Following sorting, the peeling stage was conducted exclusively for the peeled and unpeeled samples, wherein the outer seed coat—unsuitable for flour production—was removed. Subsequently, the seeds underwent washing to eliminate surface contaminants. This process entailed soaking the seeds in clean water for 15 minutes to dissolve residual mucilage, followed by thorough rinsing under running water.

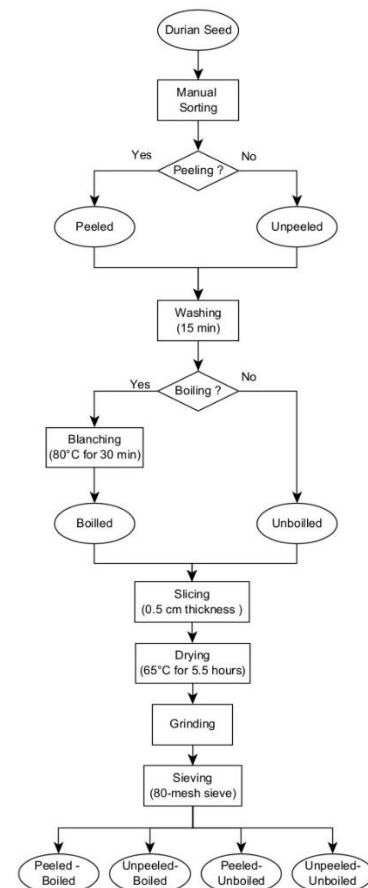


Figure 2. Durian seed flour processing

For the boiled and unboiled samples, the washed seeds were subjected to blanching at 80°C for 30 minutes (Wibawani et al., 2023). After blanching, the seeds were sliced uniformly to a thickness of 0.5 cm to optimize the subsequent drying and milling processes. The sliced seeds were then dried in an oven at 65°C for 5.5 hours (Lisa et al., 2015) to reduce moisture content and enhance shelf stability. The dried slices were pulverized using a mechanical grinder, and the resulting powder was sieved through an 80-mesh sieve to ensure particle uniformity and a fine flour consistency.

Measurement of Physical Properties

In order to find out the physical properties of durian seed flour, the density, Carr index, Hausner ratio, angle of repose, and flowability were measured. Bulk density was determined according to the method described by Puteri et al. (2017). Durian seed flour was placed into a 10 ml graduated cylinder. Then, the 10 ml flour was weighted. The bulk density was calculated by dividing the sample weight by the sample volume. For the tapped density, the cylinder was mechanically tapped for 30 times. The tapped density was calculated by dividing the sample weight by the sample volume after tapping. Carr index and Hausner ratio were calculated from bulk and tapped density (Nani and Krishnaswamy, 2021) by using equations (1) and (2).

$$\text{Carr index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \quad (1)$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad (2)$$

Flow rate and angle of repose were determined according to the method described by Khan and Saini (2016). A glass funnel was attached to a stand at a distance of 10 cm above a table surface. Durian seed flour (30 g) was poured through the funnel to create a heap, the height and diameter were then measured. The flow rate was measured by using stopwatch. In order to determine the angle of repose, the anti-tangent

of radius and height was calculated.

Measurement of Functional Properties

The solubility in water and swelling power were assessed according to the method described by Kusumayanti et al. (2025). To measure the solubility, 0.5 g of the sample was heated in 10 mL of distilled water at 60°C for 30 minutes without agitation. The samples were then centrifuged at 1600 rpm for 10 minutes. Subsequently, 5 mL of the supernatant was collected, dried, and weighed. Percentage of solubility was calculated by dividing weight of dried supernatant to the weight of sample (dry basis). For swelling power, the sample (0.1 g) was heated in 10 mL of distilled water at 60°C for 30 minutes with continuous agitation. Subsequently, the samples were centrifuged at 1600 rpm for 15 minutes, and the weight of the sedimented portion was recorded. Swelling power was calculated by dividing weight of sediment to the weight of sample (dry basis).

Foaming capacity and stability were determined according to the method described by Puteri et al. (2017). The sample solution (60 mL, 5% w/v) was homogenized for 1 minute. The solution was then placed in a 100 mL graduated cylinder and the foam volume was recorded. In addition, the foam stability was obtained as the percentage of foam volume retained after 10 minutes. The foaming capacity was expressed using Equation (3).

$$\text{Foaming capacity (\%)} = \frac{\text{volume after homogenization} - \text{initial volume}}{\text{initial volume}} \times 100\% \quad (3)$$

Color Analysis

The color profile of the sample was interpreted by Hunter Lab using a Chromameter (Konica, Minolta CR-300, Tokyo, Japan). The L* value represents lightness ranging from 0 to 100 (white). The a* value represents red (+) to green (-), while the b* value represents yellow (+) to blue (-) (Puteri et al., 2017). Moreover, the value of ΔE (total color difference to reference or black) was also calculated using Equation (4). The ΔL , Δa , and Δb values represent the differences in

lightness, red–green, and yellow–blue color components, respectively, relative to the reference sample (black).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (4)$$

Measurement of Protein Solubility

Protein solubility was measured to quantify the percentage of soluble protein using the Lowry method with modification (Dent et al., 2024). Protein supernatants were diluted tenfold and

hydrolyzed in 2 N NaOH at 100 °C for 10 minutes prior to the addition of the complex-forming reagent and the Folin–Ciocalteu phenol reagent. The absorbance of sample was measured at 750 nm using a UV-VIS spectrophotometer (Optizen Alpha, KLAB, South Korea). The result was compared with a calibration curve prepared using bovine serum albumin (BSA).

Measurement of Thermal Properties

The thermal properties were observed by using Differential Scanning Calorimetry (Mettler Toledo). The onset, peak, and endset

temperature were saved to analyse the gelatinization behavior of sample.

Statistical Analysis

The data of analyses were processed with Analysis of Variance by using the RStudio (Version 2024.12.1+563) software package. The data that had a significant effect on the observation variables were further tested using Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($\alpha = 0.05$).

Table 1. Physical properties of durian seed flour with different treatment¹

Parameters	Treatment			
	BP ²	BUP ³	UBP ⁴	UBUP ⁵
Bulk Density (g ml⁻¹)	0.56±0.01	0.56±0.01	0.57±0	0.57±0.01
Tapped Density (g ml⁻¹)	0.68±0.02	0.65±0.05	0.62±0.02	0.61±0.03
Carr Index	16.67±2.89a	13.33±5.77ab	8.33±2.89b	6.67±2.89b
Hausner Ratio	1.2±0.04a	1.16±0.08ab	1.09±0.03b	1.07±0.03b
Angle of Repose (°)	23.17±2.82a	20.30±1.83ab	19.57±3.54ab	16.26±2.01b
Flow rate (s)	5.09±1.2b	6.06±0.02b	5.06±0.49b	9.36±2.47a

¹Different letters indicate significant differences among samples within the same row ($p < 0.05$). Data are means $\pm$ standard deviation. ²Boiled and peeled. ³Boiled and unpeeled. ⁴Unboiled and peeled. ⁵Unboiled and unpeeled.

Results and Discussion

Physical Properties

The results demonstrate significant differences in the flow properties and cohesiveness of durian seed flour across pre-treatment methods, while density parameters remain stable (Table 1). This phenomenon can be explained by the unique physicochemical characteristics of durian seed starch. The higher Carr Index (16.67%) and Hausner Ratio (1.20) in boiled-peeled (BP) samples compared to unboiled-unpeeled (UBUP) samples indicate that boiling enhances cohesiveness through starch gelatinization, consistent with reports that durian seed starch gelatinizes between 67.50-79.35°C (Leemud et al., 2020). Complete gelatinization at 100°C substantially modifies the flour's particle structure.

The relatively high amylose content (28.3%) in durian seed starch (Baraheng and Karrila, 2019) contributes to strong gel matrix formation post-

gelatinization, explaining the poorer flow properties in boiled samples. However, stable bulk density (0.56-0.57 g/mL) and tapped density (0.61-0.68 g/mL) values across treatments suggest consistent bulk composition despite surface modifications. Notably, UBUP samples exhibited the lowest angle of repose (16.26°) but highest flow rate (9.36 s), suggesting bimodal particle size distribution from unpeeled seed coats, and hydrophobic components reducing static cohesion while increasing dynamic friction. These flow differences reflect structural transformations during gelatinization. In boiled samples, increased cohesiveness results from granule swelling and 30-40% surface porosity reduction during drying (Li et al., 2021), exposed hydroxyl groups enhancing hydrogen bonding (Liang et al., 2024), and strengthened van der Waals interactions from surface compaction (Koyakumar and Nakano, 2016).

Unboiled samples maintained porous structures with lower Carr Index (6.67-8.33%) and

Hausner Ratio (1.07-1.09), as intact crystalline structures and rough surfaces minimized particle contact (Yan et al., 2024). The paradoxical flow dynamics in UBUP samples reflect how hydrophobic surfaces reduce static cohesion (Thomas, 2024) while fibril particles create flow-resistant interlocking networks (Farkas et al.,

2024; Wang et al., 2019), with additional contributions from capillary forces (Nie et al., 2023). Boiling-induced transformations included gelatinized starch matrices (Zheng et al., 2024), denatured surface proteins (Yang et al., 2012), and redistributed surface components (Sibrant and Pauchard, 2017).

Tabel 2. Functional properties of durian seed flour with different treatment¹

Treatment	Parameters			
	Solubility in Water (%)	Swelling Power	Foam Capacity (%)	Foam Stability (%)
BP ²	0.13±0.01ab	8.32±0.38a	4.33±0.58a	70±8.66
BUP ³	0.14±0ab	8.7±0.62a	4.67±0.58a	80±20
UBP ⁴	0.09±0.01b	4.61±0.3c	2.67±0.58b	77.78±19.25
UBUP ⁵	0.16±0.05a	6.7±0.62b	4.67±1.15a	94.44±9.62

¹Different letters indicate significant differences among samples within the same column ($p < 0.05$). Data are means $\pm$ standard deviation. ²Boiled and peeled. ³Boiled and unpeeled. ⁴Unboiled and peeled. ⁵Unboiled and unpeeled.

Functional Properties

Our analysis revealed significant pretreatment effects on functional properties of durian seed flour ($p < 0.05$). The functional properties are portrayed in Table 2. The highest water solubility was observed in BUP (0.14%) and UBUP (0.16%) samples, substantially greater than in UBP (0.09%). This phenomenon can be attributed to two primary mechanisms: the presence of seed coats rich in hydroxyl (-OH) groups from polysaccharides such as pectin and hemicellulose (Mustofa et al., 2024), and preservation of the natural porous structure of seed coats facilitating capillary water penetration (Liu et al., 2020).

The swelling power analysis at 60°C demonstrated significantly higher values in boiled samples (BP: 8.32; BUP: 8.70) compared to unboiled samples (UBP: 4.61; UBUP: 6.70). This thermal effect results from structural transformations of starch granules during boiling at 100°C, exceeding the gelatinization temperature range of durian seed starch (67.5-79.4°C) (Leemud et al., 2020). The gelatinization process occurs through three distinct phases: initial swelling below 60°C, loss of birefringence at the onset temperature (T_o), and maximum swelling at peak temperature (T_p) during crystalline region dissociation (Muñoz et al., 2015). This pattern aligns with the temperature-dependent swelling power behavior reported for

other starches, where swelling power increases dramatically near T_p due to amylopectin helix unwinding and water absorption into the granule matrix (Li and Yeh, 2001).

The intermediate swelling power of UBUP (6.70) suggests partial swelling without boiling treatment, mediated by semi-permeable seed coat membranes enabling gradual water penetration at sub-gelatinization temperatures (Lin et al., 2020), and preserved amylose-lipid complexes that restrict swelling, accounting for the 26% lower swelling power in UBUP versus BUP (Vandeputte et al., 2003). The narrow gelatinization range ($\Delta T = T_p - T_o = 11.9^\circ\text{C}$) of durian seed starch (Leemud et al., 2020) correlates with its rapid swelling profile, consistent with the negative correlation between T_o and peak viscosity in cereal starches (Sandhu and Singh, 2007). The low T_o (67.5°C) promotes early swelling initiation, while the high amylose content (28.3%) (Baraheng and Karrila, 2019) stabilizes swollen granules against disintegration.

Foaming properties exhibited remarkable dependence on both thermal treatment and seed coat presence. BUP and UBUP showed the highest foaming capacity (4.67%), with UBUP demonstrating exceptional stability (94.44%). These effects stem from boiling-induced protein structural changes enhancing air-water interface adsorption (González-Pérez et al., 2005), though

potentially causing detrimental aggregation (Changade et al., 2009); seed coats providing natural saponins and optimally amphiphilic particles (contact angle $\theta \approx 80-85^\circ$) for foam stabilization (Rüegg et al., 2022; Wasan et al., 2004); and UBUP's unique lipoprotein network and optimal particle size distribution creating protective interfacial architectures. Conversely, seed coat removal eliminated bioactive components and altered particle morphology, impairing geometric stabilization (Rüegg et al., 2022).

Color Properties

The color properties of durian seed flour are portrayed in Table 3, while the appearance is shown in Figure 3. Our analysis revealed significant pretreatment effects on color of durian seed flour ($p < 0.05$). UBP had the highest lightness ($L^* = 87.11$), indicating that peeling, without boiling, preserved the brightest flour. Its moderate a^* (5.63) and lowest b^* (12.25) suggested minimal browning, as no heat treatment was applied to induce color changes. Meanwhile, BP exhibited the second-highest lightness ($L^* = 83.83$), suggesting that boiling and peeling helped maintain a brighter color, likely due to the removal of darker peel pigments and heat-induced degradation of color compounds. A

higher L^* value indicates white color and a lower browning activity (Buzera et al., 2022). Its low a^* value and high b^* value indicated a slight reddish-yellow hue, which may result from browning reaction during boiling. BUP and UBUP showed the lowest lightness (60.02 and 70.73, respectively), confirming that the presence of the peel contributed to a darker color. The high a^* value (9.06) and b^* value (18.61) in BUP suggests a stronger red tint and yellowness, possibly from pigments in the durian seed skin or intensified browning reactions. Moreover, UBP had the highest ΔE (88.09), showing it was the most distinct in color, likely due to its high lightness and low yellowness. Higher ΔE values indicate clearer color differences with the reference (black).

Our results suggest that peeling was crucial for a brighter flour, while boiling enhances browning, particularly when the peel was present. Similar result was found in potato flour (Buzera et al., 2022). Heat treatment, especially boiling, might reduce the lightness of the flour. Consumers generally prefer flour products that exhibit high lightness (L^* value) and low chroma (color intensity), as these characteristics are associated with purity and refined quality (Alabi et al., 2016). Therefore, peeled durian seed flours would be more acceptable by consumers.

Table 3. Color properties of durian seed flour with different treatment¹

Treatment	Parameters			
	L^*	a^*	b^*	ΔE
BP ²	83.83 $\pm$ 0.53b	4.74 $\pm$ 0.24c	18.68 $\pm$ 0.11a	85.97 $\pm$ 0.51b
BUP ³	60.02 $\pm$ 1.93d	9.06 $\pm$ 0.71a	18.61 $\pm$ 0.7a	63.95 $\pm$ 1.88d
UBP ⁴	87.11 $\pm$ 0.59a	5.63 $\pm$ 1.09bc	12.25 $\pm$ 1.53c	88.09 $\pm$ 0.78a
UBUP ⁵	70.73 $\pm$ 0.76c	6.48 $\pm$ 0.16b	15.27 $\pm$ 0.11b	72 $\pm$ 0.77c

¹Different letters indicate significant differences among samples within the same column ($p < 0.05$). Data are means $\pm$ standard deviation. ²Boiled and peeled. ³Boiled and unpeeled. ⁴Unboiled and peeled. ⁵Unboiled and unpeeled.



Figure 3. Durian seed flour with different treatment (a) BP, (b) BUP, (c) UBP, and (d) UBUP

Protein Solubility

There was no significant difference between

treatment on protein solubility of durian seed flour (Figure 4). Boiling and peeling had no effect on the solubility of protein in durian seed flour. However, the analysis method might also influence the result. The Lowry method integrates principles from the biuret assay and the Folin-

Ciocalteu reaction. Dent et al. (2024) reported that the Lowry method is most suited in measuring the solubility of hydrolyzed proteins. Therefore, further investigation on protein solubility in protein isolate from durian seed flour is needed, especially by using different method.

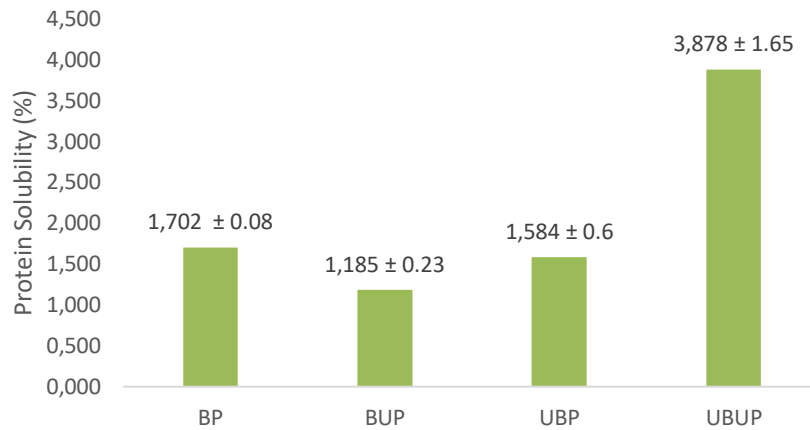


Figure 4. Protein solubility of durian seed flour with different treatment. Different letters indicate significant differences ($p < 0.05$).

Thermal Properties

The thermal properties of durian seed flour with different thermal treatments are portrayed in Table 4. Onset temperature showed the beginning of thermal transition, peak temperature showed the maximum transition rate, while endset temperature showed the temperature where the transition completes. Those temperature often corresponds to gelatinization behavior.

BP exhibited the highest thermal transition temperatures (onset: 139.37°C, peak: 144.67°C, endset: 164.24°C), suggesting that boiling and peeling enhanced starch stability, likely due to partial gelatinization and retrogradation during processing. The high peak temperature implies a more ordered, heat-resistant starch structure, possibly due to amylose reassociation or protein-starch interactions (Dang et al., 2022). BUP showed lower transition temperatures compared to BP, indicating that the presence of the peel may have interfered with starch reorganization during boiling, leading to less thermal stability. UBP had much lower transition temperatures, but showing typical native starch gelatinization. Since

the sample was not boiled, the starch granules remained intact, allowing for a more conventional gelatinization process. UBUP displayed the lowest and most unusual thermal transitions, which are far below typical starch gelatinization ranges. This suggests that the unpeeled, unboiled sample underwent significant structural changes, possibly due to enzymatic activity. The extremely low peak temperature may indicate a glass transition (T_g) rather than gelatinization, implying a highly amorphous or degraded starch structure with poor thermal stability.

