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Optimizing irrigation scheduling using a drip system to enhance water use efficiency in tomato cultivation

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Abstract

Irrigation scheduling with drip irrigation is a crucial management technique for ensuring optimal soil moisture, thereby promoting plant growth, production, and economic return while enhancing water efficiency. Tomato crops, a mass consumer product grown worldwide, face significant challenges in the Sylhet region due to inefficient water management. To ascertain the best watering schedule for tomato crops based on evapotranspiration, a field experiment was carried out utilizing a randomized complete block design (RCBD) with six treatments and four replications. The six treatments include two irrigation frequencies (daily basis, F1 and every alternate day, F2) with three water doses W1, W2, W3 (i.e., 50%ETc, 75%ETc, 100%ETc). The Penman-Monteith method was used to estimate crop water requirements, and water for farming was applied according to designated schedules. Data analysis using SPSS 23 revealed that the impacts of irrigation frequency and water doses on plant growth, fruit yield, and water use efficiency were significant, except for stem diameter. The plant height, number of fruits, number of flowers, yield, and water use efficiency were increased with increasing water doses and decreased with decreasing water doses. The maximum yield was gained by increasing irrigation frequency at F_1 and water dosage at W_3, but the highest WUE was obtained at a lower irrigation frequency under the treatment F_2, with the highest water dose at W_3. The study concluded that the irrigation scheduling F_1W_3 was the best in balancing tomato productivity, fruit quality, and WUE in this study. The findings of this study will help local farmers make sustainable decisions about their irrigation methods.

Keywords: Irrigation scheduling, Drip irrigation, Water use efficiency, Tomato, Yield

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INTRODUCTION

Water is a priceless natural asset, a fundamental requirement for human existence, and a primary natural resource (Deka et al., 2023). Water scarcity is exacerbated by global warming, climate change, and population growth, leading to increased competition for freshwater resources (Junk, 2013). In many parts of the world, water is becoming a scarce economic resource, particularly in arid or semi-arid countries (Buttaro et al., 2015). Bangladesh struggles to provide water security for agricultural production, much as many other nations. Over the past few decades, there has already been an increase in the demand for water and competition for freshwater resources (Mancosu et al., 2015; Arfanuzzaman & Rahman, 2017). Due to its large water footprint and limited water supply, crop agriculture has significant challenges in ensuring sufficient water share (Allen et at., 1998). The primary consumer of Bangladesh's scarce water resources is agriculture (Chowdhury, 2010). Tomato is an essential agricultural crop in the Solanaceae family. Its primary uses are as a cash commodity and a food crop on a global scale (Gatahi, 2020). In Bangladesh, it is a significant crop for vegetable production. Around 6% of the region is now under tomato cultivation during both the summer and winter seasons (Karim et al., 2009). Tomato cultivation in Bangladesh is estimated to be around 4.4 million metric tons, ranking fourth after potato, onion, and brinjal (BBS, 2021). Local tomato growers overwater their plants to boost productivity, which exacerbates the

imbalance between water supply and demand and produces wastewater (Li et al., 2017). The quality and storability of any fruit are impacted by numerous pre-harvest actions throughout production. One essential farming technique that impacts fruit and vegetable productivity and quality is irrigation (Agbemafle et al., 2014).

Implementing water-efficient irrigation systems may result in enhanced yields, better fruit quality, and improved efficiency of water use. To conserve water, a range of techniques have been applied to tomato cultivation, such as deficit irrigation, mulching, and trickle irrigation. Drip irrigation (DI) is a water-saving technique in which water is supplied directly to the root zone at small, frequent intervals, resulting in a significant reduction in overall water use. In contrast to conventional irrigation methods, drip irrigation has the potential to conserve water by 45–50% while sustaining an acceptable reduction in yield (Marino et al., 2014). In tomatoes and other commodities, numerous studies comparing sprinkler or furrow irrigation with DI have demonstrated that DI ultimately increased WUE and crop yields (Biswas et al., 2015). A prudent assessment of the crop water requirements is vital to scheduling irrigation and planning of agricultural irrigation systems (Mushtaq et al., 2020). For the improvement of WUE, scientists and academics are now very concerned about this (Derib et al., 2011; Eshete et al., 2020). Irrigation scheduling is the amount and frequency of water supplied to a crop; it is crucial to prevent over- or under-irrigation since this can lower crop quality, yield, and water use efficiency (WUE) (Pardossi & Incrocci, 2011). Diverse irrigation scheduling methods exist, including those that rely on plant-based sensing (Jones, 2004), soil moisture sensors (e.g., volumetric, soil moisture tension) (Ganjegunte et al., 2012) and meteorological conditions (e.g., FAO Penman–Monteith, evaporation pans) (Pardossi & Incrocci, 2011). An alternative method involves creating a daily water balance to determine ETc and plan irrigation times based on projected crop water extraction and the effective soil water storage capacity. These irrigation scheduling techniques can be highly effective (Mohammad et al., 2013). Tracking ETc losses and additions from irrigation and precipitation is the basic strategy for the ET-based method, which aims to maintain the balance of SM available to the plant at any given time (Nouri et al., 2016). For many years, the Penman–Monteith approach has been a popular choice for scientific studies (Farooque et al., 2021). Nevertheless, very few have tried to explain the Penman-Monteith model's operation in a way that farmers may utilize to help with irrigation scheduling (Her et al., 2019).

Although there exist more precise techniques for optimizing irrigation schedules (Linker, 2021) still, there is a dearth of research on irrigation scheduling in tomatoes. The impact of water levels and irrigation frequency on tomato crops has been the focus of several investigations, although most of these investigations examined both variables in isolation. Hence, to enhance both the quantity and quality of agricultural output as well as optimize irrigation efficiency, it would be advantageous to research irrigation scheduling. The goal of this study is to determine the best watering schedule for tomato crops that will maximize fruit quality and WUE while lowering production costs and water usage. We anticipate that the outcomes will equip local farmers with the necessary knowledge to make sustainable decisions about their irrigation methods.

MATERIALS AND METHODS

Site description

The study was carried out in the Sylhet Agricultural University research field (35 meters above sea level, latitude 24.89°N and longitude 91.87°E) from December 2023 to February 2024. The site is in the subtropical climate zone (BARC, 2022). Two manual soil samples were taken, covering the targeted area, following normal soil sampling procedures. Soil analysis was performed using the laboratory of the soil science department at Sylhet Agricultural University. The soil sample had a sandy loam texture with 25% field capacity.

Experimental design

The experiment was conducted in a randomized complete block design (RCBD) with six treatments and four replications. Plots and blocks were separated by 0.3 m, and each plot was 1.21 m by 1.06 m. For tomato plants, the spacing is 50 cm between plants and 60 cm between rows. The treatments are as follows:

T_1 : Evapotranspiration - Once a day (F_1), 50% of ETc (W_1)
T_2 : Evapotranspiration - Once a day (F_1), 75% of ETc (W_2)
T_3 : Evapotranspiration - Once a day (F_1), 100% of ETc (W_3)
T_4 : Evapotranspiration - Once in every alternate day (F_2), 50% of ETc (W_1)
T_5 : Evapotranspiration - Once in every alternate day (F_2), 75% of ETc (W_2)
T_6 : Evapotranspiration - Once in every alternate day (F_2), 100% of ETc (W_3)

Irrigation Setup

The drip irrigation system was designed to deliver water and fertilizers to the crops efficiently. It utilized a 0.5 HP pump connected to a 500-liter tank to ensure an ample water supply for crop needs. A filtration system was installed to avoid clogging of the drippers and other components due to the impurities of irrigation water. A venturi connected to the pump created a vacuum, enabling the mixing of fertilizers with the water flow. The drip irrigation system's water flow was controlled, and the pressure was adjusted using flow control and pressure regulating valves. A main line of 25 mm diameter served as the primary conduit, delivering water from the pump to lateral

pipes. These lateral pipes, with a diameter of 14 mm, distributed water from the main line to individual plants. Adjustable drippers were fixed to the lateral pipes, supplying water and fertilizer directly to the root zone of each plant.

Reference Evapotranspiration (ET_0) Calculation

Transpiration, the loss of water from a plant's surface and evaporation, and the loss of water from the soil surface happen simultaneously. Together, they are termed evapotranspiration (ET). The rate of ET from an imaginary crop, assuming a height of 0.12 meters, an albedo of 0.23, and a constant canopy resistance of 70 sm^{-1}, is known as reference evapotranspiration (Allen et al., 1998). The reference crop evapotranspiration ET_0 was determined using the Penman-Monteith technique (Allen et al., 1998; Boltana et al., 2023). The Bangladesh Meteorological Department (BMD) provided the hourly minimum and maximum temperature, radiation, sunlight hours, wind speed, latitude, and elevation of the field, which were used to compute the ET_0.

$$ET_0 = \frac{0.408(Rn-G)+\gamma\frac{900}{T+273}u2(es-ea)}{\Delta+\gamma(1+0.34u2)}$$

Where,

ET_0 = reference crop evapotranspiration (mm/day),

R_n = net radiation at crop surface ($MJ/m^2/day$),

G = soil heat flux ($MJ/m^2/day$),

T = average temperature (^oC),

U_2 = wind speed measured at 2 m above ground (m/s),

e_s-e_a = vapor pressure deficit(kpa)

Δ = slope vapor pressure curve ($kPa/^oC$),

γ = psychometric constant ($kPa/^oC$),

900 = conversion factor.

Crop Water Requirement Determination

The crop water requirement represents the quantity of water equivalent to the loss from a cultivated area due to evapotranspiration (ET), typically expressed as a rate in millimeters per day. Determining the crop water requirement involves estimating crop evapotranspiration (ETc), which can be computed using the following formula (Pereira et al., 2015)

$ETc = Kc * ET_0$

The amount of rainfall was collected from the Bangladesh Meteorological Department (BMD) and adjusted with crop evapotranspiration (ETc) during the experimental period.

Net irrigation requirement (NIR) = ETc – Rainfall

The volumetric water requirement for a tomato plant was computed by the following equation (Biswas et al., 2015);

V = NIR * A

The duration of operation (RT) of the drip irrigation system is dependent on the volume of water required, dripper discharge, and dripper discharge rate. In this experiment, adjustable drippers were used, and the dripper discharge rate was adjusted manually. As all the treatments were different based on the irrigation schedule, the time of operation was also different from each other. The running time (RT) of the drip irrigation system was calculated using the Equation (Simić et al., 2023);

$$RT = \frac{the\ volume\ of\ water\ applied}{\text{number of drippers} \times dripper\ discharge\ rate}$$

The volume of water applied was in m^3, and the dripper discharge rate was in m^3/s.

Where, ET_0 = reference evapotranspiration (mm/day)

ETc = Crop Evapotranspiration (mm/day)

A = wetted area (m^2)

Kc = the crop coefficient.

Crop coefficient (Kc) adjustment

This factor represents the ratio of crop evapotranspiration (ETc) to reference evapotranspiration (ET_0), encapsulating the impact of four key characteristics that distinguish the crop from reference grass. These factors include the crop-soil surface reflectance (albedo), crop height, canopy resistance, and soil evaporation. As crop ETc varies across growth stages, the crop coefficient (Kc) fluctuates throughout its developmental phases, typically categorized into four stages: initial, crop development, mid-season, and late season (Allen et al., 1998; Ewaid et al., 2019). The adjustment of the k_c value (Table 1) was carried out in compliance with the guidelines provided by the FAO (FAO Irrigation and Drainage Paper, 2006).