Compared to boiled durian seed, the onset, peak, and endset temperature of durian seed flour without boiling decreased significantly. It showed that boiling might increase thermal stability of the flour. In accordance to Zhu et al. (2020), the gelatinization temperature of starch might increase after thermal treatment, due to complex formation. Moreover, Dang et al. (2022) reported that the difference of gelatinization temperature after thermal treatments may be attributed to the change of molecular structure and granule structure of amylopectin.

Table 4. Thermal properties of durian seed flour with different treatment¹

Treatment	Parameters		
	Onset (°C)	Peak (°C)	Endset (°C)
BP ²	139.37	144.67	164.24
BUP ³	118.92	128.78	140.2
UBP ⁴	62	93.1	124.28
UBUP ⁵	25.66	26.87	34.33

¹Different letters indicate significant differences among samples within the same column ($p < 0.05$). Data are means $\pm$ standard deviation. ²Boiled and peeled. ³Boiled and unpeeled. ⁴Unboiled and peeled. ⁵Unboiled and unpeeled.

Conclusion

The study demonstrates that processing methods—boiling and peeling—significantly influence the physical, functional, color, and thermal properties of durian seed flour. Boiling enhanced cohesiveness and thermal stability due to starch gelatinization and retrogradation but reduced flowability, while peeling improved lightness and flow properties by eliminating seed skin pigments and hydrophobic components. Unpeeled flours (BUP, UBUP) exhibited superior foaming capacity and water solubility, attributed to retained bioactive compounds in seed skin, though they were darker in color. Notably, unboiled-peeled flour (UBP) emerged as the most consumer-friendly option due to its bright color and favorable flow properties, whereas boiled-unpeeled flour (BUP) showed potential for functional applications requiring high foam stability. Thermal analysis further confirmed that boiling increased starch stability, while unboiled-unpeeled flour (UBUP) displayed atypical transitions, likely due to enzymatic activity or structural degradation. These findings highlight the importance of selecting processing techniques tailored to specific industrial needs—whether for specific quality, functional performance, or thermal resistance. Future research should explore protein solubility using alternative assays and investigate the role of seed skin components in functional properties. Moreover, functional chemical analyses, such as total phenolic content and antioxidant capacity, would significantly enhance the scientific value of the future study and provide deeper insights into the functional potential of the developed product.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

N.E.P: Methodology, analysis, investigation, writing original draft, review. M.I.F: Methodology, analysis, review and editing.

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Kobalt toksisitesinin arpa (*Hordeum vulgare* L.) bitkisi üzerindeki fotokimyasal etkilerinin araştırılması

Investigation of the photochemical effects of cobalt toxicity in barley (*Hordeum vulgare* L.) plants

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ÖZ

Bu çalışmada, arpa (*Hordeum vulgare* L. cv. Kalaycı-97) bitkisinde erken fide döneminde farklı konsantrasyonlardaki kobalt [$\text{Co}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$; 0 μM (kontrol), 300 μM , 600 μM and 900 μM] uygulamalarının fotosentetik aktivite üzerindeki kısa süreli etkileri araştırılmıştır. Bu amaçla Hoagland besin çözeltisi ile hazırlanan su kültüründe yetiştirilen arpa bitkilerinde klorofil a floresansı tekniği ile fotosentetik aktivite ölçümleri yapılmıştır. Sonuçlarımız 300 μM ve 600 μM kobalt uygulamalarının arpa bitkilerinde fotosentetik aktiviteyi olumsuz etkilemediğini göstermiştir. Ancak 900 μM kobalt uygulaması arpa bitkilerinde fotosentetik elektron taşıma reaksiyonlarını önemli ölçüde inhibe etmiştir. Örneğin, düşük F_v/F_o değerleri arpa bitkilerinde Hill reaksiyonunun kobalt toksisitesine karşı çok hassas olduğunu göstermiş olup, bu da FSII'nin donör bölgesindeki oksijen üreten kompleks ile reaksiyon merkezleri arasındaki elektron hareketinin bozulduğunu doğrulamaktadır. Ancak 900 μM kobalt uygulaması altındaki arpa fidelerinde S_m parametresi etkilenmemiştir. Bu durum muhtemelen varsayımsal ve alternatif bir elektron vericisi yardımıyla reaksiyon merkezlerine doğru düzenli bir elektron transferinin belli oranda devam ettiği olasılığını akla getirmektedir. Bu olgu, kobalt toksisitesi altındaki arpa bitkileri için fotosentetik bir tolerans mekanizması olabilir ve bu durum, N parametresinin değişmemiş olmasıyla da desteklenmektedir. Ayrıca arpa bitkilerinde 900 μM kobalt toksisitesi altında F_v/F_m oranının azalması, FSII birimlerinde meydana gelen fotoinhibisyonu açıkça göstermektedir. Arpa bitkisinde 900 μM kobalt uygulaması sonucu artan F_o ve sabit F_m değerleri, FSII ünitelerinin donör bölgesinin akseptör bölgesine göre kobalt toksisitesine daha duyarlı olduğunu doğrulamıştır. TRO/RC , ETo/RC , Dlo/RC ve ϕDo parametrelerindeki değişimler, kobalt toksisitesinin yakalanan ışık enerjisinin fotokimyasal olarak kullanımını engellediğini, ancak ısı olarak dağılmasına neden olduğunu, dolayısıyla arpa bitkisinde fotosentez verimliliğini azalttığını göstermiştir. Sonuç olarak arpa bitkisinde tarımsal verimde azalmaya yol açabilecek kobalt toksisitesinin kritik konsantrasyonunun 600-900 μM arasında olduğu sonucuna varılabilir.

Anahtar Kelimeler: arpa; klorofil a floresansı; kobalt toksisitesi; *Hordeum vulgare*; JIP testi

ABSTRACT

In this study, the short-term effects of different concentrations of cobalt [$\text{Co}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$; 0 μM (control), 300 μM , 600 μM and 900 μM] applications on photosynthetic activity in barley (*Hordeum vulgare* L. cv. Kalaycı-97) plants at the early seedling stage were investigated. For this purpose, photosynthetic activity measurements were done using the chlorophyll a



fluorescence technique in barley plants grown in water culture prepared with Hoagland nutrient solution. Our results showed that 300 μM and 600 μM cobalt applications did not adversely affect photosynthetic activity in barley plants. However, 900 μM cobalt treatment significantly inhibited photosynthetic electron transport reactions in barley plants. For instance, Hill reaction was found to be very vulnerable to cobalt toxicity, as demonstrated by the lower F_v/F_o value, therefore confirming the impairment of the electron movement between oxygen-evolving complex on the donor side of PSII and reaction centers in barley plants. However, the parameter S_m remained unchanged in barley seedlings under 900 μM cobalt treatment. This may indicate a putative and regular electron transfer toward reaction centers, probably due to an alternative electron donor. This phenomenon may be a photosynthetic tolerance mechanism for barley plants under cobalt toxicity, which is also supported by the constant level of the parameter N . In addition, the decreased F_v/F_m ratio in barley plants under 900 μM cobalt toxicity clearly indicated the occurrence of the large extent of photoinhibition. The elevated F_o and the constant F_m values in barley plants due to 900 μM cobalt treatment verified that the donor side of PSII units are more susceptible to cobalt toxicity than the acceptor side. Changes in the parameters TR_o/RC , ET_o/RC , DIO/RC , and ϕDo showed that cobalt toxicity inhibited the use of the trapped light energy photochemically but induced its dissipation as heat, therefore decreasing photosynthetic efficiency in barley plants. As a result, it may be concluded that the critical concentration of cobalt toxicity in barley plants is between 600-900 μM , which may result in decrease in agricultural yield.

Key Words: barley; chlorophyll a fluorescence; cobalt toxicity; *Hordeum vulgare*; JIP test

Giriş

Atom yoğunluğu 5 g/cm³'ten fazla ve atom numarası 20'den büyük olan metal ve metaloidler ağır metal olarak tanımlanmaktadır (Sönmez ve Kılıç, 2021). Kurşun (Pb), demir (Fe), arsenik (As), civa (Hg), nikel (Ni), çinko (Zn), kadmiyum (Cd), gümüş (Ag), mangan (Mn), bakır (Cu) ve kobalt (Co) gibi ağır metaller atmosferin yanı sıra yeryüzündeki karasal ve sucul ortamların da doğal bileşenidir (Rajkumar ve ark., 2023). Bu elementlerden bazıları bitkiler için esansiyel bazıları da faydalı birer mikroelement olarak kabul edilir.

1735 yılında George Brandt tarafından keşfedilen kobalt; periyodik tabloda 8B grubunda bulunan, atom numarası 27 ve atom ağırlığı 58.93 olan bir ağır geçiş metalidir (Mahey ve ark., 2020). Yer kabuğunun yaklaşık %0.0029'unu oluşturur. Bitkilerle kobalt elementi arasındaki etkileşimin niteliği konusunda literatürde çelişkili bilgiler mevcuttur. Örneğin Lange ve ark. (2017) ve Salam ve ark. (2024) kobalt elementini bitkiler için esansiyel olmayan faydalı bir element olarak tanımlamıştır. Diğer yandan, Li ve ark. (2024) kobaltın simbiyotik azot fiksasyonu yapan bazı mavi-yeşil alg türleri ve baklagillerin köklerindeki nodüllerde bulunan Rhizobia bakterileri için gerekli bir element olmasına rağmen, bitkiler için esansiyel olarak kabul edilmediğini rapor etmiştir. Ancak yapılan bazı araştırmalardan elde edilen

sonuçlar, kobaltın çeşitli gelişmiş bitki türlerinde esansiyel bir element olduğunu kanıtlar niteliktedir. Örneğin C4 ve CAM bitkilerinde CO₂'nin bikarbonat iyonlarına dönüşümünü sağlayan rekasiyonu katalizleyen karbonik anhidraz enziminin bazı formlarının çinko yerine kobalt elementini kofaktör olarak kullanabildiği bildirilmiştir (Jensen ve ark., 2020). Kobaltın proteaz grubu enzimlerden olan karboksipeptidazların aktif bölgesine çinkoya göre daha etkili bir şekilde bağlandığı ve böylece enzim aktivitesinin etkilenmediği belirtilmiştir (Golubkina ve ark., 2024). Benzer şekilde metionin aminopeptidaz, prolidaz, peroksidaz ve üreaz enzimlerinin aktivitelerinin kobalt elementi etkisiyle arttığı ortaya çıkarılmıştır (Permyakov, 2021). Bu sonuçlar gelişmiş bitkilerde bulunan bazı proteinlerin kobalt içerdiğini ve bazı enzimlerin aktivitesinin de kobalt elementine bağımlı olduğunu göstermektedir.

Kobaltın yer kabuğundaki ortalama miktarının diğer ağır metallere göre çok daha düşük olmasına rağmen bazı antropojenik aktiviteler karasal ve sucul ortamlardaki kobalt konsantrasyonunun ciddi şekilde artmasına yol açmaktadır. Dünya genelinde üretilen toplam kobaltın yaklaşık %15'i sert metallerin işlenmesi ve imalatında kullanılmaktadır (Klasson ve ark., 2016). Tarımsal gübrelerin bilinçsiz kullanımı, atık su deşarjı, sıvı ve katı yakıt emisyonları, artan kobalt madenciliği, pigment ve boya endüstrisinde

kurutma işlemini hızlandırmak için kobaltın kullanımı, mobil piller, televizyonlar, sıvı kristal ekranlar ve bilgisayar monitörleri, çeşitli kozmetik ürünleri, çikolata, tereyağı ve balık gibi çeşitli besinler de doğadaki kobalt kontaminasyonunun temel sebepleri arasındadır (Saaltink ve ark., 2014; Bocca ve ark., 2014; Marin ve ark., 2017). Kobalt toksisitesinin bitkilerde aktif transport mekanizmasını inhibe ettiği, yaprak absisyonunu hızlandırdığı, kök ve gövde büyümesini inhibe ettiği, fotosentetik pigment ve azot metabolizmasını olumsuz yönde etkilediği, fotosentetik etkinliği azalttığı, kromozomlarda yapısal hasarlara neden olarak mitotik aktiviteyi azalttığı, mineral madde beslenmesini bozduğu ve tohum çimlenmesini inhibe ettiği de rapor edilmiştir (Begovic ve ark., 2016; Banerjee ve Bhattacharya, 2021). Ayrıca kobalt biyolojik sistemlerde yükseltgenme-indirgenme reaksiyonlarında rol oynayan bir element olduğu için kobalt toksisitesinin bitki dokularında çeşitli aktif oksijen türlerinin oluşum hızını ve buna bağlı olarak malondialdehit (MDA) ve prolin birikimini indüklediği, antioksidant enzimlerin aktivitelerinin değişmesine yol açtığı da ortaya çıkarılmıştır (Azarakhs ve ark., 2015). Buna rağmen bitkilerde kobalt toksisitesinin etkileri ve kobalt toleransı ile ilgili araştırmalar diğer elementlerle karşılaştırıldığında oldukça sınırlıdır (Salam ve ark., 2024, Nazir ve ark., 2022; Lange ve ark., 2017). Ülkemizdeki tarımsal arazilerde de kobalt miktarının doğal ve endüstriyel faktörler nedeniyle artış gösterdiği belirlenmiştir (Adiloğlu ve Sağlam, 2015; Karaca ve ark., 2014).

Fotosentez, bitki büyümesi ve verimliliği için önemli bir metabolik süreçtir. Ancak, fotosentetik reaksiyonların ağır metal toksisitesi de dahil olmak üzere abiyotik stres faktörleri için birincil hedef olduğu iyi bilinmektedir (Amari ve ark., 2017). Bu nedenle, ağır metal toksisitesinin fotosentetik süreçler üzerindeki etkisinin daha iyi anlaşılması tarımsal verimliliği artırmak için önemlidir. Günümüzde klorofil a floresansının fotosentetik aktiviteyi ölçmek için en modern ve güvenilir teknik olduğu bildirilmiştir (Doğru ve Çakırlar, 2020a ve 2020b; Doğru ve ark., 2021).

Klorofil a floresansı sinyallerinin zamana bağlı değişimlerini ifade eden OJIP eğrisi ve bu değişimler arasındaki etkileşimlerin anlaşılmasını sağlayan JIP testi, bitki biyolojisi ve tarım alanında, fotosentetik aygıtın birçok farklı çevre koşulundaki tepkilerini anlamak amacıyla kullanılmaktadır (Strasser ve ark., 2004; Yusuf ve ark., 2010). Bu test, fotosistem II (FSII)'ye giren ve çıkan enerji akışının, bazı denklemler yardımıyla doğrudan ölçülen ve hesaplanan parametreler vasıtasıyla incelenmesine olanak sağlamaktadır. JIP testinin uygulanması ile ilgili ayrıntılar Doğru (2021) tarafından belirtilmiştir.

Yapılan araştırmalar; kobalt uygulamalarının konsantrasyona bağlı olarak *Lemna minör*, (Sree ve ark., 2015; Begovic ve ark., 2016), *Arabidopsis* (Lange ve ark., 2017) ve mısır bitkilerinde (Salam ve ark., 2024) fotosentetik aktiviteyi inhibe ettiğini; bakla bitkilerinde (Wang ve ark., 2015) ise indüklediğini ortaya çıkarmıştır. Buna göre bu çalışmanın amacı, ülkemizde oldukça yüksek bir üretim potansiyeline sahip olan arpa (*Hordeum vulgare* L. cv. Kalaycı-97) bitkisinde farklı kobalt konsantrasyonlarının fotosentetik aktivite üzerindeki konsantrasyona bağlı etkilerini, düşük kobalt konsantrasyonlarının olumlu yüksek kobalt konsantrasyonlarının ise inhibe edici etkilere yol açabileceği hipotezi üzerinden araştırmaktır. Ayrıca kobalt uygulamalarının olumlu ve olumsuz etkilerinin fotosentetik elektron taşınım sisteminin hangi bileşenleri üzerinde ortaya çıktığı da araştırılmıştır.

Materyal ve Metod

Araştırmada, bitki materyali olarak arpa (*Hordeum vulgare* L.) bitkisine ait Kalaycı-97 adlı tescilli ve sertifikalı genotip kullanılmıştır. Kalaycı-97 iki sıralı, başak ve dane rengi beyaz, orta erkenci, düşük sıcaklık ve yatmaya dayanıklı bir genotiptir. Tohumlar T.C. Tarım ve Orman Bakanlığı Ankara Tarla Bitkileri Merkez Araştırma Enstitüsü Müdürlüğü'nden temin edilmiştir.

Benzer morfolojiye ve yaklaşık aynı büyüklüğe sahip tohumlar seçilerek kabuk sterilizasyonu sağlamak amacıyla %5'lik sodyum hipoklorit

(NaOCl) çözeltisinde 15 dakika bekletilmiş ve daha sonra 3 kez distile su ile yıkanarak plastik çimlendirme kaplarında bidistile su (15 ml) ile ıslatılmış filtre kağıtları arasına yerleştirilmiştir. Petri kapları 24 °C sıcaklık ve %40-50 oransal neme sahip olan iklim dolabında karanlık ortamda çimlenmeye bırakılmıştır. Üç gün sonra uniform fideler perlit ve ½ oranında seyreltilmiş Hoagland besin çözeltisi içeren 250 ml hacme sahip kaplara transfer edilerek su kültürüne alınmış; 18/25 °C sıcaklık (gece/gündüz), 16/8 saat fotoperiyot (gündüz/gece), %50±5 oransal nem ve 200 $\mu\text{mol foton m}^{-2} \text{s}^{-1}$ ışık şiddetine sahip iklim dolabına yerleştirilmiştir. Yedi günlük olan bitkiler dört gruba ayrılmıştır. Birinci gruptaki kontrol bitkileri çalışmanın sonuna kadar ½ oranında seyreltilmiş Hoagland besin çözeltisi ile sulanmıştır. İkinci, üçüncü ve dördüncü gruptaki bitkilere besin çözeltisine karıştırılarak kök yoluyla sırasıyla 300, 600 ve 900 μM kobalt nitrat [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$] çözeltisi uygulanmıştır. Bitki büyütme kaplarındaki çözeltiler, pH ve elektriksel iletkenlik değişimlerini ve alg üremesini önlemek ve yeterli oksijenasyon sağlamak amacıyla iki gün arayla değiştirilmiş ve uygulamaların başlatılmasından beş gün sonra, kobalt elementinin fotosentetik aktivite üzerindeki kısa süreli etkilerini belirlemek amacıyla deneme sonlandırılarak klorofil *a* floresansı ölçümleri gerçekleştirilmiştir.

Klorofil a floresansı ölçümleri

Klorofil *a* floresansı ölçümleri bitkilerin yapraklarında “bitki verimlilik analizatörü” (HandyPEA florometresi, Hansatech Instruments Ltd., Pentney, King’s Lynn, Norfolk, England)

kullanılarak gerçekleştirilmiştir. Bu amaçla ölçümlerde kullanılacak yapraklar yaprak klipsleri kullanılarak 45-60 dakika boyunca oda sıcaklığında karanlık adaptasyonuna maruz bırakılmıştır. Daha sonra yaprak yüzeylerine 3,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ şiddetinde ışık uygulanmış ve elde edilen parametrelerin değerlendirilmesi PeaPlus ve Biolyzer adlı programlarla uygulanan JIP testi yardımıyla yapılmıştır (Strasser ve ark., 2004; Bussotti ve ark., 2007; Yusuf ve ark., 2010). Klorofil *a* floresansı ve JIP testi ile ilgili terminoloji çizelge 1’ de verilmiştir.

İstatistiksel analizler

Deneme tesadüf blokları deneme desenine göre birbirinden bağımsız üç tekrar olarak gerçekleştirilmiştir. Elde edilen verilerin aritmetik ortalama ve standart hataları hesaplanmış, daha sonra verilere SPSS 20.0 paket programı kullanılarak, istatistiki varyans analizi (ANOVA) uygulanmıştır. Her bir bağımsız değişken için uygulamaların kontrole göre neden olduğu farkın önem kontrolü Duncan testi ile %5 düzeyinde hesaplanmıştır.

Çizelge 1. Bazı klorofil a floresansı ve JIP testi parametrelerinin tanımları (Kumar ve ark., 2020)

Table 1. Definitions of some chlorophyll a fluorescence and JIP test parameters (Kumar et al., 2020)

Parametreler Parameters	Tanımı Definitions
D_{I_0}/RC	FSII reaksiyon merkezleri başına dissipasyon enerjisi akışı
E_{T_0}/RC	FSII reaksiyon mekezleri başına Q_A 'dan sonraki bölgede maksimum elektron taşınımı
TR_0/RC	FSII reaksiyon merkezleri başına yakalanan ve Q_A 'yı indirgeyen maksimum enerji akışı
ABS/RC	FSII reaksiyon merkezleri başına anten klorofilleri için ışık absorpsiyon akışı
RC/ABS	FSII anten klorofilleri başına aktif reaksiyon merkezlerinin yoğunluğu
Alan	Klorofil floresansı eğrisinin üst kısmında bulunan, F_0 ile F_m arasında kalan ve plastokinon (PQ) havuzunun boyutunu yansıtan alan
$\Delta V/\Delta t_0$	Kapalı (indirgenmiş) reaksiyon merkezlerinin birikim hızı
F_m	Tüm FSII reaksiyon merkezlerinin kapalı olduğu durumda maksimum klorofil floresansı yoğunluğu
F_0	Tüm FSII reaksiyon merkezlerinin açık (oksidlenmiş) olduğu durumda minimum klorofil floresansı yoğunluğu
F_v/F_m	FSII'nin maksimum kuantum etkinliği
F_v/F_0	FSII'nin donör bölgesinde bulunan suyu parçalayan sistemin etkinliği (Hill reaksiyonu etkinliği)
N	Floresans yoğunluğunun maksimum değere ulaşmasına kadar geçen süre içerisinde Q_A 'nın indirgenme sayısı
PI_{abs}	Performans indeksi
SFI_{abs}	FSII'nin yapısal ve fonksiyonel durumunun indikatörü
tF_m	F_m 'ye ulaşıncaya kadar geçen süre (ms)
S_M	Tüm reaksiyon merkezlerinin kapanması için gerekli enerji miktarı
Ψ_0	Yakalanan bir ekzitronun bir elektronu Q_A 'dan elektron taşınım sistemine hareket ettirme etkinliği
ϕE_0	Q_A 'dan PQ'ya doğru gerçekleşen elektron taşınımının kuantum verimi
ϕP_0	Primer fotokimyasal olayların maksimum kuantum verimi
ϕD_0	Termal dissipasyonun kuantum verimi
$\phi_0/(1-\phi_0)$	Primer fotokimyasal olaylar için konformasyon terimi
$\Psi_0/(1-\Psi_0)$	Termal (ışığa bağımlı olmayan) reaksiyonlar için konformasyon terimi

Araştırma Bulguları ve Tartışma

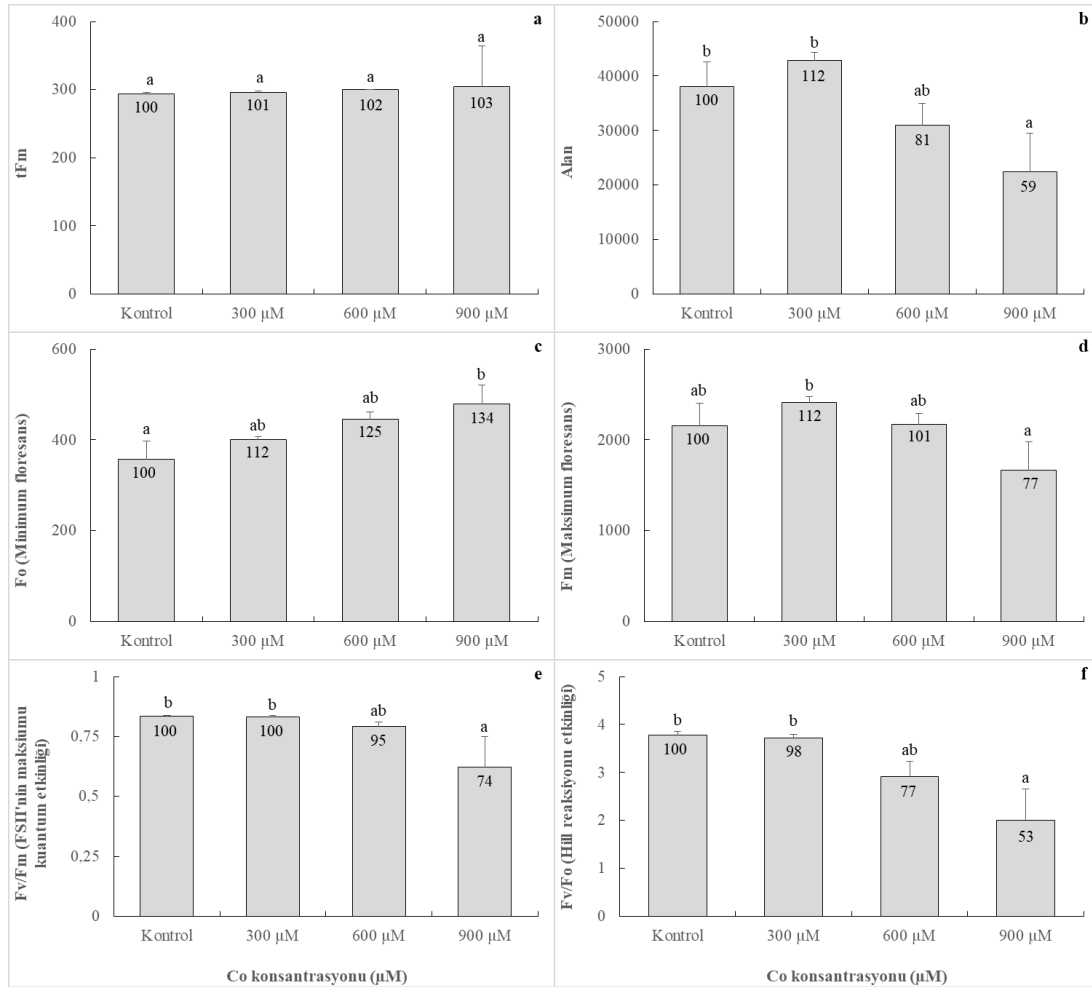
Bitkilerde fotosentetik aktivitenin değerlendirilmesi, çevresel stres faktörlerinin fiziksel varlığının ve yoğunluğunun uygulanabilir yöntemlerle izlenmesi için önemlidir. FSII' de gerçekleşen fotokimyasal reaksiyonlar hakkında önemli bilgiler sağlayan klorofil a floresansı tekniği, bitkilerin FSII' de absorblanan ışık enerjisini elektron taşınım reaksiyonları için ne kadar iyi kullandığının bir ölçüsüdür. Klorofil a floresansı ağır metal toksisitesi de dahil olmak üzere çevresel stres faktörlerinin fotosentetik ağıta verdiği hasarı ve stres altında FSII' nin durumunu ve işlevini analiz etmek için yaygın olarak kullanılan bir tekniktir (Stirbet ve ark., 2018).

Çalışmamızda 300 μM ve 600 μM kobalt $[Co(NO_3)_2 \cdot H_2O]$ uygulamalarının arpa bitkilerinde klorofil a floresansı ve JIP testi parametreleri üzerinde ilgili kontrollerle karşılaştırıldığında istatistiksel olarak anlamlı bir değişime yol

açmadığı belirlenmiştir ($P>0.05$). Ancak 900 μM kobalt uygulaması birçok klorofil a floresansı ve JIP testi parametresinde, arpa bitkilerindeki fotosentetik etkinliğin olumsuz yönde etkilendiğini gösterecek şekilde değişimlere yol açmıştır ($P<0.05$). FSII' nin maksimum kuantum etkinliğini gösteren F_v/F_m oranının uygun çevresel koşullar altında, bitki türü ve uygulanan stres tipinden bağımsız olmak üzere 0.83 civarında olduğu ve bu parametrenin bir stres indikatörü olarak kullanılabileceği bildirilmiştir (Doğru, 2021). Çalışmamızda 900 μM kobalt uygulaması sonucunda F_v/F_m oranının kontrole göre önemli derecede azalması, arpa bitkilerinde kobalt toksisitesi etkisiyle meydana gelen fotoinhibisyonu göstermektedir (Şekil 1e) (Meravi ve Prajapati, 2018). 900 μM kobalt uygulamasının ϕP_0 ve $\phi_0/(1-\phi_0)$ parametrelerinde kontrollerle karşılaştırıldığında neden olduğu azalma da FSII birimlerinde gerçekleşen elektron hareketlerinin ve primer fotokimyasal olayların belli oranda inhibe edildiğini kanıtlamaktadır (Şekil 3b, Şekil 6b).

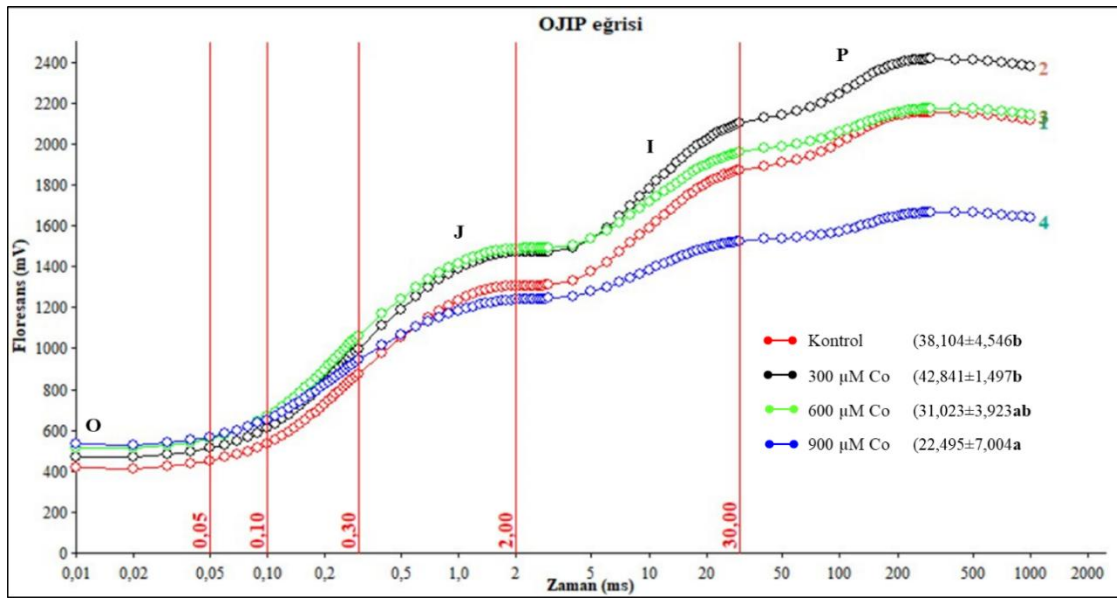
Moustaka ve ark. (2024) FSII' nin ve FSII yapısında bulunan oksijen evölüsyon kompleksinin (OEK) stres faktörlerine oldukça duyarlı olduğunu ve bitkilerde stres cevaplarının araştırılması için kullanılabileceğini bildirmiştir. Yapılan araştırmalar ağır metal toksisitesinin hem FSII aktivitesini ve elektron taşıma reaksiyonlarını hem de OEK' nın aktivitesini etkilediğini ve Fv/Fo oranını azalttığını göstermiştir (Azmat ve ark., 2022). Nitekim, çalışmada kullanılan arpa bitkilerinde Hill reaksiyonunun aktivitesini gösteren Fv/Fo oranı 900 µM kobalt uygulaması sonucunda kontrole göre belirgin derecede

azalmıştır (Şekil 1f). Bu sonuç kobalt toksisitesi etkisiyle arpa bitkilerinde OEK tarafından parçalanmış su molekülü ve açığa çıkarılan elektron miktarının azaldığını göstermektedir. Begovic ve ark. (2016) kobalt toksisitesi altındaki *Lemna minör* bitkilerinde OEK' nın parçalanarak aktivitesini kaybettiğini ortaya çıkarmışlardır. Çeşitli bitki türleri ile yapılan çalışmalar, birçok metal iyonunun OEK' nın bir bileşeni olan protein kompleksinin yapısındaki mangan iyonu ile yer değiştirebileceğini ve böylece OEK aktivitesinin inhibe olduğunu göstermiştir (Küpper ve Andresen, 2016).



Şekil 1. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 µM, 600 µM ve 900 µM] konsantrasyonlarının arpa bitkisinde (a) tFm, (b) alan (OJIP eğrisinde Fo ve Fm arasında kalan bölge), (c) Fo, (d) Fm, (e) Fv/Fm ve (f) Fv/Fo üzerine etkisi (farklı harfler uygulamaların Duncan testine göre $P < 0.05$ seviyesinde farklı olduğunu, barların içindeki rakamlar kontrole göre % değişim değerlerini göstermektedir, kontrol=100.)

Figure 1. Effect of different cobalt concentrations on (a) tFm, (b) area (the area between Fo and Fm in the OJIP curve), (c) Fo, (d) Fm, (e) Fv/Fm, and (f) Fv/Fo in barley plants [Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers inside the columns indicate % change relative to control, control=100.]

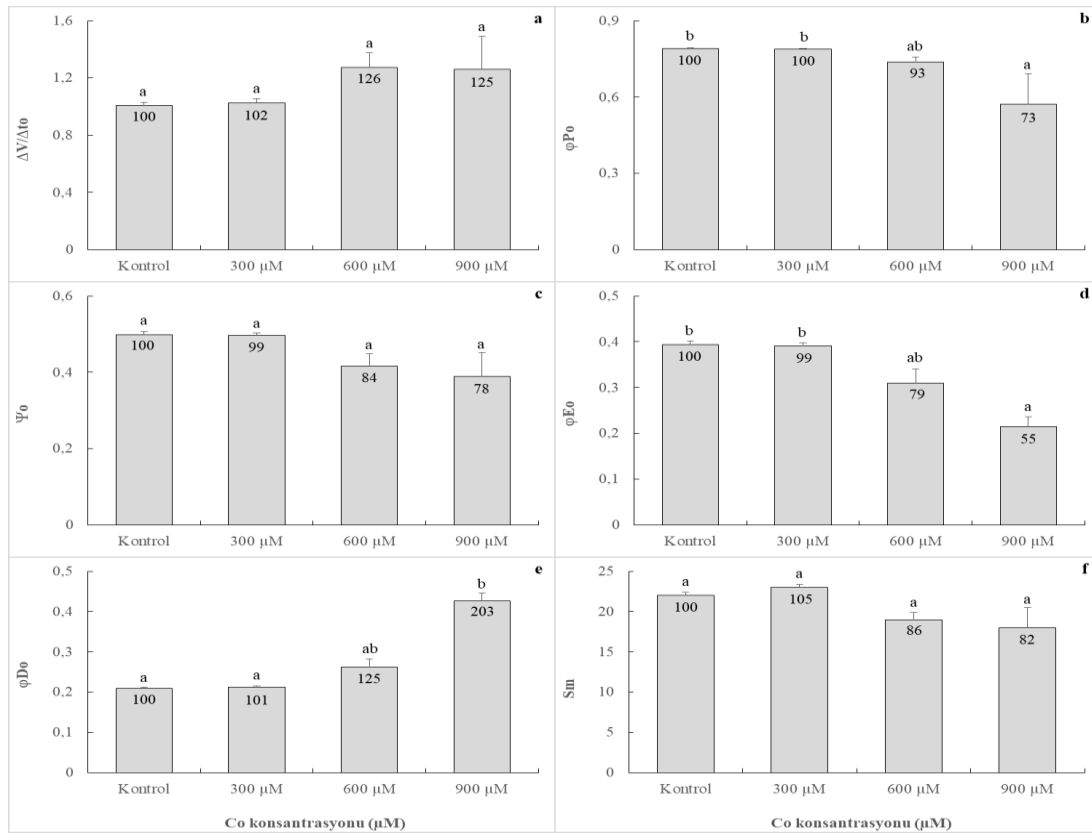


Şekil 2. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM] konsantrasyonlarının arpa bitkisinde klorofil a floresans indüksiyon (OJIP) eğrisi üzerine etkisi [Parantez içindeki rakamlar alan parametresinin (eğrideki minimum ve maksimum floresans arasındaki alan) ortalama $\pm$ standart hata değerlerini, farklı harfler Duncan testine göre uygulamalar arasındaki önemli farklılıkları ($P < 0.05$) ve sağ taraftaki koyu renkli rakamlar kontrole göre % değişimi göstermektedir, kontrol=100. "O", "J", "I" ve "P" noktaları sırasıyla 50 μs , 1 ms, 10 ms ve 100 ms zamanlarındaki floresans değerlerini ifade etmektedir.]