Table 1. Kc values for tomato used in the experiment.

Growth stage	Days after transplant	Standard value of kc (FAO)	Adjusted value of kc
Initial	1 -7	0.60	0.395
Development	8 - 29	0.60 – 1.3225	0.395 – 1.0285
Mid stage	30 - 51	1.3225	1.0285 – 1.2285
Late stage	52 - 82	0.8	1.0859 – 0.8

Agronomic Variables

Tomato seedlings were transplanted to the experimental field on 02 December 2023, 28 days after sowing, when they reached a height of 10 cm with 3 true leaves. After transplanting the seedlings, a variety of intercultural practices were undertaken to enhance the growth and development of the plants. Bamboo sticks were used to support each plant once they were firmly established. Weeding was carried out at 15, 30, 50, and 70 days after transplanting (DAT) to maintain weed-free conditions in the field and promote optimal crop establishment. The recommended doses of manures and fertilizers, such as well-decomposed cow dung, urea, TSP, and MOP, respectively, were applied (DAE). 50% cow dung and 50% TSP were applied during the final land preparation. The rest of the cow dung and TSP were applied in the pit before transplanting the seedlings. Urea and MoP were applied in two equal installments. The first half was applied 20 DAT, and the second half was applied 35 DAT. During the experiment, plant growth and physiological characteristics (such as plant height, stem diameter, number of branches, number of flowers, and number of fruits) were measured for all treatments. Fruits were picked while they were ripening and mature. Crop maturity was evaluated by the presence of red coloring on the fruits. It was possible to collect a total of five crops during the harvesting stages. The fruit parameters (fruit length, fruit weight, fruit diameter, total yield) were measured after harvesting.

Water Use Efficiency

Water use efficiency is defined as the ratio of grain yield to the total amount of irrigation water applied during the whole growing season. It was calculated by the following equation (Vikas Sharma et al., 2021);

$$\text{WUE} = \frac{Total\ yield\ (kg)}{the\ amount\ of\ water\ applied\ (m3)} \times 100$$

Statistical Analysis

The data was analyzed using SPSS 23.0 statistical software (SPSS Inc., Chicago, II, USA). The effects of various interventions were evaluated using a general linear model and descriptive and homogeneity tests in SPSSA P-value of less than 0.05 for the Duncan test indicated that differences between treatments were significant. Origin Pro software (OriginLab Corporation, MA, USA) was employed to generate analytical graphical representations of the data. The final report and the result were compiled utilizing the data that had been analyzed.

RESULTS

Weather data in the experiment

Figure 1 shows the data observed during the experiment. In daily meteorological observation, the average minimum air temperature ranged from 20.29°C to 20.10°C, while the highest air temperature ranged from 21.47°C to 21.48°C. The average relative humidity ranged from 85.50% to 69.64%, while ET_0 varied from 2.48 mm to 2.11 mm.

In every other day of weather observation, the average relative humidity ranged from 84.88% to 68.49%, and ET_0 ranged from 2.68 mm to 2.04 mm. The average minimum air temperature ranged from 20.45°C to 20.09°C, while the highest temperature ranged from 21.67°C to 21.67°C. Evapotranspiration (ETc) was highly variable since the meteorological circumstances in the research area changed dramatically day by day. Figure 1 represents how the value of ETc fluctuated with water doses with daily and every alternate day irrigation.

Crop Water Requirement

Water requirements for crops are the amount of water (or depth) required to replenish the amount gone via evapotranspiration (ETc). After transplantation, irrigation treatment started on 10 December 2023 and closed on 20 December 2023. A total of 73 irrigations and 36 irrigations were done daily and alternate-basis irrigation, respectively. No rainfall occurred during the experimental period. The amount of irrigation water that is required for various water doses and irrigation frequencies is outlined in Table 2. In the experiment, the amount of irrigation water was increased with increased water doses and with the highest irrigation frequency. It was shown that the F_1W_3 treatment resulted in the largest volume of irrigation water required, whereas the F_2W_1 treatment resulted in the lowest amount of irrigation water needed.

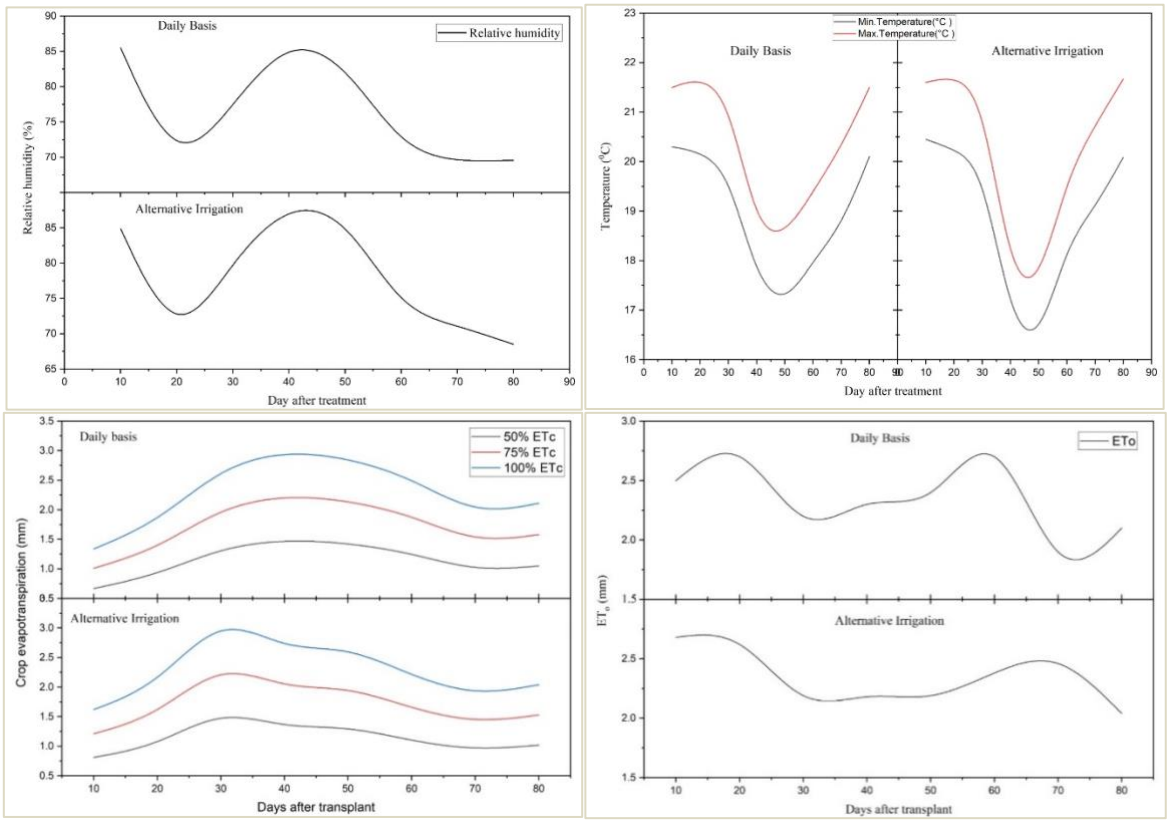


Figure 1. Trend of change of weather data and evapotranspiration during the experiment.

Table 2: Crop Water Requirement for tomato under the different treatments.

Treatment	Total NIR (mm)	Total Irrigation water (L)
F_1(Daily basis)		
W_1	83.62	24.41
W_2	125.43	36.61
W_3	167.24	48.81
F_2(Alternate day)		
W_1	40.83	11.92
W_2	61.23	17.87
W_3	81.65	23.83

W and F represent water dose and irrigation frequency, respectively. W_1: 50% ETc; W_2: 75% ETc; W_3: 100% ETc; F_1: daily basis; F_2: every alternate day.

Tomato Production and Quality

Plant Height

As shown in Table 3, the effect of water dose and irrigation frequency on plant height during the initial 15 days following transplanting was insignificant. At 30 to 80 days after transplanting, the effect of irrigation frequency and water dose on plant height was statistically significant ($p < 0.05$). As shown in Table 3, the highest water dose and the highest irrigation frequency resulted in the greatest plant height for treatment F_1W_3. Conversely, the lowest water dose and the lowest irrigation frequency produced the shortest plant height for treatment F_2W_1.

Stem diameter

The variation in stem diameter was not significantly affected by water dose and irrigation frequency ($p < 0.05$), as shown in Table 3. The greatest diameter of the stem was achieved with a moderate water dose in treatment W_2 and the highest frequency irrigation under treatment F_1. Conversely, the smallest diameter of the stem was obtained with the least water dose under treatment W_1 and the lowest frequency irrigation under treatment F_2.

Number of Flowers

The statistical analysis demonstrated that there was a significant relationship ($p < 0.05$) between water dose, irrigation frequency, and the number of flowers, as shown in Table 3. The number of flowers in the F_2 treatment significantly decreased by 13% than F_1. The quantity of flowers in the W_1 treatment was reduced by 13.7% compared to the W_3 treatment. In contrast, the W_2 treatment suffered a 9% reduction in flowers compared to the W_3 treatment. In treatment F_1W_3, the maximum water dose and highest irrigation frequency resulted in the greatest

quantity of flowers. Conversely, treatment F_2W_1, which utilized the lowest water dose and lowest irrigation frequency, produced the fewest flowers (Table 3).

Table 3. Analysis of Variance and Duncan's multiple range test of the average plant height, stem diameter and flowers per plant.

Factor	Plant Height (cm)						Stem Diameter (mm)	Flowers per Plant
	7 DAT	15 DAT	30 DAT	45 DAT	60 DAT	80 DAT		
Irrigation Frequency								
F_1	13.50	17.99	41.27 a	62.43 a	75.05 a	82.67 a	19.44	43.33 a
F_2	13.48	17.20	36.56 b	56.95 b	69.40 b	79.33 b	19.25	37.67 b
Water Dose								
W_1	13.31	17.15	35.15 b	55.79 b	68.19 b	76.50 c	18.43	37.50 b
W_2	13.50	17.61	39.04 a	60.20 a	72.41 a	80.50 b	20.38	40.50 ab
W_3	13.65	18.03	42.55 a	63.08 a	76.08 a	86.00 a	19.24	43.50 a
ANOVA								
F	ns	ns	**	***	**	*	ns	**
W	ns	ns	**	***	**	***	ns	**
F× W	ns	ns	ns	ns	ns	ns	ns	ns

W and F represent water dose and irrigation frequency, respectively. W_1: 50% ETc; W_2: 75% ETc; W3: 100% ETc; F_1: daily basis; F_2: every alternate day; DAT: days after transplant; *: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$; ns: no significant at $p < 0.05$. Values within the same columns that are accompanied by different letters vary significantly at $p < 0.05$.

Table 4. Analysis of Variance and Duncan's multiple range test of the total yield, total water, fruits per plant, total water per plant and water use efficiency.

Factor	Total Yield (kg)	Total Water (m^3)	Fruits per Plant	Total yield (kg $plant^{-1}$)	Total water (m^3 $plant^{-1}$)	WUE (kg m^{-3})
Irrigation Frequency						
F_1	34.99 a	0.5857 a	30.33 a	2.19 a	0.04 a	59.18 b
F_2	18.83 b	0.2857 b	25.00 b	1.18 b	0.02 b	64.62 a
Water Dose						
W_1	16.64 c	0.2906 c	24.00 b	1.04 c	0.02 c	57.64 c
W_2	26.80 b	0.4358 b	27.50 b	1.68 b	0.03 b	62.51 b
W_3	37.28 a	0.5808 a	31.50 a	2.33 a	0.04 a	65.56 a
ANOVA						
F	***	***	**	***	***	***
W	***	***	**	***	***	***
F× W	***	***	ns	***	***	***

W and F represent water dose and irrigation frequency and T_0 represents the farmers practice, respectively. W_1: 50% ETc; W_2: 75% ETc; W3: 100% ETc; F_1: daily basis; F_2: every alternate day; WUE: water use efficiency; *: significant At $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$; ns: no significant at $p < 0.05$. Values within the same columns that are accompanied by different letters vary significantly at $p < 0.05$.

Number of fruits

In the experiment, water dose and frequency of irrigation had a significant impact on the quantity of fruits produced by each plant. F_2 produced fewer fruits per plant by 17.5% compared to F_1. With decreased irrigation frequency, the number of fruits per plant decreased, whereas it increased with increasing water dose. According to the data presented in Table 4, the F_1W_3 treatment produced the greatest number of fruits per plant, whereas the F_2W_1 treatment yielded the fewest fruits per plant.

Yield

The yield response to water dose and frequency was significantly reduced in comparison to F_1W_3 treatment and farmer practice. In comparison to F_1, the total yield and yield per plant from the F_2 treatment were reduced by 46%. Likewise, the overall production and per-plant yield of W_1 and W_2 were reduced by 55% and 28%, respectively, in comparison to W_3. As the frequency of irrigation decreases, both the total yield and yield per plant diminish (Table 4). The experiment required the highest water volume at the maximum dose.

Water Use Efficiency

Table 4 presents the impact of watering frequency and water dose on water use efficiency was statistically significant ($p<0.05$). The water use efficiency in F_2 treatment was significantly increased by 8.4% than F_1. The W_1 treatment significantly decreased 12% water use efficiency than W_3, whereas the W_2 treatment significantly

decreased 4.6% water use efficiency than W_3. In the experiment, the maximum water use efficiency was achieved under F_2W_3 treatment, whereas the lowest water use efficiency was obtained from F_1W_1, as shown in Table 4.

Water Use-Yield Relationship

The water–yield relationship was affected by irrigation frequency and water dose (Table 4). At treatment F_1, the amount of water consumption and yield per plant dramatically increased by 50% and 54%, respectively, in comparison to treatment F_2. Instead, the least amount of yield was gained at the lowest water dose, which needed the least amount of water. The maximum yield was obtained at the highest water dose, which demanded the most water.

Table 5. Analysis of Variance and Duncan's multiple range test of the average weight per fruit, average diameter per fruit, and average length per fruit.

Factor	Average Weight Fruit^{-1}(gm)	Average Diameter Fruit^{-1}(mm))	Average Length Fruit^{-1}(mm)
Irrigation Frequency			
F_1	70.45 a	48.48 a	48.02 a
F_2	46.05 b	43.96 b	41.06 b
Water Dose			
W_1	42.41c	42.38b	41.30 b
W_2	59.74 b	45.62b	44.65 ab
W_3	72.60 a	50.67a	47.68 a
ANOVA			
F	***	**	***
W	***	**	*
F× W	ns	ns	ns

W and F represent water dose and irrigation frequency, respectively, and T represents the farmer's practice, respectively. W_1: 50% ETc; W_2: 75% ETc; W_3: 100% ETc; F_1: daily basis; F_2: every alternate day; *: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$; ns: no significant at $p < 0.05$. Values within the same columns that are accompanied by different letters vary significantly at $p < 0.05$.

Fruit Weight

Both the water dose and the frequency of irrigation had an impact on the average weight of each fruit (Table 5). Each fruit's average weight increased as the water dosage increased, but a decrease in irrigation frequency led to a decrease in the weight of each fruit. According to the results of the experiment, the treatment F_1W_3 produced the highest average weight per fruit, while the treatment F_2W_1 produced the lowest average weight per fruit.

Fruit Length

The average length per fruit was significantly affected by water dose and irrigation frequency presented in Table 5. In comparison to F_1, the mean length per fruit in F_2 was considerably reduced by 14.5%. As water quantities increased, fruit length increased, as shown in Table 5. W_3 resulted in fruit length that was 13.4% and 6.4% greater than W_1 and W_2, respectively.

Fruit Diameter

The impact of water dose and irrigation frequency on fruit diameter was found to be statistically significant ($p < 0.05$), as shown in Table 5. The average diameter per fruit in treatment F_2 was reduced by 9.2% less than treatment F_1. The mean diameter per fruit exhibited a positive correlation with escalating water dosages and irrigation frequency. The experimental results indicated that treatment F_1W_3 yielded the greatest average diameter per fruit, while treatment F_2W_1 produced the smallest average diameter per fruit.

DISCUSSION

Effect of Irrigation Schedule on Crop Water Requirement

The experiment was carried out in winter seasons (December to February), and it was low in average air temperature and high in relative humidity. The ET_0 was estimated using the Penman-Monteith method and shown in Figure 1. The variation in ET_0 values is indicative of the fluctuating meteorological parameters within the designated study area. During the winter months, evapotranspiration was diminished due to the reduced wind speed, high relative humidity, and low temperatures (Alemayehu et al., 2009). The ETc values throughout the experimental period are depicted in Figure 1, and the fluctuations seen can be attributed to the crop coefficient, as indicated in Table 1. Although the Kc fluctuated minimally, it was not consistent in any growth stage (De Azevedo et al., 2007). The ETc values for all the treatments illustrated in Figure 1 were found to be minimal at the onset and conclusion, when the crops were at their most productive stage, and increased during the mid-phases. The profundity or quantity of water required to replace the water lost by ETc is referred to as the crop water demand. There is an uneven distribution of the total amount of water needed for crop development throughout the crop's life cycle; rather, it varies according to location, climate, soil type, cultivation method, effective precipitation, and

other factors. (De Azevedo et al., 2007). In our case, two irrigation frequencies with three water doses were observed for the irrigation schedule. This was because the irrigation requirement varied at different treatments (Table 2).

Effect of Irrigation Schedule on Plant Growth

The frequency of irrigation and water dosage had a substantial impact on plant height, flower count presented in Table 3. From the results, we found that the plant height decreased as the water dose decreased and increased as irrigation frequency increased. The highest altitude was attained under F_1W_3, and the lowest height was obtained under the F_2W_1 treatment. The findings are consistent with these results and agree with Agbna et al. (2017), who observed that vegetative growth decreased in deficit irrigations. This was because significantly reduced photosynthesis by plants diminished the quantity and energy of metabolites required for healthy plant development under water-stress conditions. Wu et al. (2021) explained that water dose had an adverse influence on plant height and stem diameter. Tomato yields decreased in response to reduced irrigation amounts, these declines were accompanied by reductions in plant height and leaf count. These changes may be caused by increased water stress. In our experiment, the plant height decreased with the decrease in irrigation frequency. This result is supported by Oke et al. (2020). In this study, stem diameter was unaffected by the frequency of irrigation or water dosage, supported by Alves Souza et al. (2020), who confirmed that the values of stem diameter could not significantly be affected by one- or four-day irrigation intervals. This study indicated that the number of flowers varied with irrigation frequency and water dose. At the maximum water dosage and frequency of watering, the greatest number of blooms as each plant was produced, whereas the lowest quantity of flowers per plant was obtained at the lowest irrigation dose and highest irrigation interval indicated in Table 3. Tolerant genotypes may exhibit a reduced rate of floral abscission as a result of photosynthate translocation to the reproductive organs and maintenance of photosynthesis under drought conditions. In susceptible cultivars, the lack of available assimilates to the developing floral organs may result from a reduction in photosynthesis during times of water stress, which subsequently causes the abscission of flowers and flower buds explained by Sivakumar & Srividhya (2016). A scarcity of water during this phase would have resulted in a diminished flower production, and as hypothesized (Mahendran & Bandara, 2000), water scarcity during the flowering stage not only hinders the development of flowers but also elevates the rate of blossom shedding. Table 4 shows how the frequency of irrigation and water dosage impacted the amount of fruit produced in each plant. The quantity of fruit that each plant produced was not influenced by either water doses or irrigation frequencies explained by Colimba-Limaico et al., (2022). On the contrary, the results of Biel et al., (2021) & Wu et al., (2021) indicate that the quantity of fruits produced is influenced by the dosage of water. Tomato plants are particularly susceptible to severe water stress while blossoming and fruiting (Zegbe et al., 2006). Flower abortion can lead to a reduced fruit yield per plant. As a result, both the quantity and weight of fruits may be diminished due to water stress observed by Hao et al., (2013).

Effect of Irrigation Schedule on Yield and Yield Components

The present study showed a significant difference in fruit yield and yield components by water dose and irrigation frequency. Total yield and yield components increased with increasing water doses and irrigation frequency. The frequency of irrigation has significant in terms of statistics impact on tomato yield, which is consistent with the findings of Alves Souza et al., (2020), who examined the effects of six different irrigation frequencies (2, 3, 4, 5, 6, and 7 days interval) on tomatoes cultivated in an open field and found that the highest yields were obtained with the highest watering frequency (2 days). Tomato yield was found to be substantially impacted by water doses; these results are consistent with previous research that has demonstrated the detrimental effect of the lowest water doses on fruit yield (Agbna et al., 2017; Wu et al., 2021). Furthermore, following our findings, Sezen et al., (2010) observed that the tomato yield was greatest at the maximum water dose (150% ETc). The reason for the lower yield observed in the treatments subjected to deficit water stress can be attributed to the inadequate development of flower buds in these conditions. It is well-established that water stress can have an impact on flower bud development (Naor et al., 2008). Table 5 displays the impact of irrigation frequency and water dosage on yield components. The fruit weight, fruit length, and fruit diameter increased with increasing water doses and irrigation frequency. The decrease in the irrigation frequency might reduce the length, diameter, and weight of the tomato fruits explained by (Rebouças Neto et al., 2017).

Effect of Irrigation Schedule on Water Use Efficiency

The lowest irrigation dose produced the best WUE, according to Wu et al., 2021; Abdulhady et al., 2017 and Wang & Xing, 2017. These findings, however, differ from those of Kuscu et al., (2014) and Liu et al., (2019). These inconsistent findings could be explained by the fact that irrigation, whether excessive or insufficient, tends to reduce WUE and yield explained by Hao et al., (2013). The observation that the maximum watering frequency resulted in the highest WUE is consistent with the findings of Oke et al., (2020), which suggest that an increase in irrigation frequency leads to a greater quantity of fruits, which subsequently increases yield and enhances WUE. On the contrary, Fara et al., (2019) noted that higher irrigation intervals (7 and 9 days) resulted in the highest water use efficiency. The authors further proposed that increasing the irrigation intervals could potentially lead to more effective water usage. According to López Ordaz et al., (2011), plants that were subjected to more frequent irrigation intervals exhibited reduced water usage efficiency as a result of developing their roots more superficially.