Figure 2. Effect of different cobalt concentrations on the chlorophyll a fluorescence induction curve (OJIP) in barley plants [Numbers in the parenthesis indicate mean values of the parameter area (area between F_o and F_m in the curve) $\pm$ standart error and different letters mean significant differences between the applications according to Duncan's multiple range test ($P < 0.05$), and bold numbers at the right side indicate % change relative to control, control=100. Points "O," "J," "I," and "P" represent the fluorescence values at 50 μs , 1 ms, 10 ms, and 100 ms, respectively.]

Tüm reaksiyon merkezlerinin indirgenmesi için gerekli enerji miktarını ifade eden S_m parametresi çalışmamızda kobalt uygulamaları sonucunda kontrole göre önemli bir değişim sergilememiştir (Şekil 3f). Bu sonuç F_v/F_o parametresinde gözlenen azalma ile birlikte değerlendirildiğinde, kobalt toksisitesinin arpa bitkilerinde sistemde taşınacak elektron miktarını azaltmasına rağmen reaksiyon merkezlerine uygun şekilde elektron hareketinin devam ettiğini göstermektedir. Toth (2023) stres koşulları altındaki bitkilerde OEK' da fonksiyon kaybının gözlemlendiği bazı durumlarda reaksiyon merkezlerine doğru elektron

hareketinin devam etmesini sağlayan askorbik asit, hidroksil amin, tetrametil fenilen diamin ve Mn iyonları gibi alternatif elektron donörlerinin bulunduğunu ortaya çıkarmıştır. Bu durumda kobalt toksisitesine maruz kalan arpa bitkilerinde fotokimyasal bir tolerans mekanizması olarak FSII reaksiyon merkezlerinin elektron gereksiniminin su dışındaki bazı elektron donörleri tarafından karşılanmış olması söz konusu olabilir. Nitekim çalışmamızda kobalt toksisitesi uygulanan arpa bitkilerinde N parametresinin kontrole göre değişim göstermemesi de bu fikri desteklemektedir (Şekil 4a).

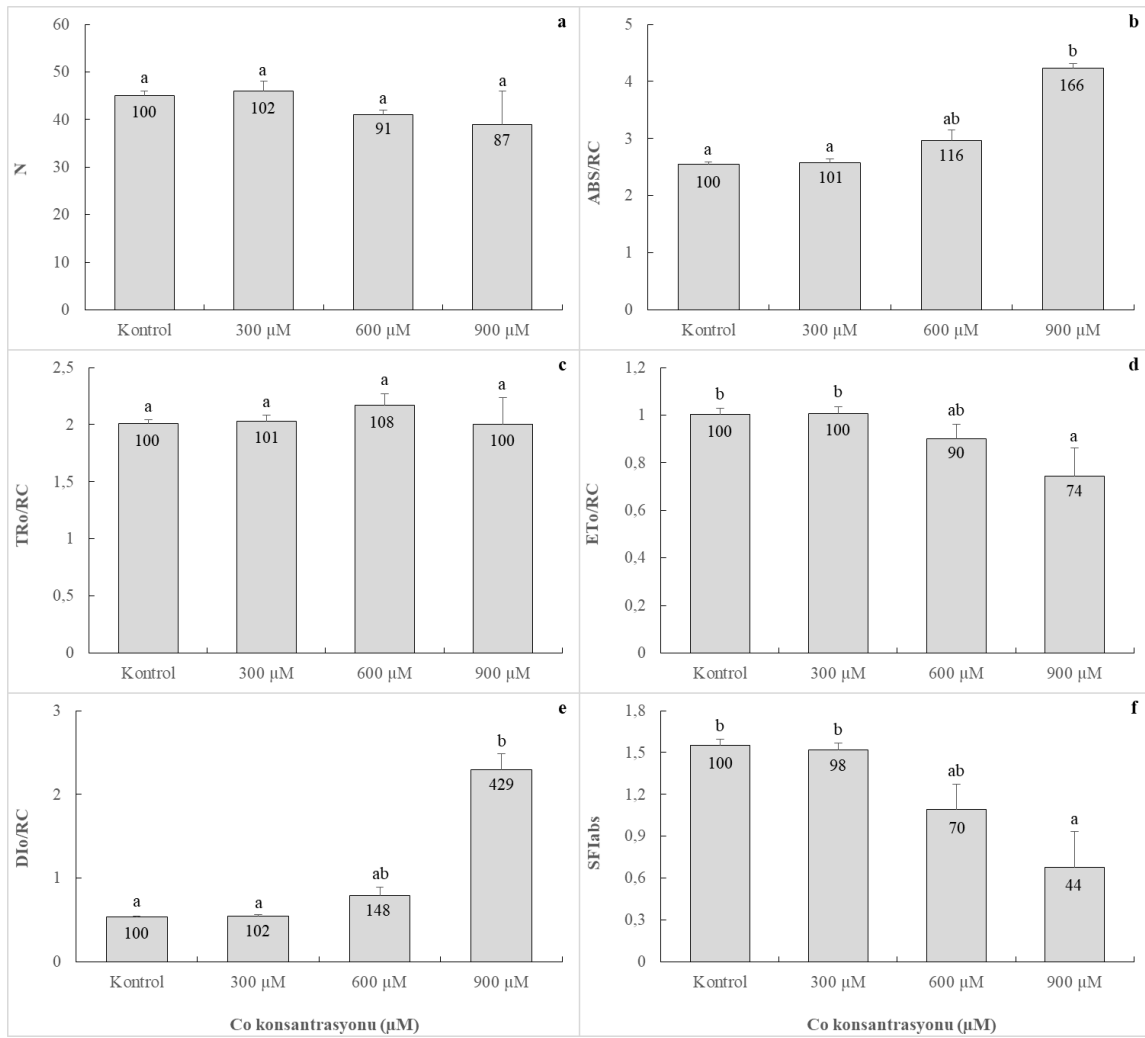


Şekil 3. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM] konsantrasyonlarının arpa bitkisinde (a) $\Delta V/\Delta t_0$, (b) ϕP_0 , (c) Ψ_0 , (d) ϕE_0 , (e) ϕD_0 ve (f) S_m üzerine etkisi (farklı harfler uygulamaların Duncan testine göre $P < 0.05$ seviyesinde farklı olduğunu, barların içindeki rakamlar kontrole göre % değişim değerlerini göstermektedir, kontrol=100.)

Figure 3. Effect of different cobalt concentrations on (a) $\Delta V/\Delta t_0$, (b) ϕP_0 , (c) Ψ_0 , (d) ϕE_0 , (e) ϕD_0 , and (f) S_m in barley plants [Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers inside the columns indicate % change relative to control, control=100.]

F_v/F_m [$F_v/F_m = (F_m - F_0)/F_m$] oranındaki değişimler F_0 ve F_m parametrelerindeki değişimlerle orantılıdır. Çeşitli stres faktörlerinin F_0 parametresindeki artıştan sorumlu olabileceği bildirilmiştir (Doğru, 2021). Örneğin Meravi ve

Prajapati (2018), inaktif durumdaki FSII reaksiyon merkezlerinin oranındaki artışın F_0 parametresinin artmasına yol açabileceğini rapor etmiştir.



Şekil 4. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM] konsantrasyonlarının arpa bitkisinde (a) N, (b) ABS/RC, (c) TRo/RC, (d) ETTo/RC, (e) Dlo/RC ve (f) SFlabs üzerine etkisi (farklı harfler uygulamaların Duncan testine göre $P < 0.05$ seviyesinde farklı olduğunu, barların içindeki rakamlar kontrole göre % değişim değerlerini göstermektedir, kontrol=100.)

Figure 4. Effect of different cobalt concentrations on (a) N, (b) ABS/RC, (c) TRo/RC, (d) ETTo/RC, (e) Dlo/RC, and (f) SFlabs in barley plants [Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers inside the columns indicate % change relative to control, control=100.]

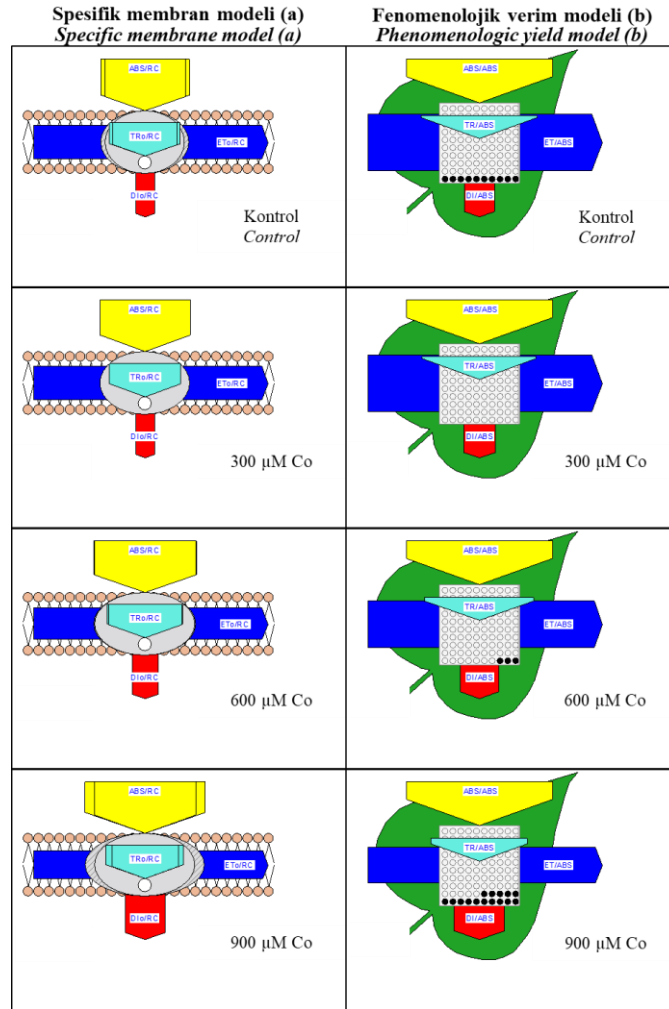
Doğru (2021) ise, kinon A (Q_A)' dan kinon B (Q_B)' ye doğru gerçekleşen elektron taşınım hızının yavaşlaması ve/veya FSII'nin ışık enerjisini yakalama etkinliğinin azalması sonucu F_o değerinin arttığını göstermiştir. Çalışmamızda 900 μM kobalt uygulaması sonucunda arpa yapraklarındaki F_o değerinin kontrole göre önemli derecede arttığı belirlenmiştir (Şekil 1c). Buna göre 900 μM kobalt uygulamasının arpa bitkisinde FSII reaksiyon merkezlerine zarar verdiği ve elektron taşınımının kuantum verimini azalttığı söylenebilir. Çalışmamızda 900 μM kobalt uygulaması sonucu arpa bitkisinin yapraklarında ϕE_o ve RC/ABS parametrelerinde gözlenen azalma ve ABS/RC parametresinde gözlenen artış da bu fikri desteklemektedir (Şekil 3d, Şekil 6a,

Şekil 4b ve Şekil 5b).

Bunun dışında FSII' nin yapısında bulunan kapalı (indirgenmiş) reaksiyon merkezlerinin birikim hızını ifade eden $\Delta V/\Delta t_o$ parametresi çalışmada uygulanan kobalt konsantrasyonlarından kontrole karşılaştırıldığında önemli derecede etkilenmemiştir (Şekil 3a). Bu durumda 900 μM kobalt uygulanan arpa bitkilerinde FSII reaksiyon merkezlerinin oksidasyon/redüksiyon döngüsünün belirli oranda devam ettiği sonucuna varılabilir. Ancak genel olarak değerlendirildiğinde, çalışmada kullanılan arpa bitkilerinde FSII' nin donör bölgesindeki elektron taşınım reaksiyonlarının 900 μM ' lık kobalt toksisitesinden olumsuz yönde etkilendiği

söylenbilir. Çalışmamızda kullanılan kobalt konsantrasyonları arpa bitkilerinde Fm parametresini kontrol bitkileriyle karşılaştırıldığında istatistiksel olarak etkilememiştir (Şekil 1d). Stres koşulları altındaki bitkilerde Fm parametresinde gözlenen azalmanın FSII' nin akseptör bölgesindeki elektron

taşınımının belli derecede inhibe edilmesinden kaynaklandığı bildirilmiştir (Doğru ve Çakırlar, 2020b). Buna göre çalışmada kullanılan arpa bitkilerinin FSII birimlerinin akseptör bölgelerinin yapısal ve fonksiyonel olarak kobalt toksisitesine belli oranda toleranslı olduğu sonucu çıkarılabilir.



Şekil 5. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM] konsantrasyonlarının arpa bitkisinde bazı JIP testi parametreleri üzerine etkisinin (a) spesifik membran modeli ve (b) fenomenolojik verim modeli ile gösterimi [(b)' de boş daireler hasar görmemiş, koyu renkli daireler ise hasar görmüş reaksiyon merkezlerini göstermektedir.]

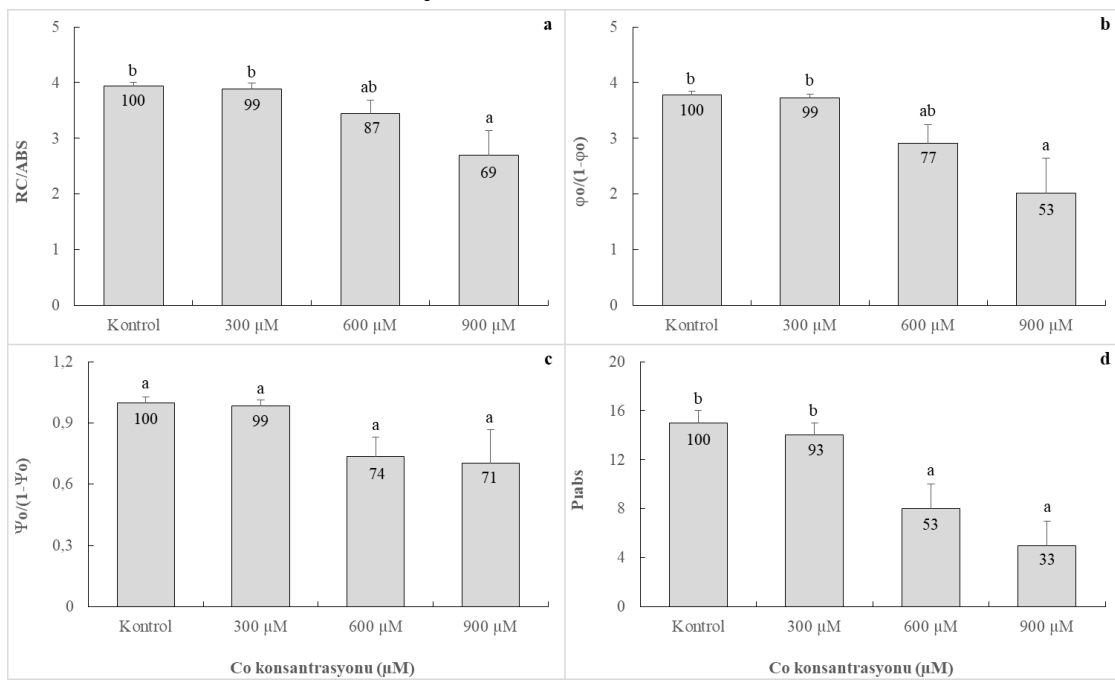
Figure 5. Presentation of some JIP test parameters by (a) specific membrane model and (b) phenomenological yield model in barley plants under cobalt toxicity [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM]. [In (b), empty circles indicate the intact reaction centers, while dark circles indicate the damaged reaction centers.]

Çalışmamızda 900 μM kobalt uygulaması arpa bitkilerinde alan parametresinin kontrole göre önemli derecede azalmasına yol açmış, tFm parametresini ise etkilememiştir (Şekil 1a ve b, Şekil 2). Buna göre 900 μM kobalt uygulanan arpa bitkilerinde OEK' dan PQ havuzuna doğru gerçekleşen elektron hareketinin yavaşladığı söylenebilir. Oukarroum ve ark. (2015) de *Lemna minör* bitkisinde yaptıkları çalışmada benzer

sonuçlar elde etmişlerdir. Çalışmamızda ilgili kontrol değerleri ile karşılaştırıldığında kobalt uygulamaları arpa bitkisinde TRo/RC parametresini etkilememiş (Şekil 4c ve Şekil 5a), 900 μM kobalt uygulaması ise ETo/RC parametresini azaltırken (Şekil 4d ve Şekil 5a), ϕDo ve D1o/RC parametrelerini artırmıştır (Şekil 3e, Şekil 4e ve Şekil 5a). Bu sonuçlar kobalt uygulamalarının arpa bitkisinde yakalanan ışık

enerjisi miktarını değiştirmedigini, elektron taşınım reaksiyonlarında kullanılan ışık enerjisi miktarını azaltıp ısı olarak ortama verilen ışık enerjisi miktarını artırdığını göstermektedir. Buna göre 900 μM kobalt uygulamasının arpa bitkisinde ışık enerjisinin fotokimyasal mekanizmalarda kullanım oranını azalttığı ve fotosentetik verimi azalttığı söylenebilir. Diğer yandan kobalt uygulamalarının arpa bitkisinde $\Psi_o/(1-\Psi_o)$ parametresi üzerinde istatistiksel bir değişime neden olmaması, ışığa bağımlı olmayan CO_2 fiksasyon reaksiyonlarının kobalt toksisitesinden etkilenmediğini göstermiştir (Şekil 6c). Plabs; RC/ABS, ϕPo ve Ψ_o parametreleri yardımıyla hesaplanan ve fotosentetik elektron taşınım

reaksiyonlarının genel fizyolojik durumunu ifade eden bir parametredir. Çalışmada sadece 600 μM ve 900 μM kobalt uygulamaları, Plabs parametresinin kontrole göre belirgin derecede azalmasına neden olmuştur (Şekil 6d). Bu sonuç, kobalt toksisitesi altında Plabs parametresinde gözlenen azalmanın öncelikli olarak primer fotokimyasal olaylardaki inhibisyon kaynaklandığını göstermektedir. SFlabs parametresinde meydana gelen değişimlerden 900 μM kobalt uygulamasının arpa bitkilerindeki FSII birimlerinde yapısal ve fonksiyonel deformasyonlara yol açtığı sonucuna varılabilir (Şekil 4f).