However, in our case, the lowest irrigation frequency and maximum water dose resulted in the highest water use efficiency under the 100% ETc treatment every other day.

The Optimal Irrigation Scheduling

The optimal scheduling of irrigation must consider fruit quality, yield, and water use efficiency. However, because of the complicated interactions they involve, establishing the correct balance between them is difficult. It is, therefore, necessary to examine their quantitative relationship (Liu et al., 2019). By balancing yield, fruit quality, and water use efficiency in an open-field tomato producer, our results may be capable of approximating the optimal frequency and dose of irrigation. To attain equilibrium between tomato production, fruit quality, and water use efficiency, this study demonstrates that the local producer dose is not advisable. In the study, the local dose increases yield but decreases fruit size and WUE in comparison to the other dosages. The 100% ETc water dose achieves a greater yield and WUE than the 50% ETc and 75% ETc water doses. According to the experimental findings, the optimal combination of watering frequency, in addition to amount, at F_1 + W_3 produced the most comprehensive outcomes. Considering the prevailing weather conditions, this irrigation schedule may be the most logical for tomato production.

CONCLUSION

Tomato evapotranspiration varied slightly on every alternate day thus, that would be wise to make daily schedules for irrigation to better regulate water dosages. Under the experimental conditions, plant growth, fruit production, and water usage efficiency were significantly impacted by irrigation frequency and water doses, except for stem diameter. The highest yield was obtained from the highest irrigation frequency at F_1 and the highest water dose at W_3, but the highest WUE was obtained at a lower irrigation frequency under the treatment F_2 and the highest water dose at W_3. The irrigation water requirement was highest at the highest irrigation frequency with the highest water dose at F_1W_3, whereas the irrigation requirement was lowest at lower irrigation frequency with the lower water dose at F_2W_1.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors state there is no competing interest.

Author contribution

Tahmina Akter: Wrote the main manuscript text, analyzed the results and discussion, and reviewed the manuscript.
Nargis Akter: Collected the data from the field, analyzed the results and discussion, and reviewed the manuscript.
Ashutus Singha: Analyzed the results and discussion, reviewed the manuscript.
M.N.N. Mazumder: Interpreting the results, reviewed the manuscript.

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Characterization of local Besni pepper (*Capsicum annuum* L.) genotypes with SRAP and ISSR markers

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Abstract

Türkiye has great plant diversity due to its geographical location and suitable climatic and soil conditions. This genetic richness will be meaningful with the studies to be done on it. Characterization studies carried out on local varieties are important in determining the characteristics of the variety and thus including those with superior characteristics in breeding studies. This study aimed to characterize the Besni Pepper genotypes a local variety with molecular (DNA) markers. In the study, 29 pepper genotypes were screened with 10 ISSR and four SRAP primers, and 62 scorable bands were produced. While the polymorphism rate was determined as 76% (47/62), the average number of polymorphic fragments for each primer was calculated as 3.3. According to the created dendrogram, 29 pepper genotypes formed 2 main groups, and these groups were divided into subgroups. The genetic similarity rate was found to be between 0.68-0.95. While the B1 and C3 genotypes were the most distant from each other, the G2 and G3 genotypes were the closest to each other. The study shows that even pepper genotypes from a narrow area have about 32% variation that can be used in future breeding studies. This variation is due to both the fertilization biology (%3-30 cross-pollination) of pepper and seed mobility between farmers. After selection studies for yield, quality, and stress conditions, genotypes can be included in breeding programs.

Keywords: *Capsicum annum*, Molecular characterization, SRAP, ISSR, Polymorphism

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INTRODUCTION

The genus *Capsicum* is native to South America and it is accepted that both Central America and Mexico are considered as a diversity region for the taxa and it is classified as an important genetic resource for agriculture and food. *Capsicum annuum* L., belonging to the genus *Capsicum* of the *Solanaceae* (nightshade) family, is commonly known by names such as sweet pepper, cayenne pepper, hot pepper, paprika, red pepper, Christmas pepper, and bell pepper (Zhigila et al., 2014). Pepper is an important species that is grown as an annual in temperate climates and as a perennial in tropical climates and can be grown both in open fields and in protective cultivation (Vural et al., 2000: Singh et al., 2011). Pepper is a fruit-bearing vegetable species and both its green unripe fruits and its ripe fruits of different colors are consumed in different ways, either fresh or processed (Swamy, 2023). One of the oldest cultivated crops, peppers were domesticated in parts of Mexico about 6,000 years ago (García-Gaytán et al., 2017). Pepper, which are native to South America and domesticated in Mexico, is of great commercial interest today and is consumed in different ways by a significant portion of the world's population (Barboza et al., 2022). Peppers have attracted attention not only for their importance in human nutrition but also for their potential as medicinal plants. This is due to the high phytochemical content of both ripe and unripe fruits that have been shown to be beneficial to human health. Beneficial phytochemicals contained in peppers include carotenoids, flavonoids, ascorbic acid and phenolic compounds with antioxidant properties, and capsaicinoids, which give peppers their spicy flavor (Bosland, 2000; Crosby et al., 2005). Studies have shown that capsaicin can reduce pain, protect cells from damage, and reduce inflammation (Fattori et al., 2016). Peppers are also an important source of vitamin A,

vitamin C, vitamin B, vitamin E, and folic acid (Wahyuni et al., 2011). Recent studies have added 5 new species to the 38 species in the *Capsicum* genus, bringing the total number to 43 (Barboza et al., 2019). Heiser and Smith (1953) reported that there are five economically important pepper species: *C. annuum*, *C. frustescens*, *C. pendulum*, *C. baccaum,* and *C. pubescens* (Samos and Kundt, 1984; Roy, 2016). A diploid and self-fertile species with 2n=24 chromosomes within the genus *Capsicum*, *C. annuum* is one of the major vegetable species grown in large quantities in Türkiye and the world (FAO, 2023). *Capsicum,* originating from the New World tropics and subtropics, has a genetic diversity center in and around Mexico. Peppers were introduced to Spain from the Americas by Columbus, who returned from one of his journeys to the Caribbean in the late 15th century. Pepper cultivation spread from the Mediterranean region to England in the mid-16th century and to Central Europe towards the end of the 16th century. The Portuguese brought it to India from Brazil in the last quarter of the 19th century. Cultivation began in China in the late 1700s (Vidhi, 2023). According to FAO (2023) statistics, approximately 38 million tons of fresh (green and red) and 5.8 million (M) tons of dried pepper were produced in the world, and the world's major pepper-producing countries are China (17 M tons), Mexico (3.7 M tons), Indonesia (3.1 M tons) and Turkiye (3.1 M tons), respectively. It was brought to Istanbul in the 16th century with the improvement of relations between the Ottoman Empire and European countries (Vural et al., 2000). There are also sources reporting that pepper was brought to Turkiye via the Middle East (Andrews, 1999)

Türkiye, located at the intersection of the Mediterranean, European, and Near Eastern gene centers, exhibits significant regional diversity in climate and soil characteristics due to both its location and topographic structure. In addition, as one of the oldest regions in the world where agriculture was first practiced, it has caused many plant species to show diversity and to be a micro gene center (Tan and Inal, 2003). As mentioned above, pepper production is carried out in almost all regions of Türkiye, one of the important pepper-producing countries (3.4 M tons) (TUIK, 2024). Over time, significant genetic diversity has occurred due to new introductions and cross-pollination, and many local pepper varieties have emerged in different regions of Turkiye. Besni pepper, the subject of this study, is one of them. Genetic diversity depends on the genetic characteristics and fertilization biology of the species (Figdore et al., 1988). Pepper tends to cross-pollinate up to 30% depending on the flower structure (heterostyle) and pollinator activity (Rego et al., 2012).

Plant germplasm is an indispensable resource for the development of high-yielding/high-quality cultivars that are tolerant or resistant to environmental (biotic, abiotic) stress factors to secure the long-term sustainability of agricultural production. Local populations consisting of landraces that have adapted to their environmental conditions for long periods of time and have survived against some abiotic and biotic stress factors are important resources for plant breeding. Türkiye, one of the world's major vegetable-producing countries, has unique resources in terms of locally cultivated plant varieties developed by the selection of local farmers and still exhibiting significant diversity. Climate change, replacement of local varieties with modern varieties, forest fires, increased land clearance, erosion, urbanization, road and dam construction, alternations in plant production and plant protection methods (intensive use of agricultural chemicals), and the continuous collection of bulbous plants from nature cause the diversity of plant genetic resources to gradually decrease. Countries and their governments that are aware of this important problem in plant germplasm have long begun to work on the collection, characterization, evaluation, and protection (*in-situ* and *ex-situ*) of resources (Tan, 1992; Sahin et al., 2022). Characterization of plant resources can be done with both classical and molecular/DNA markers. Genetic markers used in the characterization of organisms are generally classified into two categories: classical and DNA/molecular markers. While morphological, cytological, and biochemical markers are classified as classical markers, examples of molecular markers are PAPD (Randomly Amplified Polymorphic DNAs), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplification Fragment Length Polymorphism), SSRs (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats), SNP (Single Nucleotide Polymorphism) and DarT (Diversity Array Technology markers) (Jiang, 2013; Hulse-Kemp et al., 2016; Karataş et al., 2017). Molecular markers are preferred because they are not affected by environmental conditions, like morphological and biochemical markers, and can be repeated in different places and conditions.

With the development of biotechnology, genetic similarities and differences can be clearly revealed as a result of molecular characterization analyses performed on plants (Geleta et al., 2005). As with many other plant species, many molecular characterizations have been carried out in pepper. Lijun and Xuexiao (2012) screened 28 pepper lines from 5 pepper varieties with 13 ISSR primers to examine genetic diversity within and between species. Of the 135 bands produced, 105 were found to be polymorphic, and the polymorphism rate was calculated as 78%. They reported that pepper varieties were divided into 2 main groups in the dendrogram. They reported that Shannon's information index (I) was between 0.06 and 0.13, and Nei's genetic diversity index (H) was between 0.03 and 0.08. In another study where 16 genotypes belonging to Cırgalan pepper (*C. annuum*), one of the local varieties of Türkiye, were characterized with ISSR markers, 12 of the 16 ISSR primers gave results, and the polymorphism rate was 22% (23/104). The genotypes were divided into two main groups, 10 in the first cluster and 6 in the second cluster. Researchers reported that the genetic similarity rate among Cırgalan pepper genotypes varied from 0.95 to 0.99, and genetic diversity was low (Pınar et al., 2017). In a study in which 26 pepper genotypes were characterized with 26 SRAP primers, while no amplification was observed in 8 SRAP primers, 90

polymorphic bands were produced with 18 SRAP primers and evaluated. The genetic variation between genotypes was between 24 and 72%, and the PIC (Polymorphic Information Content) value was between 0.09-0.99 (Adalı 2017). The genetic relationship of 30 pepper varieties, including capia, elongate, Charleston, and bell peppers, was studied with six SSR markers and it was found that genetic diversity was low in SSR loci (Şeker 2018). In the study conducted by Cesur et al. (2020) with the SSR maker on 10 pepper genotypes, it was determined that the genotypes were divided into two main groups; the total allele number was 162, the specific allele number was 60, and the band size varied between 164 and 294 bp. The PIC ranged from 0.04 to 0.89. In a study where *C. annuum* var. *acuminatum*, *C. annuum* var. *grossum*, *C. annuum* var. *abbreviatum*, *C. frutescens* var. *baccatum* species and varieties were used as plant material in West Africa, 75 loci were identified, and 14 of them were recorded as polymorphic. The PIC value was determined as 0.67, and heterozygosity (He) was 0.78. Based on the results obtained, the authors suggested that *C. frutescens* var. *baccatum* should be considered as a variety of *C. annuum* (Olatunji and Afolayan, 2019). Tatar (2022) characterized Gaziantep pepper genotypes using 31 SRAP and 13 ISSR primers. The polymorphism rates were reported as 83% (211/254) in the SRAP method and 85% (110/128) in the ISSR method, respectively. According to the SRAP and ISSR methods, the similarity rate was determined to be 0.40-1.00 and 0.48-0.80, respectively.

Local varieties preferred by the local people and grown in limited areas are part of the sustainable local economy and are indispensable for countries. Along with the changing world, the characteristics of the products demanded by the consumer are also changing. For this reason, it is important to determine the characteristics of local varieties and include the superior ones in breeding studies. Furthermore, determining the genetic relationship between the genotypes with determined characteristics is also extremely important for the breeders. This study aimed at the molecular characterization of Besni pepper genotypes grown in Besni district of Adıyaman province, whose morphological characterization was performed by Sahin et al. (2022).

MATERIAL AND METHODS

Experimental site and plant materials

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

Table 1. Pepper genotypes used in the study and locations where they are collected

Genotypes	Supplied location	Genotypes	Supplied location
B_1	Besni-Oyratlı	B_{16}	Besni-Oyratlı
B_2	Besni-Oyratlı	B_{17}	Besni-Oyratlı
B_3	Besni-Oyratlı	B_{18}	Besni-Oyratlı
B_4	Besni-Oyratlı	B_{19}	Besni-Oyratlı
B_5	Besni-Oyratlı	B_{20}	Besni-Toklu
B_6	Besni-Oyratlı	G_1	Gölbaşı-City Center
B7	Besni-Oyratlı	G_2	Gölbaşı-City Center
B_8	Besni-Oyratlı	G_3	Gölbaşı-City Center
B_9	Besni-Oyratlı	G_4	Gölbaşı-Maltepe
B_{10}	Besni-City Center	G_5	Gölbaşı-Maltepe
B_{11}	Besni-Oyratlı	G_6	Gölbaşı-Maltepe Village
B_{12}	Besni-Oyratlı	C_1 (Cırgalan)	ERU Faculty ofAgriculture
B_{13}	Besni-Çamurcu	C_2 (Yalova Çorbacı)	ERU Faculty of Agriculture
B_{14}	Besni-Çamurcu	C_3 (Sera Demre)	ERU Faculty of Agriculture
B_{15}	Besni-Oyratlı		

B: Besni; G: Gölbaşı; C: Control; ERU: Erciyes University

Cultivation of plants

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

Molecular characterization

PCR-based ISSR and SRAP marker analysis methods were used for molecular characterization. Ten ISSR primers (Table 2) and four SRAP primer pairs (Table 4) were used in the study. Isolation of pepper DNA was performed using 50-60 mg of young leaf material using the CTAB extraction protocol reported by Doyle (1991). The PCR mixture was prepared using 30 ng of template DNA, 1U Taq DNA polymerase enzyme, 0.25 mM each dNTP, 1 µM primer, 1.5 µl 10X PCR buffer solution, 1.5 mM $MgCl_2$, and H_2O, with a total volume of 20 µl. The PCR cycles (Table 3) were performed according to Uzun (2009).

Table 2. ISSR primers used in molecular characterization

Pimer name	Primer sequence 5'-3'
AGC6G	AGCGGGGGG
AG7YC	AGAGAGAGAGAGAGYC
TCC5RY	TCCTCCTCCTCCTCCRY
CA6AC	CACACACACACAAC
CAC3GC	CACCACCACGC
GA8Y9	GAYGYGYGYGYGYGYGYG
GACA4	GACAGACAGACAGACA
AG8T	AGAGAGAGAGAGAGAGT
CA8R	CACACACACACACACAR
BDBCA7C	BDBCACACACACACACAC

Table 3. PCR conditions for ISSR primers used in the study

Step		Temperature	Time	Number of cycles
1	Initial denaturation	94 ˚C	3 min	1
	Denaturation	94 ˚C	1 min	35
2	Annealing	53 ˚C	50 s	35
	Extension	72 ˚C	2 min	35
3	Final extension	72 ˚C	7 min	1
	Storage	4 ˚C	∞	1

In the SRAP method, PCR mixture was prepared using a total volume of 20 µl, 30 ng Template DNA, 1U Taq DNA polymerase enzyme, 0.25 mM each dNTP, 1 µM primer, 1.5 µl 10X PCR buffer solution, 1.5 mM $MgCl_2$, and H_2O. The PCR cycles (Table 3) were performed according to Uzun (2009).

Table 4. SRAP primer pairs used in molecular characterization

Primer name	Primer sequence 5'-3'	
	Forward	Reverse
Em11-Me2	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGAGC
Em11-Me6	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGACA
Em5-Me12	GACTGCGTACGAATTAAC–	TGAGTCCAAACCGGAGA
Em11-Me12	GACTGCGTACGAATTCTA –	TGAGTCCAAACCGGAGA

Table 5. PCR conditions for SRAP primers used in the study (Uzun, 2009)

Step		Temperature	Time	Number of cycles
1	Initial denaturation	95 ˚C	3 min	1
	Denaturation	94 ˚C	45 s	5
2	Annealing	35 ˚C	1 min	5
3	Elongation	72 ˚C	1 min	5
4	Denaturation	94 ˚C	45 s	35
5	Annealing	50 ˚C	1 min	35
6	Elongation	72 ˚C	1 min	35
	Last elongation	72 ˚C	5 s	1
7	Storage	4 ˚C	∞	1

Statistical analysis

In the study, the bands in the gel images obtained from the ISSR and SRAP marker methods were scored as present (1) and absent (0). The data were analyzed in the NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pcversion 2.11, Exeter Software, Setauket, N.Y., USA, Rohlf, 2000) computer package program. Similarity indices were calculated according to the DICE (1945) method and the dendrogram was created according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method. Key metrics such as the total number of bands, number of polymorphic bands, average polymorphism, effective number of alleles (Ne), allele frequency, Shannon's information index (I), expected heterozygosity (He), and the ratio of unbiased expected heterozygosity (uHe) were determined. Principal Component Analysis (PCA) was done based on the

variance-covariance matrix. The polymorphism rates of the primers were calculated according to the formula below. Polymorphism rate = (Number of polymorphic bands/total number of bands) x 100.

RESULTS

The data generated from the ISSR and SRAP marker systems used in the study were analyzed together and a similarity dendrogram was created with these data. In this study, scorable images (Figure 1) were obtained from 10 primers out of 16 ISSR primers and four primer pairs out of 18 SRAP primer pairs tested for 29 pepper genotypes. A total of 14 primers, 10 ISSR, and four SRAP, were evaluated and data were generated. For the data produced by ISSR and SRAP marker methods for 29 pepper genotypes, primer band length (bp), number of bands (number), polymorphic band number (number), polymorphism rate (%), monomorphic band number (number), and monomorphism rate (%) were presented in Table 6. A total of 62 bands were obtained, with an average of 4.4 bands per primer, and band lengths ranged from 150 to 1300 bp (Table 6). It was recorded that 47 of these bands were polymorphic, the average number of polymorphic bands was calculated as 3.3, while the average polymorphism rate was calculated as 66.3%. Primers with 100% polymorphism rate were BDBCA7C, Em11-Me2, and Em11-Me6, while primer combinations Em5-Me12 and Em11-Me12 produced monomorphic bands. A total of 15 monomorphic bands were obtained and the average number of monomorphic bands was calculated as 1.07.

Table 6. Band profiles and percent polymorphism rates of ISSR and SRAP primers in 29 pepper genotypes

Primers	Primer sequence 5'-3'	Band length	Number of band	Polymorphic band number	Polymorphism rate (%)	Monomorphic band number	Monomorphism rate (%)
AGC6G	AGCGGGGGG	200-1200	9	8	% 88	1	% 11
AG7YC	AGAGAGAGAGAGAGYC	550-1000	2	1	% 50	1	% 50
TCC5RY	TCCTCCTCCTCCTCCRY	370-1020	5	4	% 80	1	% 20
CA6AC	CACACACACACAAC	560-1100	4	2	% 50	2	% 50
CAC3GC	CACCACCACGC	300-1300	7	6	% 85	1	% 14
GA8Y9	GAYGYGYGYGYGYGYGYG	150-1000	5	3	% 60	2	% 40
GACA4	GACAGACAGACAGACA	350-1200	6	4	% 66	2	% 33
AG8T	AGAGAGAGAGAGAGAGT	200-1200	8	6	% 75	2	% 25
CA8R	CACACACACACACACAR	450-1020	4	3	% 75	1	% 25
BDBCA7C	BDBCACACACACACACAC	400-1020	6	6	% 100	0	% 0
Em11-Me2	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGAGC (R)	150-300	2	2	% 100	0	% 0
Em11-Me6	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGACA (R)	300-430	2	2	% 100	0	% 0
Em5-Me12	GACTGCGTACGAATTAAC (F) TGAGTCCAAACCGGAGA (R)	350	1	0	% 0	1	% 100
Em11-Me12	GACTGCGTACGAATTCTA (F) TGAGTCCAAACCGGAGA (R)	200	1	0	% 0	1	% 100
Total			62	47		15	
Average			4.4	3.3	%66.3	1.07	%33.4

Table 7. Polymorphism values of studied primers

Primers	p	q	Ne	I	He	uHe
AGC6G	0.248	0.752	1.287	0.307	0.187	0.191
AG7YC	0.661	0.339	1.389	0.315	0.219	0.223
TCC5RY	0.557	0.443	1.464	0.432	0.287	0.292
CA6AC	0.791	0.209	1.473	0.340	0.243	0.249
CAC3GC	0.379	0.621	1.370	0.398	0.250	0.257
GA8Y9	0.543	0.457	1.340	0.325	0.215	0.219
GACA4	0.568	0.432	1.381	0.355	0.235	0.240
AG8T	0.579	0.421	1.488	0.424	0.286	0.292
CA8R	0.686	0.314	1.580	0.461	0.320	0.326
BDBCA7C	0.489	0.511	1.921	0.672	0.479	0.488
Em11-Me2	0.700	0.300	1.697	0.587	0.400	0.408
Em11-Me6	0.437	0.563	1.586	0.547	0.363	0.372
Em5-Me12	1.000	0.000	1.000	0.000	0.000	0.000
Em11-me12	1.000	0.000	1.000	0.000	0.000	0.000
Average	0.617	0.383	1.426	0.368	0.248	0.254

p and q: Allele frequency; Ne: Effective number of alleles; I: Shannon's information index; He: Expected heterozygosity; uHe: Unbiased expected heterozygosity.