Şekil 6. Farklı kobalt [$\text{Co}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$; 300 μM , 600 μM ve 900 μM] konsantrasyonlarının arpa bitkisinde (a) RC/ABS, (b) $\phi\text{Po}/(1-\phi\text{Po})$, (c) $\Psi_o/(1-\Psi_o)$ ve (d) Plabs üzerine etkisi (farklı harfler uygulamaların Duncan testine göre $P < 0.05$ seviyesinde farklı olduğunu, barların içindeki rakamlar kontrole göre % değişim değerlerini göstermektedir, kontrol=100)

Figure 6. Effect of different cobalt concentrations on (a) RC/ABS, (b) $\phi\text{Po}/(1-\phi\text{Po})$, (c) $\Psi_o/(1-\Psi_o)$, and (d) Plabs in barley plants [Columns with different letters mean significant differences between the treatments according to Duncan's multiple range test ($P < 0.05$) and numbers inside the columns indicate % change relative to control, control=100.]

JIP testi, OJIP klorofil a floresans geçişlerinin zamana bağlı ve nicel olarak analiz edilmesini ve fotosentetik elektron taşınım reaksiyonlarının belirli zaman noktalarındaki değişikliklerin daha iyi anlaşılmasını sağlamaktadır. Çalışmamızda kontrol, 300 ve 600 μM kobalt uygulanan arpa bitkileri klorofil a floresan geçişleri (OJIP eğrisi) açısından tipik bir polifazik artış göstermiştir (Şekil 2). OJIP eğrisinde "O-J" fazı, fotosentetik elektron

taşınım reaksiyonlarında primer elektron akseptörü olan Q_A 'da meydana gelen elektron akışını; "J-I" fazı Hill reaksiyonunun etkinliğini; "I-P" fazı ise ferredoksinin indirgenme durumu ile FSI ve FSII miktarının birbirine oranını göstermektedir (Kumar ve ark., 2020; Lotfi ve ark., 2020). Kontrol bitkileri ile 300 μM kobalt uygulanan arpa bitkilerinde OJIP eğrisi ve farklı fazların morfolojileri çok büyük benzerlik

göstermekte ancak sadece sayısal olarak ve istatistiksel anlamda önemli olmayan bir farklılık sergilemektedir (Şekil 2). Ancak 600 μ M kobalt uygulanan arpa bitkilerinde sadece “I-P” fazı kontrolle karşılaştırıldığında morfolojik olarak değişmiş, “I” ve “P” pikleri zayıflamıştır. Bu sonuç, istatistiksel bir fark gözlenmesi de, 600 μ M kobalt uygulaması sonucunda arpa bitkilerindeki Hill reaksiyonu etkinliğinin azalmaya başladığını göstermektedir. 900 μ M kobalt uygulaması ise arpa bitkilerinde OJIP eğrisinin “J-I” ve “I-P” fazlarının morfolojik olarak kontrol bitkilerine göre değişmesine, özellikle “I” ve “P” piklerinin zayıflamasına neden olmuştur (Şekil 2). Bu sonuçlar 900 μ M kobalt uygulamasının arpa bitkilerinde Hill reaksiyonu etkinliğinin azalmasına ve buna bağlı olarak elektron taşınımının FSII’ den sonraki basamaklarını ve FSI yapısındaki ferredoksinin indirgenme oranını olumsuz yönde etkilediğini göstermektedir.

Sonuçlar

Sonuç olarak, çalışmamızda kullandığımız 300 ve 600 μ M kobalt konsantrasyonlarının arpa bitkilerinde genel olarak fotosentetik aktivite üzerinde olumsuz bir etki yapmadığı, gözlenmiştir. Ancak 900 μ M kobalt uygulaması arpa bitkilerinde primer fotokimyasal olayları, Hill reaksiyonunun etkinliğini ve Q_A ’ya doğru ve Q_A ’dan sonraki elektron taşınım reaksiyonlarını belli oranda inhibe etmiştir. Ayrıca reaksiyon merkezlerinde yapısal hasar yol açarak ve absorblanan ışık enerjisinin elektron taşınım reaksiyonları yerine fotokimyasal olmayan mekanizmalarla tüketilmesine neden olarak fotosentetik aktiviteyi olumsuz yönde etkilediği belirlenmiştir. Ancak kobalt toksisitesi altında arpa bitkilerinde fotokimyasal olmayan mekanizmaların aktifleşmesi savunma mekanizmasının bir bileşeni de olabilir. Bu noktanın aydınlatılması için farklı fizyolojik ve biyokimyasal analizlerin yapılması gerekmektedir. Son olarak, çalışmada kullanılan arpa bitkileri için kobalt toksisitesi eşik değerinin 600-900 μ M arasında bir konsantrasyona karşılık geldiği söylenebilir. Bu değer Poacea familyası

üyeleri olan diğer tahıl türleri için de yaklaşık olarak bir referans değeri olarak kullanılabileceğini düşünmekteyiz. Ancak bu önermenin yapılacak çeşitli fizyolojik ve biyokimyasal analiz ve ölçümlerle test edilmesi gereklidir.

Çıkar çatışması

Makale yazarları arasında herhangi bir çıkar çatışması bulunmamaktadır.

Yazar katkısı

Makale yazarları çalışmanın tüm aşamalarında eşit oranda katkı sağlamıştır.

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Effects of compost application on sunflower (*Helianthus annuus* L.) yield and some soil enzyme activities in heavy metal-contaminated soils

Ağır metal içeren topraklarda kompost kullanımının ayçiçeği (*Helianthus annuus* L.) verimi ve bazı toprak enzim aktivitesine etkisi

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ABSTRACT

Heavy metal accumulation in soils causes environmental pollution, negatively affecting soil microbiota and limiting plant growth and development. This study investigated the effects of different compost doses (0, 1.5%, 3%, and 4.5%) on sunflower (*Helianthus annuus* L.) growth and soil enzyme activities under high heavy metal content soil conditions. The experiment was conducted with three replications, and morphological plant characteristics (plant height, number of nodes, root length, fresh and dry weights), and soil enzyme activities (catalase, urease, dehydrogenase), were analyzed. The results showed that compost applications significantly affected plant growth and soil enzyme activities. The 1.5% compost dose yielded the best results for plant height, root length, and fresh and dry weights, whereas root length, number of nodes, root weights, and catalase and dehydrogenase enzyme activities reached their highest levels at the 4.5% compost dose. These findings suggest that compost can enhance plant growth and improve soil biological activity in heavy metal-contaminated soils. Specifically, the 1.5% compost dose promoted plant growth, while the 4.5% dose increased soil enzyme activities.

Keywords: Compost, Sunflower, Heavy metal, Soil enzyme activity, Plant growth

Öz

Ağır metal birikimi topraklarda çevresel kirliliğe yol açmakta, toprak mikrobiyotasını olumsuz etkilemekte, bitki büyümesini ve gelişimini sınırlamaktadır. Bu çalışmada, yüksek ağır metal içeriğine sahip toprak koşullarında ayçiçeği (*Helianthus annuus* L.) büyümesi ve toprak enzim aktiviteleri üzerine farklı kompost dozlarının (0, %1.5, %3 ve %4.5) etkileri incelenmiştir. Deneme, üç tekerrürlü olarak gerçekleştirilmiş ve morfolojik bitki özellikleri (bitki boyu, boğum sayısı, kök uzunluğu, yaş ve kuru ağırlıklar) ile toprak enzim aktiviteleri (katalaz, üreaz, dehidrogenaz) analiz edilmiştir. Sonuçlar, kompost uygulamalarının hem bitki büyümesini hem de toprak enzim aktivitelerini önemli ölçüde etkilediğini göstermiştir. %1.5 kompost dozu, bitki boyu, kök uzunluğu ve yaş ile kuru ağırlıklar için en iyi sonuçları verirken, kök uzunluğu, boğum sayısı, kök ağırlıkları ve katalaz ile dehidrogenaz enzim aktiviteleri %4.5 kompost dozunda en yüksek seviyelere ulaşmıştır. Bu bulgular, kompostun ağır metal ile kirlenmiş topraklarda bitki büyümesini artırabileceğini ve toprak biyolojik aktivitelerini iyileştirebileceğini göstermektedir. Özellikle %1.5 kompost dozu bitki büyümesini teşvik ederken, %4.5 dozunun toprak enzim aktivitelerini artırdığı görülmüştür.

Anahtar Kelimeler: Kompost, Ayçiçeği, Ağır metal, Toprak enzim aktivitesi, Bitki büyümesi

Introduction

Phosphate mining worldwide leads to the formation of hazardous waste types such as low-grade phosphorites, acid mine drainage, and phosphogypsum. These wastes are among the most environmentally harmful materials due to their content of heavy metals, including cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), nickel (Ni), uranium (U), thorium (Th), and radium-226 (^{226}Ra) (Tayibi et al., 2009). These toxic elements accumulate in the soil and, under intense chemical weathering, leach into surrounding water bodies, causing toxicity in plants, animals, and humans (Reta et al., 2018; Afshan et al., 2019; Khelifi et al., 2020). Contaminated irrigation water further exacerbates this issue, as heavy metals like Cd and Pb directly inhibit seed germination and early seedling growth in crops (Uslu et al., 2025). The primary source of toxic metals in agricultural soils is phosphate fertilizers. The type and concentration of heavy metals in phosphate fertilizers depend on the metal content of the raw phosphate rock used (Tayibi et al., 2009). In nature, phosphate rock is predominantly found in the form of the apatite mineral, which is classified into different types based on its anion and cation composition, including carbonate apatite, fluorapatite, chlorapatite, hydroxyapatite, and sulfate apatite. Among these, fluorapatite is the most commonly used type in phosphate fertilizer production (Sezen, 1995).

In this regard, sunflower (*Helianthus annuus* L.), a widely cultivated crop in agricultural production, is a crucial plant for investigating the effects of phosphate fertilizer applications. Due to its broad phenotypic adaptability, sunflower is extensively grown in several countries, including Russia, Ukraine, the United States, Argentina, and China (Kostenkova et al., 2019). Its seeds are particularly valued for their high nutritional content, especially in terms of oil composition, and they also exhibit medicinal properties (Adeleke and Babalola, 2020). Previous studies show that Phosphorus (P) fertilization of

sunflowers accelerates plant growth and increases sunflower yield (Abbadi and Gerendás, 2011; Sadozai et al., 2013). Phosphorus is particularly essential for the plant until the seed-filling stage, with remobilization rates from leaves and stems to maturing achenes ranging between 30% and 60%. Notably, phosphorus deficiency at the onset of the vegetative growth stage leads to adverse effects such as growth retardation, delayed flowering, poor achene filling, and reduced oil content (Mello Prado and Moreira Leal, 2006).

However, considering the environmental impacts of heavy metals contained in phosphate fertilizers, the use of organic materials as an alternative to mineral fertilizers is becoming increasingly important for enhancing soil fertility in sunflower cultivation. In addition to improving crop productivity, organic-based fertilizers play a crucial role in sustainable agriculture by enhancing soil health and mitigating environmental effects (Ramazanoglu, 2024). In this context, compost is recognized as a significant nutrient source for various crops. As an organic amendment, compost is a valuable resource that improves soil quality and enhances agricultural production (Tian et al., 2016).

This study aims to investigate the effects of compost application on the growth and development of sunflower plants cultivated in soils obtained from phosphate mining sites, as well as its impact on selected soil enzyme activities.

Materials and Methods

Experimental site and design

This research was carried out in a greenhouse environment within the research and application area of the Field Crops Department at Harran University's Faculty of Agriculture. The experimental setup was initiated on July 15, 2024, and continued until the plants reached the flowering stage, at which point they were harvested. The study followed a randomized complete block design with three replications,

utilizing plastic pots for the cultivation process.

Soil collection and characteristics

The soil used in the experiment was collected from an agricultural field located within the Mazıdağı phosphate mining site in Karataş village, Mazıdağı district, Mardin Province (37°29'49.02"

N, 40°20'35.70" E) (Figure 1). Due to limited irrigation in the region, this land is primarily used for wheat cultivation during the winter months. The chemical properties of the experimental soil were analyzed, and the results are presented in Table 1.

Table 1. Some chemical analysis results of the study soil.

pH (1:2.5 v/w)	EC (1:5 v/w) (dS m ⁻¹)	Lime (%)	Organic Matter (%)
7.68	0.15	13.44	0.72

The soil pH was determined as 7.68, indicating a slightly alkaline reaction. The electrical conductivity (EC) was measured as 0.15 dS m⁻¹, showing that the soil had no salinity issues. The lime content was determined as 13.44%, classifying the soil as calcareous. The organic matter content was measured as 0.72%, indicating a low organic matter level. The total

heavy metal concentrations in the experimental soil were analyzed, and the results are presented in Table 2. When compared to the limit values set by the World Health Organization (WHO), it was observed that the concentrations of Cd (6.47 mg kg⁻¹), Cr (113.34 mg kg⁻¹), and Ni (100.17 mg kg⁻¹) exceeded the WHO threshold limits.

Table 2. Total heavy metal concentration of the experimental soil (mg kg⁻¹).

	Cd	Cr	Ni	Cu	Pb
Soil	6.47	113.34	100.17	37.45	9.03
WHO limit Value	3	100	50	100	100

WHO: World Healty Orrganizasyon.

In particular, the cadmium (Cd) concentration exceeded the WHO limit of 3 mg kg⁻¹ by more than twofold, while chromium (Cr) and nickel (Ni) concentrations were also found to be above the WHO limits of 100 mg kg⁻¹ and 50 mg kg⁻¹, respectively. On the other hand, the

concentrations of copper (Cu) and lead (Pb) remained below the WHO limit of 100 mg kg⁻¹. These findings indicate that the experimental soil has particularly high accumulations of cadmium, chromium, and nickel.

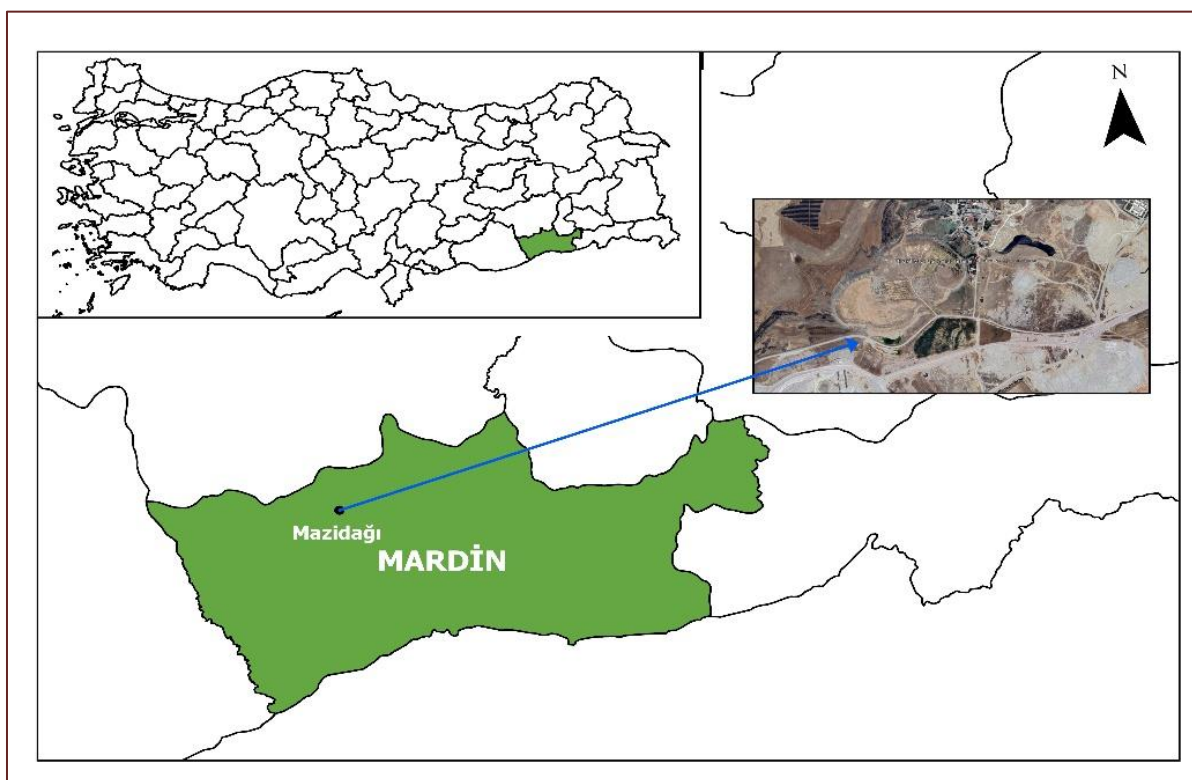


Figure 1. Sampling location of the soil used in the experiment.

Plant material and fertilization

The Suomi sunflower variety was used as the plant material. Fertilization was performed at the time of sowing with 10 kg P da⁻¹ (18% N – 46% P DAP), while 15 kg N da⁻¹ (46% N Urea) was applied as a topdressing fertilizer.

Experimental setup

The collected soil was placed into 5 kg capacity plastic pots, and compost was applied at rates of 1.5%, 3%, and 4.5%, except for the control treatment, at the time of sowing. Five seeds were sown in each pot, and after emergence, thinning was performed to leave one plant per pot.

Measured parameters

At the flowering stage, the experiment was terminated, and various plant growth parameters were analyzed. These parameters included plant height (cm), number of nodes, root length (cm), fresh biomass of the plant and root (g), and dry biomass of the plant and root (g). Additionally, soil enzyme activities, including catalase, urease, and dehydrogenase, were measured to assess the effects of compost application on soil biological activity.

Soil analysis

The soil reaction (pH) was analyzed using a 1:2.5 soil-to-water suspension, whereas the electrical conductivity (EC) was assessed in a 1:5 soil-to-water mixture. The pH measurement was performed using a pH meter after the suspension was shaken for a specific period and allowed to settle (Jackson, 1958). EC measurement was conducted using a conductivity meter (EC meter) after filtering the suspension. Calcium carbonate (CaCO₃) content was determined using the Scheibler calcimeter method, in which the soil sample reacts with hydrochloric acid (HCl), and the amount of carbon dioxide (CO₂) gas released is measured to calculate the lime content in the soil (Allison and Moodie, 1965). Organic matter content was determined using the wet combustion method. In this method, soil organic matter is oxidized using a mixture of potassium dichromate (K₂Cr₂O₇) and sulfuric acid (H₂SO₄). The resulting color change in the solution was analyzed by titration to calculate the organic matter content (Nelson and Sommer, 1982).

Enzyme analysis

Soil enzyme activity assessments were

performed to evaluate dehydrogenase, urease, and catalase activities. Dehydrogenase activity was quantified using a spectrophotometric method, measuring triphenyl formazan (TPF) production following a 24-hour incubation at 25°C, with absorbance recorded at 485 nm (Tabatabai, 1982). Urease activity was determined based on the spectrophotometric detection of ammonium ions (NH_4^+) released as a result of urea hydrolysis, following a one-hour incubation at 37°C (Tabatabai and Bremner, 1972). Catalase activity was analyzed by measuring the oxygen released from the reaction of soil samples with hydrogen peroxide (H_2O_2) using spectrophotometric methods. These enzyme activities were assessed to evaluate soil microbial activity and biochemical processes (Beck, 1971).

Statistical analysis

The experimental parameters were reported as the arithmetic mean of three replicates. To identify statistically significant differences among

the three treatments, ANOVA tests were applied at a significance level of $P < 0.05$. All statistical evaluations were carried out using the SPSS software package (version 20).

Results and Discussion

Plant height (cm)

In the study, statistically significant differences were observed among treatments in terms of plant height ($P < 0.05$). The control group (57.0 cm) had the lowest plant height among all treatments (Figure 2). Among the compost treatments, the shortest plant height was recorded in the 4.5% compost application (76.33 cm), while the tallest plant height was observed in the 1.5% compost application (81.0 cm). Compared to the control group, the increase in plant height due to compost applications was 42.10% for the 1.5% compost treatment, 35.66% for the 3% compost treatment, and 33.91% for the 4.5% compost treatment (Figure 3).



Figure 2. Comparison of plant growth under different treatments after harvest.

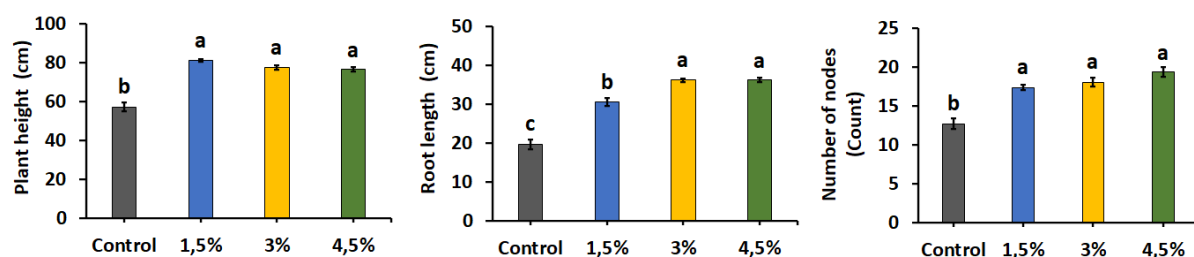


Figure 3. Effects of different compost application rates on plant height, root length, and number of nodes.

Root length (cm)

Statistical analyses revealed significant differences among compost treatments in terms of root length ($P < 0.05$). The control group (19.66 cm) had shorter root lengths compared to the compost-treated groups. Among the compost applications, the shortest root length was recorded in the 1.5% compost treatment (30.50 cm), while the longest root length was observed in the 4.5% compost treatment (36.16 cm). Compared to the control group, the increase in root length due to compost applications was 55.14% for the 1.5% compost treatment, 83.62% for the 3% compost treatment, and 83.93% for the 4.5% compost treatment (Figure 3).

Number of nodes (count)

Statistical analyses showed significant differences among compost treatments in terms of the number of nodes ($P < 0.05$). The control group (12.66 count) had the lowest number of nodes among all treatments. Among the compost applications, the lowest number of nodes was recorded in the 1.5% compost treatment (17.33 count), while the highest number of nodes was observed in the 4.5% compost treatment (19.33 count). Compared to the control group, the increase in the number of nodes due to compost applications was 36.89% for the 1.5% compost treatment, 42.18% for the 3% compost treatment, and 52.69% for the 4.5% compost treatment (Figure 3).

Plant fresh weight (g)

Statistical analyses revealed significant

differences among compost treatments in terms of plant fresh weight ($P < 0.05$). Sunflower plants grown in the control group (33.14 g) had a lower fresh weight compared to those in the compost-treated groups. Among the compost applications, the lowest plant fresh weight was recorded in the 4.5% compost treatment (41.34 g), while the highest plant fresh weight was observed in the 1.5% compost treatment (49.08 g) (Figure 4). Compared to the control group, the increase in plant fresh weight due to compost applications was 48.10% for the 1.5% compost treatment, 37.15% for the 3% compost treatment, and 24.74% for the 4.5% compost treatment (Figure 4).

Plant dry weight (g)

Statistical analyses revealed significant differences among compost treatments in terms of plant dry weight ($P < 0.05$). Sunflower plants grown in the control group (5.77 g) had a lower dry weight compared to those in the compost-treated groups. Among the compost applications, the lowest plant dry weight was recorded in the 4.5% compost treatment (7.75 g), while the highest dry weight was observed in the 1.5% compost treatment (8.86 g) (Figure 4). Compared to the control group, the increase in plant dry weight due to compost applications was 53.55% for the 1.5% compost treatment, 40.38% for the 3% compost treatment, and 34.32% for the 4.5% compost treatment. The highest increase in dry weight was observed in the 1.5% compost treatment, while the lowest increase was recorded in the 4.5% compost treatment (Figure 4).

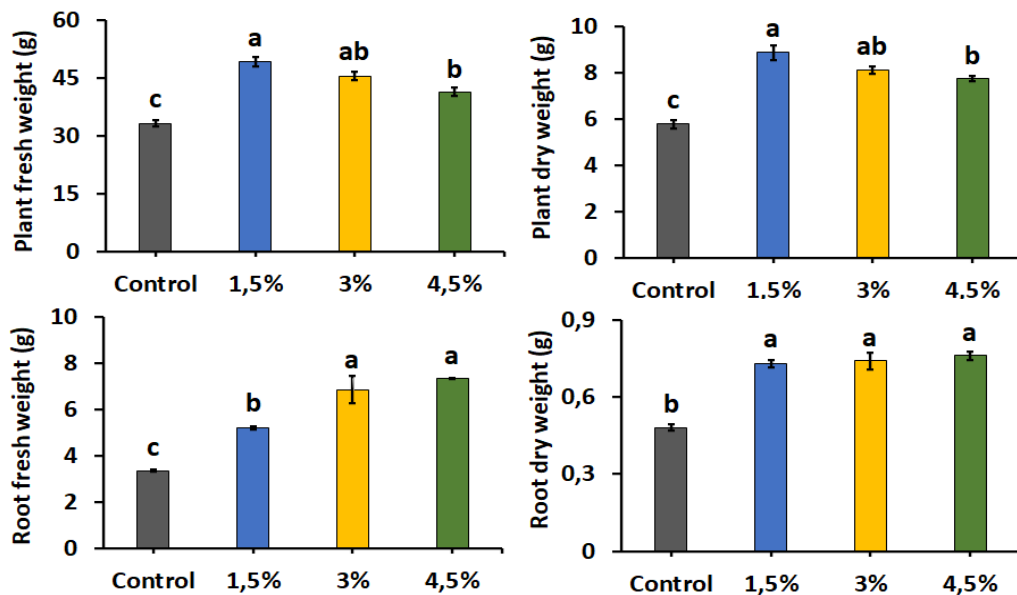


Figure 4. Effects of different compost application rates on plant fresh and dry weight, as well as root fresh and dry weight.

Root fresh weight (g)

Statistical analyses revealed significant differences among compost treatments in terms of root fresh weight ($P < 0.05$). The control group (3.33 g) had a lower root fresh weight compared to the compost-treated groups. Among the compost applications, the lowest root fresh weight was recorded in the 1.5% compost treatment (5.20 g), while the highest value was observed in the 4.5% compost treatment (7.33 g) (Figure 4). Compared to the control group, the increase in root fresh weight due to compost applications was 56.16% for the 1.5% compost treatment, 105.71% for the 3% compost treatment, and 120.12% for the 4.5% compost treatment (Figure 4).

Root dry weight (g)

Statistical analyses revealed significant differences among compost treatments in terms of root dry weight ($P < 0.05$). The control group (0.48 g) had the lowest root dry weight among all treatments. Among the compost applications, the lowest root dry weight was recorded in the 1.5% compost treatment (0.73 g), while the highest value was observed in the 4.5% compost treatment (0.76 g) (Figure 4). Compared to the control group, the increase in root dry weight due

to compost applications was 52.08% for the 1.5% compost treatment, 54.17% for the 3% compost treatment, and 58.33% for the 4.5% compost treatment (Figure 4).

Catalase enzyme activity

Statistical analyses revealed significant differences among compost treatments in terms of catalase (CAT) enzyme activity ($P < 0.05$). The control group ($143.33 \text{ ml O}_2 \text{ g}^{-1} \text{ dm } 5 \text{ min}^{-1}$) exhibited the lowest catalase enzyme activity among all treatments. Among the compost applications, the lowest catalase enzyme activity was recorded in the 1.5% compost treatment ($160.0 \text{ ml O}_2 \text{ g}^{-1} \text{ dm } 5 \text{ min}^{-1}$), while the highest value was observed in the 4.5% compost treatment ($163.33 \text{ ml O}_2 \text{ g}^{-1} \text{ dm } 5 \text{ min}^{-1}$) (Figure 5). Compared to the control group, the increase in catalase enzyme activity due to compost applications was 11.63% for the 1.5% compost treatment, 12.10% for the 3% compost treatment, and 13.95% for the 4.5% compost treatment. The highest increase in catalase enzyme activity was observed in the 4.5% compost treatment, while the lowest increase was recorded in the 1.5% compost treatment (Figure 5).

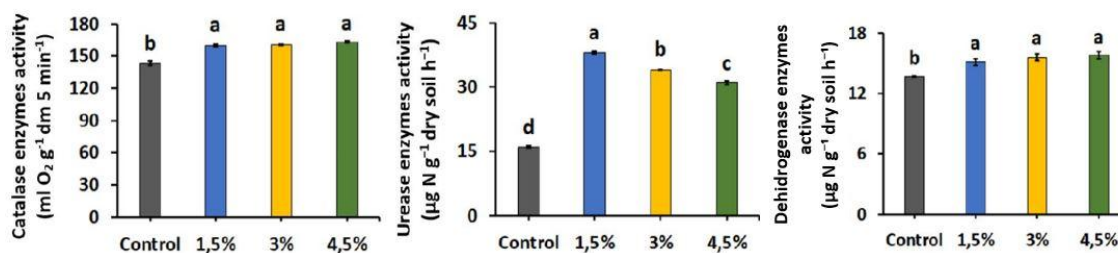


Figure 5. Effects of different compost application rates on soil catalase, urease, and dehydrogenase enzyme activities.

Urease enzyme activity

Statistical analyses revealed significant differences among compost treatments in terms of urease enzyme activity ($P < 0.05$). The control group ($16.03 \mu\text{g N g}^{-1} \text{ dry soil h}^{-1}$) exhibited the lowest urease enzyme activity compared to the compost-treated groups. Among the compost applications, the lowest urease enzyme activity was recorded in the 4.5% compost treatment ($31.06 \mu\text{g N g}^{-1} \text{ dry soil h}^{-1}$), while the highest value was observed in the 1.5% compost treatment ($38.08 \mu\text{g N g}^{-1} \text{ dry soil h}^{-1}$) (Figure 5). Compared to the control group, the increase in urease enzyme activity due to compost applications was 137.55% for the 1.5% compost treatment, 112.60% for the 3% compost treatment, and 93.76% for the 4.5% compost treatment. The highest increase in urease enzyme activity was observed in the 1.5% compost treatment, while the lowest increase was recorded in the 4.5% compost treatment (Figure 5).

Dehydrogenase enzyme activity

Statistical analysis demonstrated significant variations in dehydrogenase enzyme activity across the compost treatments ($P < 0.05$). The control group exhibited the lowest dehydrogenase activity ($13.68 \mu\text{g TPF g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$), whereas compost applications resulted in increased enzyme activity. Among the compost treatments, the lowest dehydrogenase activity was detected in the 1.5% compost application ($15.13 \mu\text{g TPF g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$), while the highest activity was measured in the 4.5% compost

treatment ($15.80 \mu\text{g TPF g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$) (Figure 5). When compared to the control, dehydrogenase activity increased by 10.60% in the 1.5% compost treatment, 13.89% in the 3% compost treatment, and 15.50% in the 4.5% compost treatment. The greatest enhancement in enzyme activity was observed in the 4.5% compost treatment, whereas the smallest increase was recorded in the 1.5% compost treatment (Figure 5).

Effects of application levels on parameters:

Heatmap

The heatmap, created to evaluate the effects of compost applications on plant growth parameters and soil enzyme activities, visualizes the impact of different compost doses on various growth and biochemical parameters (Figure 6). The heatmap includes plant height, root fresh and dry weight, plant fresh and dry weight, number of nodes, root length, as well as catalase, urease, and dehydrogenase enzyme activities. In the heatmap, the color scale represents values; blue tones indicate low levels, while green tones represent high levels. Accordingly, the variations in parameters under different compost ratios were assessed.

First, the control group had the lowest values in all parameters, which is represented by blue tones. This indicates that plant growth and soil biological activity were at lower levels in soils without compost application. In contrast, compost-treated groups exhibited higher values, particularly in plant growth and soil enzyme activities, supporting the idea that compost

enhances soil fertility and biological activity.

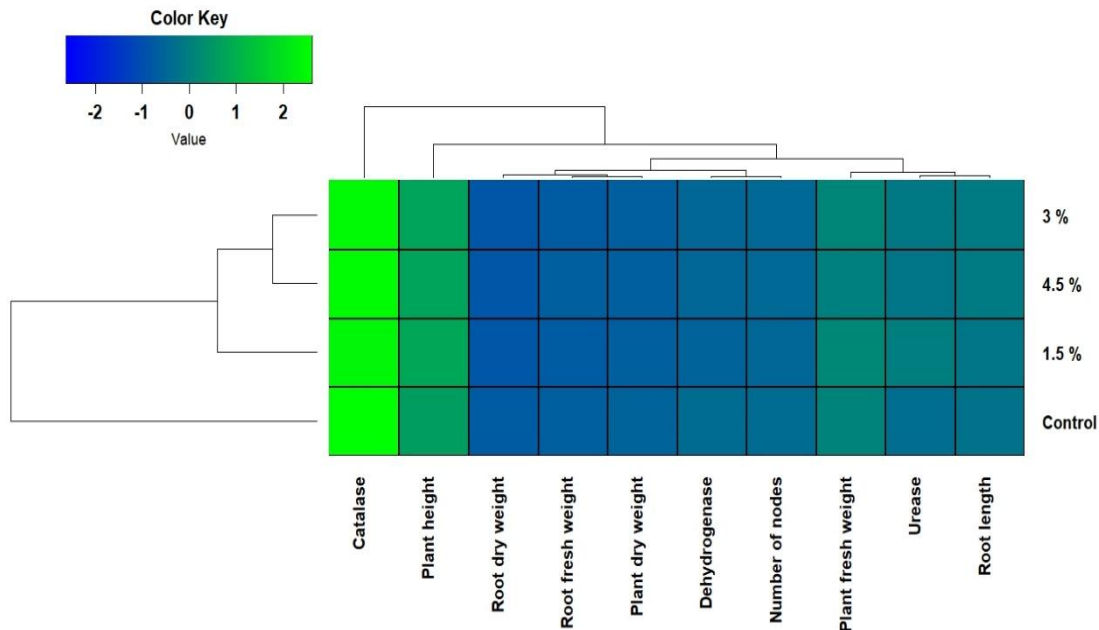


Figure 6. Heatmap showing the correlation coefficients between the analyzed parameters.

Especially, catalase enzyme activity was measured at higher levels compared to other parameters and was represented by green tones in the heatmap. This indicates that compost applications enhanced soil microbial activity and contributed to the reduction of oxidative stress in the soil (Tartoura et al., 2014). Comparisons among compost treatments revealed that growth parameters such as plant height, root length, and number of nodes increased with compost applications. However, the highest compost application rate (4.5%) did not always yield the best results. For instance, parameters such as plant fresh and dry weight, root fresh and dry weight showed higher values in the 1.5% and 3% compost treatments (Adugna 2016). This suggests that as the compost rate increases, some growth parameters may be suppressed, or the benefits of compost may diminish beyond a certain level.

From the perspective of soil enzyme activities, dehydrogenase and urease enzyme activities also increased with compost applications. Dehydrogenase enzyme serves as an indicator of microbial respiration and biological activity in the soil, and significant increases were recorded with compost treatments. Similarly, urease enzyme,

which plays a crucial role in the nitrogen cycle, demonstrated that compost applications accelerated soil biochemical processes. Overall, these findings indicate that compost applications promote plant growth, enhance soil enzyme activities, and improve soil biological productivity. However, an increase in compost quantity did not always result in a directly proportional improvement across all parameters, as lower compost rates (1.5% and 3%) produced better results for certain growth parameters.

Data clustering technique: Correlation graphs

A strong positive correlation was found between plant height and root length ($r = 0.84$; $p < 0.01$). This relationship indicates that the growth of stems and leaves during the plant's development process is directly linked to the elongation of the root system. This finding suggests that under conditions where the plant exhibits uniform growth, the root system also develops in parallel (Forde and Lorenzo, 2001; Gregory 2006). Consequently, an increase in plant height may serve as a significant determinant of root development.