Expected and observed allelic frequency values (p and q) ranged from 0.248 to 1.0 and from 0.00 to 0.752 (Table 7). The effective number of alleles (Ne) of the genotypes varied from 1.0 to 1.921 with a mean of 1.426, whereas the mean Shannon's Information index (I) was 0.368 with individual values ranging from 0.0 to 0.672. The expected heterozygosity (He) was found to be between 0.0 and 0.479 (average 0.248), and the unbiased expected heterozygosity (uHe) value varied from 0.0 to 0.488. The expected heterozygosity for all primers except Em5-Me12 and Em11-me12 was found to be lower than the unbiased expected heterozygosity.

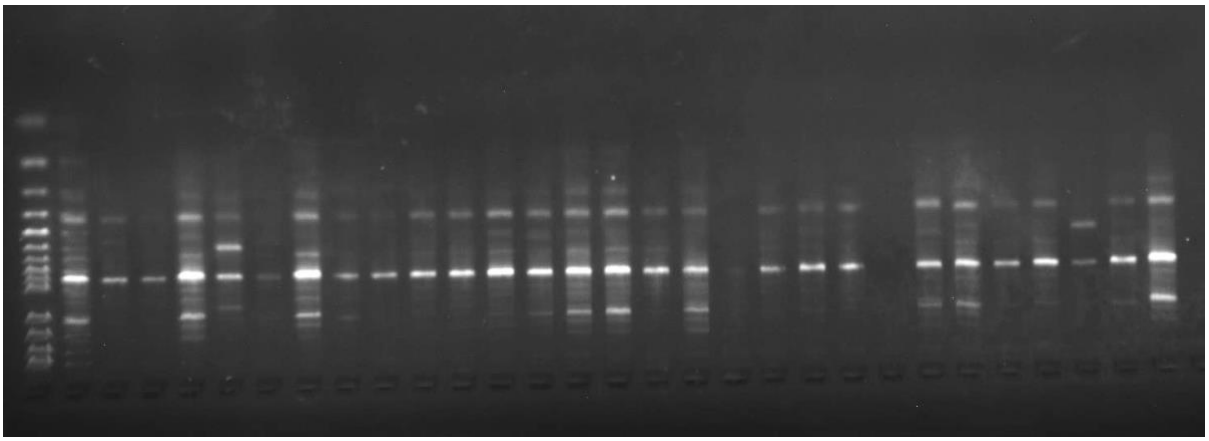


Figure 1. Gel image of AGC6G primer.

In the dendrogram created according to the Dice similarity matrix, the genetic similarity rate of Besni pepper varies between 0.68 and 0.95. According to the dendrogram, the genotypes were divided into two main groups at a similarity level of 68%: a large group with 24 genotypes and a small group with five genotypes. These groups also formed subgroups among themselves (Figure 2). Genotypes G5, G6, C1, C2, and C3 were included in the second (II) group, and the other 24 genotypes were grouped in the first (I) group. The large group was divided into two subgroups at 80% similarity level, and 11 genotypes collected from Besni district were grouped in the first subgroup (I-1), while the second subgroup (I-2) included 9 genotypes taken from Besni district and 4 genotypes taken from Gölbaşı district. I-1 and I-2 subgroups were divided into two groups (I-1-i and I-1-ii; I-2-i and I-2-ii) at 82% similarity level. When the dendrogram is examined, it is seen that B1 and C3 are the most distant genotypes, while genotypes G2 and G3 show 95% similarity and are the closest genotypes to each other among the other genotypes. Samples taken from the same village are located close to each other in the dendrogram.

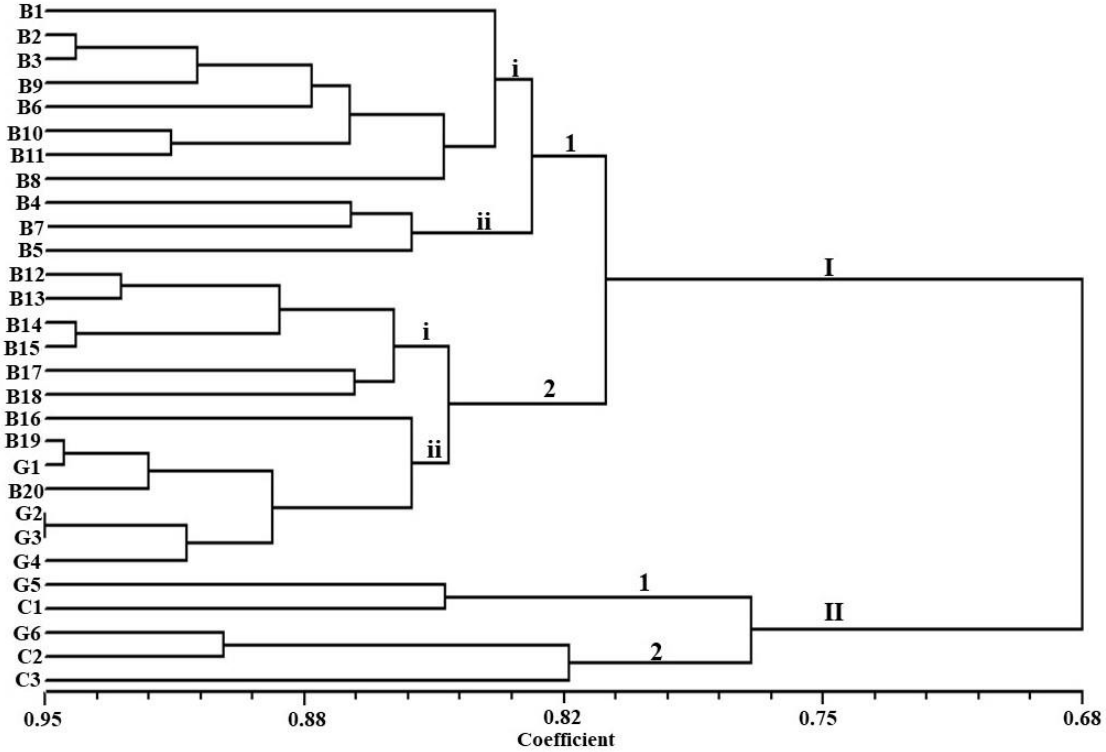


Figure 2. Dendrogram of the 29 genotypes using UPGMA method produced from SRAP and SSR markers.

According to the Two-Dimensional Principal Component Analysis graph, 29 pepper genotypes formed two different groups that were not close to each other. While one group consisted of genotypes taken from Besni district, the other group consisted of genotypes taken from Gölbaşı district and control varieties. Genotypes B1 (1) and B18 (18) collected from Oyratlı village were located in a different place than the other genotypes. It was observed that the genotypes collected from Oyratlı, Toklu, and Çamurcu villages of Besni district were separated from the other genotypes. In addition, it was observed that the genotypes obtained from Gölbaşı district were grouped with the genotypes used as control (Figure 3). In the Three-Dimensional Principal Component Analysis graph, it is seen that the genotypes form two groups. As in the Two-Dimensional Principal Component Analysis graph, it is seen that the genotype numbered B18 (18) is separated from the other genotypes. At the same time, the genotypes collected from Gölbaşı district and the control varieties are located close to each other and form a group (Figure 4).

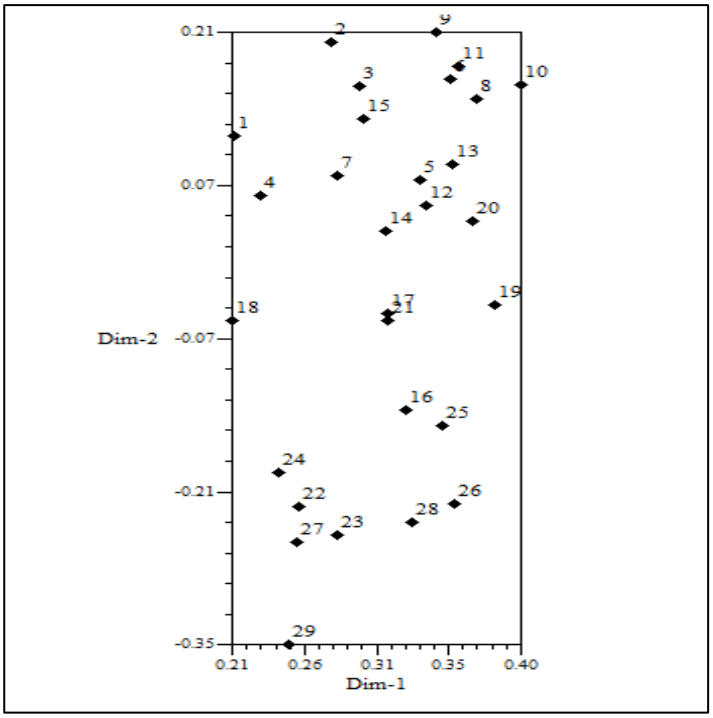


Figure 3. Two-dimensional graph created as a result of Principal Component Analysis with ISSR and SRAP data on 29 pepper genotypes.

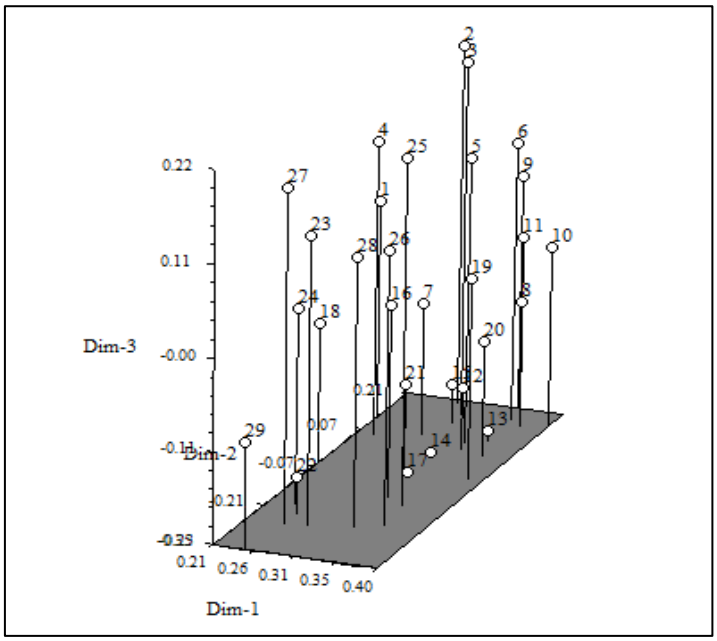


Figure 4. Three-dimensional graph created as a result of Principal Component Analysis with ISSR and SRAP data on 29 pepper genotypes.

DISCUSSION

In this study, the variation rate was determined in a total of 29 pepper genotypes, 26 Besni pepper genotypes, and 3 control varieties, using ISSR and SRAP marker methods. A total of 62 bands were obtained in the study, 15 of which were monomorphic and 47 were polymorphic. A variation between genotypes was found between 0.68-0.95 (32%). The groupings depending on the variation are shown in Figure 1, Figure 2, and Figure 3.