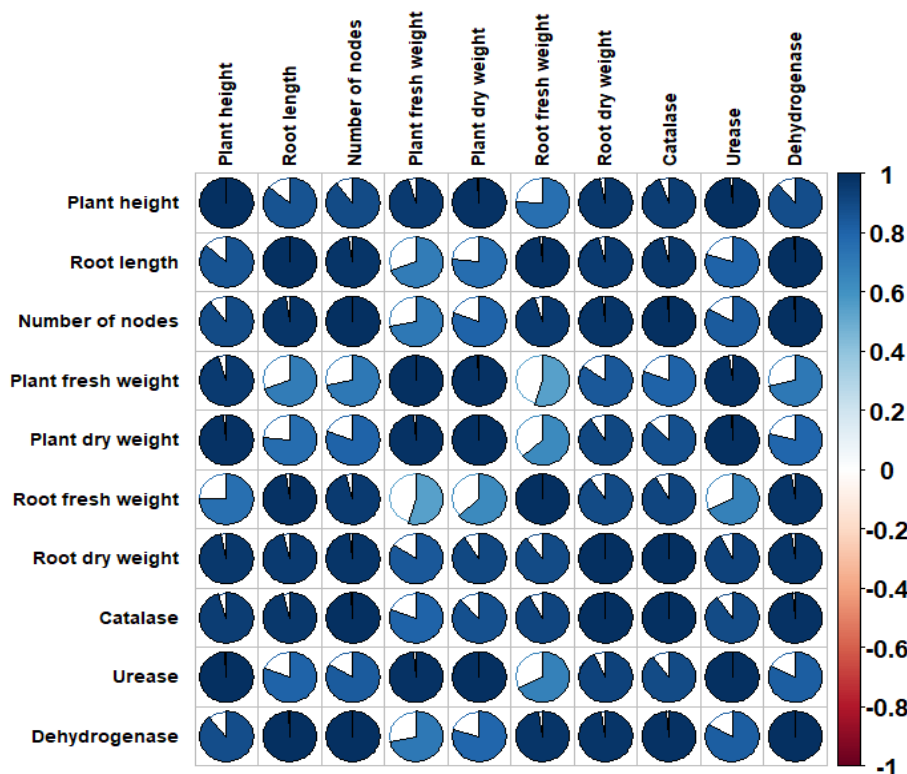


Figure 7. Correlation graph showing the relationships between the analyzed parameters. (Blue color represents positive correlations, while red and orange colors indicate negative correlations. The intensity of the colors represents the strength of the correlation, and the size of the circles indicates statistical significance.)

A strong positive correlation was found between plant fresh weight and dry weight ($r = 0.97$; $p < 0.01$) as well as between root fresh weight and dry weight ($r = 0.89$; $p < 0.01$). This result indicates that as plant and root fresh weights increase, dry matter content also increases accordingly. In other words, plants with higher fresh weights are expected to yield greater dry matter content after drying. In terms of root length, a significant correlation was observed with both root fresh and dry weights. As root length increased, root biomass also increased, suggesting that plants with more developed root systems could extend further into the soil and store more water and nutrients (Figure 7).

When examining soil biochemical properties, catalase, urease, and dehydrogenase enzyme activities were found to have strong correlations with plant growth parameters. In particular, catalase enzyme activity showed a strong correlation with plant height ($r = 0.92$; $p < 0.01$) and root length ($r = 0.94$; $p < 0.01$). Additionally, a positive correlation was identified between urease enzyme activity and plant height ($r = 0.97$;

$p < 0.01$). Regarding dehydrogenase enzyme activity, a significant correlation with root length ($r = 0.90$; $p < 0.01$) was observed. These findings suggest that increased soil enzyme activity is directly linked to enhanced plant height, root length, and overall biomass accumulation (Figure 7).

Mining activities have generated an estimated 5 to 14 million tons of mining waste in recent years (Schoenberger, 2016). These wastes are characterized by low organic matter content, insufficient nutrient levels, high metal concentrations, and acidic pH, which collectively exacerbate soil degradation. Consequently, these adverse conditions promote erosion and negatively impact vegetation and soil microbial activity (Lima et al., 2016). Given the increasing concerns regarding soil contamination and degradation, the application of organic amendments has gained prominence as an effective remediation strategy. In particular, compost has been recognized as a cost-effective and sustainable soil amendment. Numerous studies have demonstrated that compost

enhances soil quality by increasing organic matter content and improving plant growth conditions (Lu et al., 2014; Białobrzewski et al., 2015).

In this study, compost was applied to sunflower plants at rates of 1.5%, 3%, and 4.5%, and its effects on plant growth were evaluated. The findings indicate statistically significant differences ($p < 0.05$) among the treatments in terms of plant height, root length, fresh and dry biomass of both shoots and roots. Additionally, a significant variation in the number of nodes was observed ($p < 0.05$). The highest plant height was recorded at the 1.5% compost application rate, while the longest root length and highest number of nodes were observed at the 4.5% compost application rate. These results suggest that lower compost doses create more favorable conditions for overall plant growth, whereas higher compost doses promote root development and internode formation. However, excessive compost application may limit plant growth, potentially due to nutrient oversupply or the redistribution of resources toward root development rather than shoot elongation.

Compost applications are widely recognized for not only promoting plant growth but also contributing significantly to the immobilization of heavy metals. Owing to its rich organic matter content, compost interacts with heavy metals to form stable complexes, thereby lowering their bioavailability (Irfan et al., 2021). Consequently, the potential toxicity of heavy metals, which could otherwise impede plant development is effectively mitigated. Moreover, compost has been shown to elevate soil pH, leading to a reduction in heavy metal solubility and promoting their conversion into more stable, less mobile forms (Liang et al., 2017). This transformation significantly restricts heavy metal mobility, thereby limiting their absorption by plants (Beesley et al., 2010). Additionally, the combination of compost with biochar has been reported to enhance its metal-binding efficiency, resulting in greater stabilization and improved nutrient dynamics in the soil (Tang et al., 2014; Uslu et al., 2020).

The positive effects of compost on plant growth have been confirmed by numerous studies. Canellas and Olivares (2014) reported that organic materials, particularly compost, support biochemical, morphological, and physiological processes due to their rich nutrient content. It has been documented that sunflower growth significantly improves in soils rich in organic matter (Beesley et al., 2010). Similarly, Zaman et al. (2018) demonstrated that compost applications increase both fresh and dry biomass in sunflowers. Furthermore, various studies have verified that compost ensures a consistent supply of nutrients to plant tissues and contributes to a significant increase in biomass production (Abdella et al., 2018; Lwin et al., 2018; Nwamezie, 2018).

From a soil biochemical perspective, significant differences were observed among catalase, urease, and dehydrogenase enzyme activities ($p < 0.05$). The highest catalase and dehydrogenase enzyme activities were recorded at the 4.5% compost application rate, whereas the highest urease activity was observed at the 1.5% compost dose. It is well established that enzymatic activity in soil is directly related to the existing microbial population, the availability of nutrients, and root exudates (Caldwell, 2005; Moreno-Espíndola et al., 2018). Compost applications were found to enhance catalase and dehydrogenase enzyme activities; however, a distinct trend was noted for urease activity. Specifically, urease activity was higher at lower compost application rates, whereas increasing compost doses led to a reduction in its activity. This finding suggests that while higher compost doses promote certain enzymatic activities, they may suppress urease activity to a certain extent (García-Gil et al., 2000; Lakhdar et al., 2010).

In conclusion, compost applications have significant effects on sunflower growth, soil microbial activity, and heavy metal immobilization. The influence of different compost doses on plant growth and enzymatic activities varies. While lower compost doses are advantageous in terms of promoting plant height

and urease activity, higher doses support root development as well as catalase and dehydrogenase activities. Therefore, determining and applying appropriate compost doses in areas affected by mining activities is crucial, as compost serves as an effective soil amendment that enhances both plant growth and soil quality.

Conclusion

This study demonstrated that compost application positively affects sunflower growth and soil enzyme activities in heavy metal-contaminated soils. The 1.5% compost dose improved plant height and biomass, while the 4.5% dose enhanced root development and catalase and dehydrogenase enzyme activities. Compost also contributed to heavy metal immobilization by forming stable complexes with metals, thereby reducing their bioavailability and potential toxicity. However, excessive compost doses did not always yield greater benefits, indicating the need for optimized application rates. Overall, compost is an effective soil amendment for improving plant growth and soil health in contaminated soils. Future research should explore long-term applications to further understand its effects on soil quality and plant development.

Conflict of Interest

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

Author contributions

The authors declare that they contributed equally to the article.

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HARRAN TARIM ve GIDA BİLİMLERİ DERGİSİ

YAZIM KURALLARI

1. Makale, **Microsoft Word programında, Calibri** yazı karakterinde, **1.15 satır aralığında, 12 punto** düz metin ve tek sütun olarak yazılmalıdır.
2. Kenar boşlukları; **sol, sağ, alt ve üst- 3 cm** bırakılarak, her satıra ardışık olarak **satır numarası** verilerek hazırlanmalıdır.
3. Yazar(lar) makalenin ne türde bir yazı (**Araştırma makalesi, derleme, teknik not vb.**) olduğunu belirtmelidir.
4. **Türkçe başlık 14 punto (koyu ve ortalı)** küçük harflerle (Başlığın sadece ilk kelimesinin baş harfi büyük) ve düz yazılmalıdır. **İngilizce başlık 12 punto** ve ortalı yazılmalıdır.
5. Yazar isimleri **Adı SOYADI** kuralına göre Türkçe başlık sonrası **12 punto (koyu, ortalı ve düz)** ve bir boşluk bırakılarak yazılmalı, yazar isimlerinin sonuna adres için üst simge olarak rakam, sorumlu yazarı belirtmek için ise * simgesi verilmelidir. **Adres satırı** yazar isimleri sonrasında 1 boşluk bırakılarak **10 punto (normal, düz ve ortalı)** yazılmalıdır.
6. Adres satırından sonra 1 boşluk bırakılarak yazarların ORCID numaraları yazılmalıdır. ORCID satırının altına, sorumlu yazar e-posta adresi belirtilmelidir.
7. Metin genel olarak;
 - **Öz,**
 - **Abstract,**
 - **Giriş,**
 - **Materyal ve Metot,**
 - **Araştırma Bulguları ve Tartışma,**
 - **Sonuçlar,**
 - **Ekler**
 - **Kaynaklar** şeklinde olmalıdır.
8. Ana başlıkların yazımında koyu olarak kelimelerin sadece baş harfleri büyük yazılmalıdır. İkincil ve üçüncül başlıklarda sadece ilk kelimenin baş harfi büyük, diğer kelimeler küçük, koyu değil ve italik yazılmalıdır. Metin ana başlıkları, metin başlangıcı ve sonunda olmak üzere 1' er boşluk bırakılmalıdır. Alt başlıklardan önce 1 boşluk bırakılmalı, ancak, sonrasında boşluk bırakılmamalıdır. Tüm başlıklar girinti verilmeden sola yaslı olarak yazılmalıdır.
9. **Metin içerisinde kaynak gösterimi (Yazar, yıl)** esasına göre yapılmalıdır. Metin içerisinde iki yazarlı bir kaynağın gösteriminde, metin Türkçe ise **(ilk yazar soyadı ve ikinci yazar soyadı, yıl)** kuralı uygulanmalıdır. İkiiden fazla yazarın bulunduğu kaynakların gösteriminde **(ilk yazarın soyadı ve ark., yıl)** kuralı uygulanmalıdır.
Örneğin: (Mamay, 2020), (İkinci ve Bolat, 2018); (Söylemez ve ark., 2019),
10. Makale İngilizce olarak yazılacaksa **(ilk yazar and ikinci yazar, yıl)** ve **(ilk yazarın soyadı et al., yıl)** kuralı uygulanmalıdır.
Örneğin: (Söylemez, 2018), (Bolat and Mamay, 2015), (Mamay et al., 2010).
11. Metin içerisinde birden fazla kaynağa aynı anda atıf yapılacak ise; kaynaklar yayımlandıkları yıl dikkate alınarak kronolojik olarak sıralanmalıdır.
12. **ÖZ (ABSTRACT):** Başlık sola yaslı olmalı, 10 punto, koyu, paragraf başında girinti verilmemelidir. Türkçe ve İngilizce metin 300 kelimayı aşmayacak şekilde, 10 punto ve 1 satır aralığında yazılmalıdır. Öz ile Anahtar Kelimeler ve Abstract ile Key Words arasında tek

satır boşluk (10 punto, düz) bırakılarak metnin hemen altında en fazla 5 adet **Anahtar Kelimeler (Key Words)** yazılmalıdır. Key Words ile ana metin (Giriş) arasında iki satır boşluk bırakılmalıdır.

13. Makalelerde fotoğraf, grafik, çizim vb. “**Şekil**” olarak, Tablolar ise “**Çizelge**” olarak ifade edilmelidir.
14. Çizelge ve Şekiller ardışık olarak numaralandırılmalıdır (Şekil 1. veya Çizelge 1.). “Şekil” ve “Çizelge” içerikleri 1 satır aralıklı ve **10 punto** olarak hazırlanmalıdır.
15. Çizelge başlıkları çizelgenin üstünde, şekil başlıkları ise şekillerin altında ilk harf büyük olacak şekilde 1 satır aralıklı **10 punto** olarak yazılmalıdır.
16. **Türkçe yazılmış makalelerde Şekil ve Çizelge başlıklarının İngilizceleri, Türkçe başlığın hemen altında *italik* olarak yazılmalıdır.** (Makale İngilizce olarak yazılmışsa, Şekil ve Çizelge başlıklarının Türkçe karşılıkları yazılmayacaktır)

Şekil 1. Araştırma bahçesinde tespit edilen ortalama sıcaklık, ortalama nispi nem ve aylık yağış miktarı ortalaması değerleri (2007-2011 yılları ortalaması)

Figure 1. The average temperature, average relative humidity and average monthly rainfall data detected in the research garden (average of the years 2007-2011)

Çizelge 2. Şeftali çeşitlerinin 2007 - 2011 yılları arasındaki fenolojik gözlem sonuçları

Table 2. Phenological observation results of peach cultivars for between 2007 and 2011

Türkçe yazılmış makalelerde Çizelge ile Şekillerin içerisinde bulunan parametrelerin İngilizce karşılıkları bu parametrelerin hemen altına ***italik*** olarak yazılmalıdır. (Makale İngilizce olarak yazılmışsa, Şekil ve Çizelgelerin içerisinde belirtilen parametrelerin Türkçe karşılıkları yazılmayacaktır.)

Çizelge 3. Denemede yer alan şeftali çeşitlerinin bazı pomolojik özellikleri

Table 3. Some pomological properties of peach varieties

Çeşitler Varieties	Meyve ağırlığı(g) Fruit weight (g)	Meyve eni (mm) Fruit width (mm)	Meyve boyu(mm) Fruit length (mm)	Çekirdek ağırlığı (g) Kernel weight (g)
Cardinal	78.19 f	50.73 d	48.48 c	5.06 d
Cresthaven	129.58 b	61.69 bc	59.56 b	8.31 bc

17. Makale metni ve Çizelge-Şekil içerisinde bildirilen ondalık rakamlar, **nokta** ile ayrılmalıdır. (123.87; 0.987 vb.).

18. Çizelge-Şekillerden önce ve sonra **bir satır boşluk** bırakılmalıdır.

19. Makale yazımında “**Uluslararası Birim Sistemi**” (SI)’ye uyulmalıdır. Buna göre; g/l yerine **g l⁻¹**, mg/l yerine **mg l⁻¹** ya da **ppm** kullanılmalıdır. Yüzde ile belirtilen ifadeler açıklayıcı olmalıdır. Örneğin; %3 yerine **%3 (w/v)**, **%3 (v/v)**, **%3 (w/w)** şeklinde belirtilmelidir.

20. **Harran Tarım ve Gıda Bilimleri Dergisi Kaynaklar listesinin bildirişinde APA Formatını kullanmaktadır.** Buna göre **kaynaklar listesi** aşağıdaki kurallar çerçevesinde hazırlanmalıdır.

1. DERGİ YAYINLARINA ATIF VERME

1.1. Tek yazarlı makale

Mamay, M. (2015). Nar yaprakbiti [*Aphis punicae* Passerini (Hemiptera: Aphididae)]’nin Şanlıurfa ili nar bahçelerindeki bulaşıklık haritası. *Türkiye Entomoloji Bülteni*, 5(3), 159-166.

1.2. İki yazarlı makale

Soylomez, S., & Pakyurek, A. Y. (2017). Responses of rootstocks to nutrient induced high EC levels on yield and fruit quality of grafted tomato cultivars in greenhouse conditions. *Applied ecology and environmental research*, 15(3), 759-770. DOI: http://dx.doi.org/10.15666/aeer/1503_759770

1.3. İkidenden fazla yazarlı makale

Mamay, M., Ünlü, L., Yanık, E., Doğramacı, M., & İkinci, A. (2016). Efficacy of mating disruption technique against carob moth, *Apomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) in pomegranate orchards in Southeast Turkey (Şanlıurfa). *International Journal of Pest Management*, 62(4), 295-299.

İkinci, A., Mamay, M., Unlu, L., Bolat, I., & Ercisli, S. (2014). Determination of heat requirements and effective heat summations of some pomegranate cultivars grown in Southern Anatolia. *Erwerbs-Obstbau*, 56(4), 131-138. DOI: <https://doi.org/10.1007/s10341-014-0220-8>

2. KİTAPLARI KAYNAK GÖSTERME

2.1. Kaynak kitap ise,

Mohsenin, N. N. (1970). *Physical Properties of Plant and Animal Materials*. New York: Gordon and Breach Science Publishers.

2.2. Kaynak kitaptan bir bölüm ise,

Author, A. A. (Year). Chapter title. In E. E. Editor (Ed.), *Title of book: And subtitle* (pp. pages). Place: Publisher.

2.3. Editörlü kitap

Yeşilyaprak, B. (Ed.). (2003). *Gelişim ve öğrenme psikolojisi*. Ankara: Pegema Yayıncılık.

2.4. Yazarı bilinmeyen kaynakları veya internet kaynaklarını kaynak olarak gösterme;

Anonymous (2005). Tereyağı, diğer süt yağı esaslı sürülebilir ürünler ve sadeyağ tebliği. Türk Gıda Kodeksi, Tebliğ No: 2005/19, Ankara.

FAO, (2015). Statistical data of FAO. Retrieved from: <http://faostat.fao.org/site/567/default.asp>.

3. YÜKSEK LİSANS ve DOKTORA TEZLERİNE ATIF VERME

Doktora ya da yüksek lisans tezlerine elektronik veri tabanlarından, kurumsal arşivlerden ve kişisel web sayfalarından erişilebilir. Eğer bir teze ProQuest doktora ve yüksek lisans tezleri veri tabanından ya da diğer bir kaynaktan erişildiyse, atıfta bu bilgi verilmelidir. Bir veri tabanı servisinde mevcut olan bir doktora ya da yüksek lisans tezi için aşağıdaki kaynak gösterme biçimi kullanılır:

3.1. Yayınlanmamış tez

Mamay, M. (2013). *Determination of population development and infestation ratio of carob moth [Apomyelois ceratoniae Zell. (Lepidoptera:Pyralidae) in pomegranate orchards in Sanliurfa province and using mating disruption technique for its control* (Yayımlanmamış doktora tezi). Harran Üniversitesi Fen Bilimleri Enstitüsü, Şanlıurfa.