Numerous molecular characterization studies have been reported on peppers, with different results reported depending on the populations and primer systems used. There are several reports on the effectiveness of PCR-based techniques, including ISSR as well as other marker methods such as SRAP, in assessing the relationship or variability among different *Capsicum* genotypes (Ibarra-Torres et al., 2014; Ekbiç and Okay, 2024). In general, all ISSR markers used in the study produced clear and reproducible amplification profiles. When the marker efficiency was further quantified by heterozygosity values, it was shown that ISSR primers were effective in assessing genetic relatedness in cultivated *Capsicum* species cultivars. Oblatunji and Afloyan (2019) compared pepper genotypes and found higher He values (0.32 to 0.88) than our study. This is because they used genotypes from different *Capsicum* species while we used only *C. annuum* genotypes. Similarly, Cesur et al. (2020) and Ekbiç and Okay (2024) reported similar heterozygosity rates to our study using only *C. annum* genotypes. Yang et al. (2005) found a lower polymorphism rate than our study in their study in which 11 pepper genotypes were screened with 12 ISSR primers. In a study conducted using 18 SRAP primer pairs in 26 pepper genotypes, it was recorded that 90 polymorphic bands were obtained. The genetic similarity rate between all genotypes varied between 72% and 24%. In the study, it was reported that a high genetic diversity was observed in *C. annuum* genotypes (Adalı, 2017). In our study, lower rates of variation and polymorphic were detected. The difference in the number of polymorphic bands between two studies using approximately the same size population can be attributed to the number of SRAP combinations and genotypic differences (genotypes collected from a wider area and selected from different backcross populations). Similarly, in the study conducted by Okay (2019) with more genotype and primer combinations, the number of groups (5) and variation rates (35%-97%) were found to be higher than in our study. We thought that this difference was because the genotypes in our study were collected from a narrower area and the number of genotypes used was low. On the other hand, in a study conducted by Rana et al. (2014), 24 pepper genotypes were screened with 12 ISSR primers. A total of 59 bands were obtained, 29 of which were reported as polymorphic. While this study is parallel to our study in terms of the total number of bands obtained, the number of polymorphic bands was found to be higher in the Besni pepper population. This difference may be due to the higher number of genotypes used in our study. Bozokalfa et al. (2017) reported similar results to our study (0.62-0.94) in their study where 94 pepper genotypes were analyzed with 33 SRAP combinations. Although they used more genotypes and SRAP combinations in their studies, the fact that lower variation was reported compared to the studies given above can be explained by the low genetic diversity.

Similar to Besni pepper, Cırgalan local pepper variety, grown in limited areas in Kayseri province and some districts was also characterized with 16 ISSR primers, and it was found that 23% of the bands were polymorphic and the genetic similarity ratio was between 0.95-0.99. The reason for the low genetic diversity (5%) among Cırgalan pepper genotypes may be that it was grown in a very narrow area, pepper cultivation is low in the region, and the flower structure of Cırgalan pepper (Pınar et al., 2017). Ahmed (2013) reported that six hybrid pepper lines were characterized with 10 ISSR primers, and 60% (57) of the 87 bands produced were found to be polymorphic. In a study conducted in India with ISSR primers on 30 Bhut jolokia peppers, it was reported that pepper genotypes formed 3 groups and genetic differences were between 57% and 92% (Hazarika and Neog, 2014). López-Espinosa et al. (2018) reported that a total of 32 bands were obtained as a result of screening 60 Habareno peppers native to the Yucatan Peninsula of Mexico with 3 ISSR primers. The polymorphism rate was 98%.

As seen in the studies given above, ISSR and SRAP marker systems can be used to detect variations in pepper populations. They were also used successfully in our study and produced descriptive results. As a result of the analysis, although there were some minor exceptions, pepper genotypes were grouped according to where they were taken and fruit shape (Shain et al., 2022). As seen in the literature given, different variation rates were detected in different populations. These differences can be attributed to genotypic differences, the number of primers used, and the flower structure of the genotypes. Depending on the heterostyly observed in pepper species, cross-pollination or self-pollination can be encouraged. In populations where self-pollination is encouraged by flower structure, pollinating agents, and climatic conditions, diversity decreases, while in opposite conditions, cross-pollination is encouraged, and diversity increases (Zhang et al., 2023).

CONCLUSION

In this study, 26 Besni pepper genotypes and three cultivars that were morphologically characterized in our previous study were molecularly characterized. As a result of the analysis, a total of 62 bands were produced, 52 of which were polymorphic. In the dendrogram created, it was seen that the genotypes were divided into two main groups consisting of two subgroups. While the genotypes G2 and G3 were the most similar to each other, the two most dissimilar genotypes were B1 and C3. It was noted that the genotypes were grouped according to where they were collected from and shape of the fruit. Genotypes obtained from the same village but from different farmers

were mostly in the same group. This may be because farmers multiplied the seeds of genotypes whose characteristics they preferred and exchanged them among themselves. Molecular studies on local genotypes are an effective method to strengthen morphological studies. At the same time, the information produced about the genetic relationships (similarities and differences) of the studied material will provide important opportunities for plant breeders in breeding studies. Identifying genotypes with unique characteristics among local pepper genotypes, adding them to the base population, and protecting them are of vital importance for the sustainability of genetic diversity and plant production. The current population has not been evaluated in terms of yield, quality, and tolerance to stress conditions (biotic and abiotic). For these purposes, the Besni pepper population should be evaluated and prominent genotypes should be included in appropriate breeding programs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Competing Interest

All authors have no conflicts of interest to disclose.

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Assessing determinants of livelihood vulnerability to climate change impact in rural communities context in arsi and east shewa zones of oromia region, Ethiopia

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Abstract

Livelihoods vulnerability is compounded effect of socio-economic and environmental factors that interact under a set of social systems that can be managed with proper strategic measures. Accordingly, Ethiopia has already adopted various responsive measures to maintain sustainable livelihoods. However, studies reveal that most of the strategies have been designed without adequate information on the vulnerability level and determining factors. This study identified the livelihoods vulnerability status and determinants in context of climate change impacts. Farmers' perceived vulnerability, ranking technique and IPCC-LVI model were employed to a wide-ranging of primary data collected from 410 farmers sampled randomly from four districts of Arsi and East Shewa zones of Oromia region. The results revealed that the climate structures have been significantly changing over time imposing challenges on livelihoods sustainability, while government has introduced various response measures. In this regard, community level livelihoods vulnerability rating score was 49% and 66.7% in highland and lowland, respectively, indicating more adverse situation under lowland community, relative to highland areas. Additionally, the perceived sensitivity rating score was 45% in highland community and 74% in context of lowland community, while the corresponding perceived community exposure are 54.5% and 77.3% in highland and lowland community. Contrarily, adaptive capacity rating score indicate about average level with slightly small difference that is 53% and 52% for highland and lowland agro-ecology respectively showing moderate level adaptive capacity of the study community to manage prevailing climate change impacts associated risks. These results demonstrate a need to strengthen adequate information on community level livelihoods vulnerability, exposure, sensitivity and adaptive capacity. Therefore, policy makers should redesign appropriate regional policy, strategy and extension approaches based on well identified community vulnerability. This ensures environmentally suitable rural development in smallholders farming system.

Keywords: Climate, change, Impacts, Livelihoods, Vulnerability, Smallholder and Farmers

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INTRODUCTION

The developing countries have been already vulnerable to climate change impacts which commonly manifested through extreme weather events (IPCC, 2007). In the context, developing countries are most vulnerable to frequent climate change impacts as the result of scarcity of capital to promote adaptation measures and high possibility of exposure to disasters (World Bank 2009). According to various findings, weather risks which commonly influence livelihood vulnerability are the most pressing consequences of climate change at global and community level. In

this regard, with a 0.6°C global temperature change, 2-3% increase in rainfall was projected in tropical and 3% decrease in subtropical latitudes; were observed in the end of 20th century, while an increase of 1.4°C and 5.8°C global temperature projected by the end of the 21st century inducing vulnerability (Udin, 2014).

Under Ethiopian circumstance, climate change related impacts are apparent through increased frequency of droughts and floods which required adequate research prior to promotion of adaptation strategies (Asfaw and Lipper, 2011, Howden; 2007 and Morton, 2007). As a result, Ethiopia has been identified among the utmost vulnerable countries to direct impacts of climate change and indirect negative effects on environmental sustainability (The World Bank Group, 2011). Apparently, in Ethiopia, more than 83% of the population entirely depends on rain-fed agriculture and thus, any small change in seasonal rainfall patterns and temperature has a direct impact on agricultural productivity (MoANR, 2017) which entirely increase the livelihoods vulnerability to climate change.

In a more detail, vulnerability is a resultant effect of three major factors: namely exposure, sensitivity and adaptive capacity as well as their relationship in the system that can be described as a set of factors that categorize certain individuals/groups as "vulnerable" to certain natural disasters. Vulnerability is also defined as the degree to what extent a particular system is prone to adverse impacts of natural disasters (Arega, 2013 and Adger, 2006). Generally, understanding vulnerability with its components helps to evaluate the nature and magnitude of climate change impact, identify sources of vulnerability and to design climate smart adaptation responses (Marshall et al, 2009).

In Ethiopia, a range of improved technologies and practices have been developed and disseminated to the broader farming communities to manage vulnerability to climate change impacts (Rosegrant, et al., 2008). Consequently, this study has been designed to generate adequate information on livelihoods vulnerability at community level and provides the proper recommendation for policy level and technical managers in respective of Oromia Region's rural community in general and study community in particular. Additionally, households' food self-sufficiency in rural communities and national food security are significantly subjective to normal performance of seasonal rainfall reliant farming systems which is naturally sensitive to climate change and weather variability in Ethiopia in general and the study areas in particular. However, the extent studies, like Muluneh (2015), indicate that Ethiopian Central Rift Valley (CRV) which embraces the study areas is under continuous threat due to frequent environmental degradation, leading to structural changes in climatic condition in the form of recurrent drought and seasonal weather variability.

On the other hand, except some few areas, location specific studies on estimating the overall impacts of climate change in context of introduced strategies are inadequate. According to studies, for instance, Mulatu et al., 2016 and FAO,2016, this is a notable gap that is yet to be sufficiently researched in the country. This research is significant because it will identify the location specific factors that are responsible for the livelihoods vulnerability in comparison between highland and lowland agro-ecologies emphasizing on four districts and twelve Villages of East Shewa and Arsi zones of Oromia region. Consequently, this study has been designed to generate adequate information on livelihoods vulnerability at community level and provides the proper recommendation for policy level and technical managers in respective of Oromia Region's rural community in general and study community in particular.

METHODS AND MATERIALS

General Context of the Areas Selected for the Study

The Areas selected for this study are situated in Oromia Regional State along the Great Central Rift Valley (CRV), where risks associated with climate change and weather variability are common and affecting livelihoods of the community. Commonly, mixed farming and agro-pastoral farming system are regularly conducted in each of highland, mid and lowlands. In this context, four districts, of which Bosat and Dudga districts were selected from East Shewa, while Hetossa and Tiyo districts were selected from the Arsi Zone. Most of the villages in the districts are situated along the Awash river catchment, where recurrent adverse impacts of climate change are severe as the result of frequent drought (Figure 1).