Söylemez, S. (2014). *Effects of nutrient induced salinity levels and rootstocks on plant growing, yield and some fruit quality features at soilless grown grafted tomatoes* (Yayımlanmamış doktora tezi). Harran Üniversitesi Fen Bilimleri Enstitüsü, Şanlıurfa.

3.2. Yayınlanmış tez

May, B. (2007). *A survey of radial velocities in the zodiacal dust cloud*. Bristol, UK: Canopus Publishing.

4. SEMPOZYUM VE TOPLANTI BİLDİRİLERİNE ATIF VERME

Mamay, M. (2017). Population density of overwintering larvae of Carob Moth [*Apomyelois* (= *Ectomyelois*) *ceratoniae* Zell. (Lepidoptera: Pyralidae)] in pomegranate orchards in Southeastern Anatolia. SEAB 2017. *Proceedings of the 3rd International Symposium on EuroAsian Biodiversity*, (pp. 235), 05-08 July 2017, Minsk, Belarus.

İkinci, A. & Mamay, M. (2017). Effects of fruit thinning on morphological, physico-chemical properties, bioactive compounds, antioxidant activity and pest & disease control in pomegranate fruit (*Punica granatum* L.) *International Conference on Agriculture, Forest, Food Sciences and Technologies*, (pp. 642), 15-17 May 2017, Cappadocia, Turkey.

Sönmez, C., Mamay, M. & Söylemez, S. (2019). Determination of the effect of different hydroponic culture and different NH₄:NO₃ ratio on the density of aphid [*Aphis* spp. (Hemiptera: Aphididae)] population in greenhouse lettuce. *1st International Gobeklitepe Agriculture Congress (IGAC-2019)*, (pp. 599-604), 25-27 November, Şanlıurfa, Turkey.

Not: Yukarıda yer alan kaynak gösterimlerde bulamadığınız farklı materyal veya konu başlıklarındaki kaynak bildirişleri için internetteki APA Kaynak Gösterimi ile ilgili web sayfalarından ya da aşağıdaki linkteki bilgilerden yararlanabilirsiniz.

https://libguides.library.usyd.edu.au/ld.php?content_id=47913440

Şencan, İ., ve Doğan, G. (2017). Bilimsel yayınlarda kaynak gösterme, tablo ve şekil oluşturma rehberi: APA 6 Kuralları. *Türk Kütüphaneciliği Dergisi*, Ankara.

https://www.tk.org.tr/APA/apa_2.pdf

HARRAN TARIM ve GIDA BİLİMLERİ DERGİSİ

YAZAR REHBERİ

1. Harran Tarım ve Gıda Bilimleri Dergisi'ne gönderilen makaleler Dergi Yayın Kurulu tarafından belirlenen yazım kurallarına göre yazılmalıdır.
2. Makaleler, **Dergipark Sistemi üzerinden online** olarak yüklenmelidir.
3. Tüm yazarlar tarafından imzalanan **Telif Hakkı Devir Sözleşmesi** ve **Makale Kontrol Listesi** (sorumlu yazar tarafından imzalanacak) makale ile birlikte sisteme yüklenmelidir.
4. **iThenticate Programı Benzerlik Raporu** (**%20**'yi geçmemelidir) ve gerekli ise **Etik Kurul Kararı** makale ile birlikte sisteme yüklenmelidir.
5. Hazırlanacak olan makale metni genel olarak;

- Öz,
- Abstract,
- Giriş,
- Materyal ve Metot,
- Araştırma Bulguları ve Tartışma,
- Sonuçlar,
- Ekler,
- Beyanlar
 - Çıkar Çatışması
 - Yazar Katkısı
- Kaynaklar bölümlerinden oluşmalıdır.

6. **Başlık:** Kısa ve açıklayıcı olmalı, **Calibri** yazı karakterinde, **14 punto, koyu**, düz, ortalanarak ve küçük harflerle (Başlığın sadece ilk kelimesinin baş harfi büyük) yazılmalıdır. Başlık tercihen 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 12 punto ve koyu yazılmalıdır.

7. Harran Tarım ve Gıda Bilimleri Dergisi'ne yayınlanması için makalenin ilk gönderiminde **yazar isimleri, kurum isimleri, adresleri, ORCID numaraları ve e-posta bilgileri yer almamalıdır.**

8. Makalenin hakem değerlendirmesi tamamlandıktan ve makale Yayın Kurulu tarafından kabul edildikten sonra, 7. maddede yer alan yazar isimleri ve diğer bilgiler, hakem önerilerine göre yeniden düzenlenmiş olan makale sayfası üzerine yazıldıktan sonra, Dergi web sayfasında yer alan düzenlenmiş makaleyi gönder sayfasından Dergi sistemine yüklenmelidir. **Kontrol edilmiş veya düzeltilmiş olan makale, yeni bir makale gibi Dergi web sayfasından yüklenmemelidir.**

9. Yazar isimleri **Adı SOYADI** kuralına göre Türkçe başlık sonrası **12 punto (koyu, ortalı ve düz)** ve bir boşluk bırakılarak yazılmalı, yazar isimlerinin sonuna adres için üst simge olarak rakam, sorumlu yazarı belirtmek için ise * simgesi verilmelidir. Adres satırı yazar isimleri sonrasında 1 boşluk bırakılarak **10 punto (normal, düz ve ortalı)** yazılmalıdır. Adres satırından sonra 1 boşluk

bırakılarak yazarların ORCID numaraları yazılmalıdır. ORCID satırının altına sorumlu yazar e-posta adresi belirtilmelidir.

10. **ÖZ:** Çalışmanın yürütüldüğü yer ve zamanını, amacını, yöntemini ve sonuçları içermelidir. Sola yaslı, 10 punto, koyu, paragraf başında girinti verilmemelidir. Türkçe ve İngilizce metin 300 kelimeyi aşmayacak şekilde 10 punto ve 1 satır aralığında yazılmalıdır. Öz ile Anahtar Kelimeler ve Abstract ile Key Words arasında tek satır boşluk (10 punto, düz) bırakılarak, metnin hemen altında en fazla 5 adet **Anahtar Kelimeler (Key Words)** yazılmalıdır. Key Words ile ana metin (Giriş) arasında iki satır boşluk bırakılmalıdır.

11. **Giriş:** Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir. Bu bölümde; çalışmanın konusu özetlenmeli, konu hakkındaki mevcut bilgi doğrudan ilişkili önceki çalışmalarla değerlendirilmeli ve bilgi üretimine ihtiyaç duyulan hususlar vurgulanıp çalışma ile ilişkilendirilmelidir. Son olarak çalışmanın amacı net ve açık bir şekilde ifade edilmelidir.

12. **Materyal ve Metot:** Bu bölümde; çalışmada kullanılan canlı ve cansız materyaller, uygulanan yöntemler, değerlendirilen ölçütler, uygulanan deneme desenleri veya örnekleme yöntemleri ile istatistiksel analizler gerektiğinde kaynaklarla da desteklenerek, açık ve net biçimde anlatılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır. Bu amaçla gerektiğinde alt başlık kullanılmalıdır.

13. **Araştırma Bulguları ve Tartışma:** Çalışmada elde edilen bulgular şekil ve çizelgeler yardımıyla ve istatistiksel analizlere dayalı olarak açık ve net bir biçimde verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. Aynı veriler hem grafik hem de çizelge ile verilmemeli, konuya en uygun araç seçilmeli, anlatımda tekrarlayan cümle ve ifadelerden kaçınılmalıdır. Tartışma kısmında, uyum ve zıtlık açısından önceki çalışmalarla karşılaştırılmalı, doldurduğu bilgi açığı vurgulanmalı, önceki bölümlerdeki ifadelerin olduğu gibi tekrarından kaçınılmalıdır.

14. **Sonuçlar:** Bu bölümde; elde edilen nihai sonuçlar ve varsa öneriler, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir.

15. **Ekler:** Çalışmayı destekleyen kurum ve kuruluşlar ile çalışmaya katkı sağlayanlar bu kısımda ifade edilmelidir. Ayrıca, makalenin lisansüstü tezlerden üretilip üretilmediği, abstract olarak kongre ve sempozyumlarda sunulup sunulmadığı da Ekler bölümünde belirtilmelidir.

16. **Beyanlar (Declarations)**

Çıkar Çatışması: Kişiler makalelerin etik ilkeler çerçevesinde değerlendirilebilmesi ve bağımsız bir süreç yürütülebilmesi için olası çıkar çatışmaları ile ilgili olarak yayın kurulunu bilgilendirmelidir. Ekonomik veya kişisel fayda sağlanan durumlar çıkar çatışmasını meydana getirir. Bilimsel sürecin ve yayınlanan makalelerin güvenilirliği; bilimsel çalışmanın planlanması, uygulanması, yazılması, değerlendirilmesi, düzenlenmesi ve yayınlanması sırasında çıkar çatışmalarının objektif bir şekilde ele alınmasıyla doğrudan ilişkilidir. Makale ile ilgili çıkar çatışması söz konusu değilse, **“makale yazarları, aralarında herhangi bir çıkar çatışması olmadığını beyan eder”** ifadesi yazılmalıdır.

Yazar Katkısı: Çalışmanın tasarlanması, planlanması, kurulması, yürütülmesi, verilerin analizi ve

makalenin yazılmasında içeriğe bilimsel açıdan katkı sağlayan her bir yazarın makaleye katkı şekli belirtilmelidir. Yazar katkıları, örnek olarak “**MM çalışmayı tasarlayarak denemeleri kurmuş, MM ve AA çalışmayı yürütmüş, BB verileri analiz etmiş, MM, AA ve BB makaleyi yazmıştır**” şeklinde ifade edilebilir.

17. **Kaynaklar:** Makalede atıfta bulunulan literatürlere Harran Tarım ve Gıda Bilimleri Dergisi Yayın Kurulu tarafından belirlenen **yazım kurallarına göre** yazılmalıdır.

Harran Tarım ve Gıda Bilimleri Dergisi **Yazım Kuralları** için ...

18. **Kısaltmalar ve Semboller:** Makale başlığı ve başlıklarda kısaltma kullanılmamalıdır. Gerekli olan kısaltmalar kavramların ilk geçtiği yerde parantez içinde verilmelidir. Kısaltmalarda ve sembollerin kullanımında ilgili alanın evrensel kurallarına uyulması zorunludur.

19. **Formüller:** Makalelerde formüller “Eşitlik” olarak adlandırılmalı ve italik olarak yazılmalıdır. Makalede birden fazla eşitlik varsa numaralandırılmalı, numara formülün yanında sağa dayalı olarak parantez içinde gösterilmelidir.

20. Makaleye ardışık olarak **satır ve sayfa numarası** verilmelidir.

21. **Calibri** karakterinde, **12 punto** ve **1.15 satır** aralıklı yazılan makale **20 sayfayı** geçmemelidir.

22. Yayınlanmasına karar verilen eserler, sadece şekilsel olarak, yukarıda yer alan bilgiler doğrultusunda yeniden düzenlenmeli, yazar(lar)ca herhangi bir eklenti ya da çıkartma yapılmamalıdır.

23. Makale içerisinde, dergi basıldığı haliyle görünen hataların sorumluluğu yazarlara aittir. Yayın Kurulundan kaynaklanan basım hataları için ise düzeltme yayınlanabilir.

24. Harran Tarım ve Gıda Bilimleri Dergisi; yazarlardan makale gönderimi, değerlendirilmesi ve basım aşamalarında herhangi bir basım ücreti almamaktadır.

MANUSCRIPT WRITING RULES

1. The manuscript should be written in Microsoft Word program, in Calibri font, **1.15** line spacing, **12** pt. plain text and a single column.
2. Margins; **Left, right, bottom and top 3 cm** should be left, and each row should be prepared consecutively by giving the line number.
3. Author (s) should indicate the type of manuscript (**Research Manuscript, Review, Technical Note** etc.).
4. **The English title should be written in 14 pt (bold and centered)** lowercase letters (only the first word of the title is capitalized) and in plain text. The Turkish title should be written in 12 font size and centered.
5. Author names should be written in **12 pt. (Bold, centered and plain)** and a space after the title according to the Name SURNAME rule, followed by a number as superscript for the address and a * symbol to indicate the corresponding author. Address line should be written after the author names, leaving **1 space and 10 pt (normal, straight and centered)**.
6. Authors' ORCID numbers should be written, leaving 1 space after the address line. Under the ORCID line, the responsible author e-mail address must be specified.
7. The text should generally be in the following form;
 - **Abstract**
 - **Introduction**
 - **Material and Method,**
 - **Results and Discussion,**
 - **Conclusions**
 - **Acknowledgement**
 - **References**
8. In the writing of main titles, only the initials of the words should be capitalized in bold. In secondary and tertiary titles, only the first letter of the first word should be capitalized, other words should be in small, not bold and italic. There should be 1 space each, including the main headings of the text, the beginning and the end of the text. 1 space should be left before subtitles, but no spaces should be left after them. All titles should be left justified without indenting.
9. Reference should be cited **in the text based on (Author, year) rule**. In the presentation of a reference with two authors in the text, the rule (**first author's surname and second author's surname, year**) should be applied. In the display of sources with more than two authors (**first author's surname et al., year**) rule must be applied.

For example; (Bilgili, 2020), (Bilgili and vanEs, 2018); (Bilgili et al., 2019).

10. If more than one reference will be cited at the same time in the text; Referencens should be ordered chronologically, considering the year they were published.

11. **ABSTRACT:** Title should be left justified, 10 pt, bold, not indented at the beginning of the paragraph. Turkish and English texts should be written in 10 font size and 1 line spacing, not exceeding 300 words. **A maximum of 5 Key Words** should be written just below the text, leaving a single line space (10 pt., Plain) between Abstract and Keywords, and Öz (Turkish Abstract) and Key Words. Two lines of space should be left between Key Words and the main text.

12. Photographs, graphics, drawings, etc. should be expressed as "Figure" and Tables as "Tables".

13. Tables and Figures should be numbered consecutively (Figure 1. or Table 1.). Contents of "Figure" and "Table" should be prepared with 1 line spacing and 10 pt.

14. Table titles should be written above the table, and figure titles should be written below the figures in 10 pt, 1 line spacing with the first letter capital.

15. Figure and Table titles should be written in italics;

Figure 1. The average temperature, average relative humidity and average monthly rainfall data detected in the research garden (average of the years 2007-2011) Table 2. Phenological observation results of peach cultivars for between 2007 and 2011

16. Decimal numbers in the manuscript text and Table-Figure should be separated by **a period**. (123.87; 0.987 etc.).

17. One blank line should be left before and after the table-figures.

18. Manuscript writing should comply with the "International Unit System" (SI). According to this; Use g l-1 instead of g / l, and mg l-1 or ppm instead of mg / l. Percentages should be descriptive. For example; It should be specified as 3% (w / v), 3% (v / v), 3% (w / w) instead of 3%.

19. Harran Journal of Agriculture and Food Sciences uses **APA Style** in the submission of the sources list. Accordingly, the list of references should be prepared in accordance with the following rules.

19.1. **Citation to journal publications;**

19.1.1. Single author manuscripts;

Mamay, M. (2015). Infestation map of pomegranate aphid [*Aphis punicae* Passerini (Hemiptera: Aphididae)] in Şanlıurfa province pomegranate orchards. Turkey Entomology Bulletin, 5(3), 159-166.

19.1.2. Two-author manuscripts;

Soylemez, S., & Pakyurek, A. Y. (2017). Responses of rootstocks to nutrient induced high EC levels on yield and fruit quality of grafted tomato cultivars in greenhouse conditions. Applied Ecology and Environmental Research, 15(3), 759-770. DOI: http://dx.doi.org/10.15666/aeer/1503_759770

19.1.3. Manuscripts with more than two authors;

İkinci, A., Mamay, M., Unlu, L., Bolat, I., & Ercisli, S. (2014). Determination of heat requirements and effective heat summations of some pomegranate cultivars grown in Southern Anatolia. Erwerbs-Obstbau, 56(4), 131-138. DOI: <https://doi.org/10.1007/s10341-014-0220-8>.

19.2. Referencing Books;

19.2.1. If the source is a book;

Mohsenin, N. N. (1970). Physical Properties of Plant and Animal Materials. New York: Gordon and Breach Science Publishers.

19.2.2. If it is a chapter from the source book;

Author, A. A. (Year). Chapter title. In E. E. Editor (Ed.), Title of book: And subtitle (pp. pages). Place: Publisher.

19.2.3. Edited book;

Yeşilyaprak, B. (Ed.). (2003). Development and learning psychology. Ankara: Pegema Publishing.

19.3. Citing sources of unknown author or internet sources;

Anonymous (2005). Butter, other milk fat-based spreads and plain butter notification. Turkish Food Codex, Communiqué No: 2005/19, Ankara.

FAO, (2015). Statistical data of FAO. Retrieved from: <http://faostat.fao.org/site/567/default.asp>.

19.4. Citing Master's and Doctoral theses;

Doctorate or master theses can be accessed from electronic databases, corporate archives and personal web pages. If a dissertation is accessed from the ProQuest database of doctoral and master's theses or any other source, this information should be provided in the reference. For a doctorate or master thesis available in a database service, the following citation format is used;

Unpublished thesis;

Mamay, M. (2013). *Determination of population development and infestation ratio of carob moth [Apomyelois ceratoniae Zell. (Lepidoptera:Pyralidae)] in pomegranate orchards in Sanliurfa province and using mating disruption*

technique for its control (Unpublished doctoral dissertation). Harran University, Graduate School, Şanlıurfa.

Söylemez, S. (2014). *Effects of nutrient induced salinity levels and rootstocks on plant growing, yield and some fruit quality features at soilless grown grafted tomatoes* (Unpublished doctoral dissertation). Harran University, Graduate School, Şanlıurfa.

Published thesis; May, B. (2007). A survey of radial velocities in the zodiacal dust cloud. Bristol, UK: Canopus Publishing.

19.5. Citing Symposium and Meeting Papers

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Note: You can use the web pages related to **APA Referencing Style** on the internet.

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