Generally, an altitude of the study areas ranged from 500-3500 meters above sea level (masl). More specifically, the study has been conducted in the east shewa zone representing the low land areas and Arsi Zone, representing the highland parts. The highland (2300 to 3200 masl) with cold climate, while the midland (1500 to 2300 masl), experiencing warm climate and the lowlands (500 to 1500 masl) having hot with arid land areas (Tamirat,2019 and Tamirat 2018). Despite the persistent drought in the lowlands, there are three distinct seasons; with two rainy seasons and one dry season (World Bank Group, 2011).

East Shewa zone covers a total of 971,159.21ha land area with twelve administrative districts (10 rural and 2 urban) in the central lowland of Oromia Region. Agro-ecologically, the zone constitutes 18.7% highland, 27.5% midland and 53.8% lowland with rainfall 1150mm (Asfaw, et al., 2020). As to this author, of total land size, about 12.6% is arable land, of which arable land 47.31% is currently under cultivation practices in every year. Similarly, Arsi the largest Zone of the region is situated in central Oromia Regional State covering an area of 19,825.22 km^2 and consist of 25 administrative districts (WIKIPEDIA, 2023). Generally, due to diverse set of agro-ecological

zonation, Arsi zone is predominantly characterized by moderately cool (40%) followed by cool (34%) annual temperature (Addisu, et al., 2020).

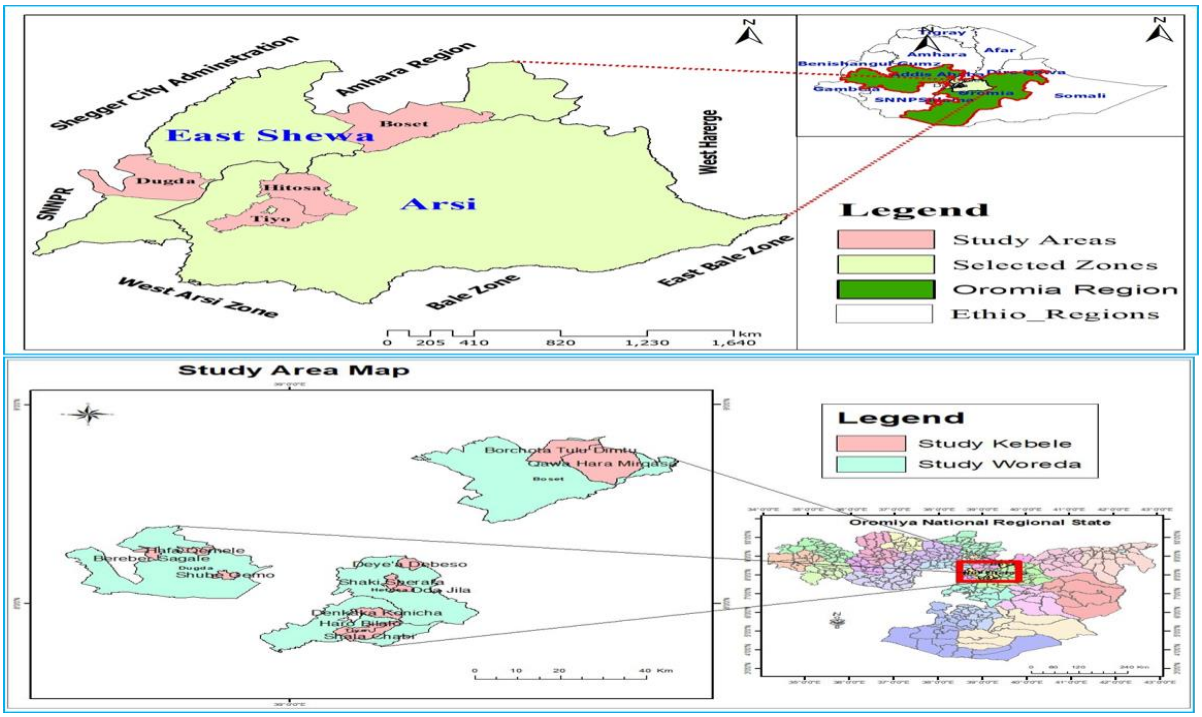


Figure 1. Study area map and location (**Source**: Developed based on google File)

Data collection

A total of 410 farm households were sampled through structured questionnaire interviews across the two agro-ecology of the farming systems in the two zones. To attain the required standard and good quality data, the structured interview questionnaires, formats for secondary data collection and checklist (interview guide) for guiding the discussion process have been developed thus enabling relevant data collection from primary (individual farmers through enumerators managed interviews) and secondary sources (published source documents) for the analytical and crosschecking of the information. Based on the experience of Abate (2009) and Thornton (2010), who listed multi-dimensional vulnerability determinant variables, essential data were identified, where the selected indicators included in the data collection checklist.

The primary data such as socio-demographic (educational level, age, sex, marital status, social networks, farming experience, etc) economic and resources (landholding, livestock holding, crops and cropping systems, seasonal incomes, source of incomes, water availability, sources of livelihoods etc) were collected. Furthermore, infrastructures and services (extension services, public facilities, access to markets, credit and mass media services) and natural disasters (emphasized on drought frequency and weather variability) and strategies (adaptation and coping strategies related data) were collected. The whole spectrum of data were analyzed using Statistical Package for Social Sciences (SPSS) sub-program. Furthermore, participatory discussions and direct observations procedures are intensely synchronized with other data collection techniques with the intention to triangulate the collected information including the study findings. Based on the nature of the data, appropriate analytical techniques were selected and specified against study variables and objective, where descriptive statistical techniques were employed.

On the other hand, livelihoods vulnerability indicators and components were identified to define the required data and relevant information. To delineate the livelihoods vulnerability indicators and components, adequate literature review was conducted. In this regard, the selected exposure impact indicators include; frequency of climate variability, water resource availability, shortage of irrigation water sources, increased drought frequency, increased major agricultural disease and pest occurrence. For adaptive capacity six indicators were used i.e. agricultural livelihoods diversification, non-farm income diversification, wealth and yearly income, use of improved agricultural technology, technical capacity to manage climate change, exposure to technical and Technological supports. Again, sensitivity component indicators include, Seasonal productive crop Land decrease (abandoned land), Crops productivity and production decrease over time, Significant loss of crops varieties over time, decline of livestock number, productivity and production over years and food sufficiency reduced (below yearly 1.8 Qt/person) and increased climate change impact associated Risks.

Data analysis

Descriptive analysis

The identified and collected data were analyzed using descriptive statistics; which includes frequency, percentage and mean emphasizing on the aspect of the data that helps to compare results with ground reality and some other study evidences. In this regard, primary data that are related to perceived impact of climate change were analyzed using descriptive statistical tools across the selected two agro-ecologies based on some selected vulnerability criterion, where the processed data were summarized and presented in tabular formats.

Indexing and Inferential Methods

Index Model for Vulnerability Components Analysis

This section highlights the approaches that were adopted to compute the vulnerability major components (sensitivity, exposure and adaptive capacity) separately before the aggregated vulnerability analyses. In this context, the collected data were analyzed based on the index formula suggested by Nasir et al. (2014), which was employed to determine the adaptation of introduced strategies and innovations in the study community. The model specification is a composite of the summarized components and variables shown below (Formula 1):

$$ASI = ASn \times 0 + \text{Asl} \times + \text{Asm} \times 2 + \text{Ash} \times 3 \quad (1)$$

Where, ASI = Aggregated Adaptation Strategy Index

Asn = Frequency of farmers rating the strategy as having none ……. (0)

Asl = Frequency of farmers rating the strategy as having low level ……. (1)

Asm = Frequency of farmers rating the strategy as having medium level ……. (2)

Ash = Frequency of farmers rating the strategy as having high level ……. (3)

The respondents have been managed to rate present-day status comparing with previous times situation, to generate information over time and they were expected to assign a scoring value on a four-point scale for each indicator in respective of each major component and strategy in viewpoint of ranking. The four-point scales represent as high, moderate, low and negligible in which these ranking levels were represented by 3,2,1 and 0 numerical values, respectively. The acquired data were assessed quantitatively to produces a description related to major vulnerability components from the viewpoint of the respondents which can be presented for each respondent and in aggregate form. The collected data were properly coded and analyzed quantitatively and the results interpreted to draw relevant conclusion that enable the investigator to generate the reliable evidence to encompass the policy recommendations. In this aspect, the score of each variable rating level were summarized and finally aggregated from which the percentage was calculated considering the assumed highest score if all respondents (farmers) rate a particular indicator three (3 point) which is simply 600 for highland and 630 points for lowland agro-ecology, respectively for strategies and variables ranking.

Index Model for Livelihoods' Vulnerability Analysis

In several contexts, climate impact vulnerability is an important aspect of climate change studies which stimulated the selection of Vulnerability analytical model for current study. Based on Adu (2013), IPCC Livelihood Vulnerability Index (IPCC-LVI) technique was used to determine the overall livelihoods vulnerability to climate change impacts. The below (formula 2) is a working equation which indicates the function of the major components:

***Vulnerability = f(Exposure, Sensitivity, Adaptive Capacity*)** **(2)**

The frequently employed procedure to assess vulnerability is the vulnerability index grounded on specifically defined combinations of indicators (Adu, 2013). Accordingly, each of the vulnerability's major component (exposure, sensitivity, and adaptive capacity) usually have sub-components and these components were standardized where average weight of the components were used. To analyze the index of each indicator, it is required to standardize the measurements of each indicator (making unit free), where the following model has been selected and employed to execute the standardization of particular measurement (equation 3).

$$Index_{shi} = \frac{S_h - S_{min}}{S_{Max} - S_{min}} \quad (3)$$

Where S_h is an observed sub-component of particular indicator for *I* household and S_{min} and S_{max} are identified minimum and maximum values of indicators, respectively for specific sub-component. After each indicator has been standardized, the sub-component indicators were averaged using below equation (formula 4).

$$M_h = \frac{\sum_{k=0}^{n} Indexshi}{n} \quad (4)$$

where M_h is one of the eight major components of vulnerability [Livelihood Strategies (LS), Socio-Demographic Profile (SDP), Health (H), Social Network (SN), Water (W), Food (F) and Natural Disaster (ND), or Climate Variability (CV)] for household and the $index_{shi}$ were assumed to characterize sub-components which is commonly indexed by i, that makes up each major component, and n is the number of sub-components in each major component (Hahn, 2008). Then, the working equation to estimate the livelihoods vulnerability based on the IPCC framework is:

LVI-IPCC*= (*Exposure-Adaptive capacity) * Sensitivity ***(5)***

Generally, livelihoods vulnerability components (major and sub-components) and indexing procedures for each component were adapted from IPCC framework. Commonly, several researchers converge to common components and indexing approach adopted from IPCC, where eight major components with 35 sub-components were identified for the study of livelihoods vulnerability. Specifically, Thi et al. (2024), Tran, et al. (2023) and Septri et al. (2024) approaches were adopted as the reference in this particular study to determine overall livelihoods' vulnerability in the study community.

Tobit Regression Model to Assess Determinants of Livelihood Vulnerability

The determinants of livelihood vulnerability to climate change impacts were analyzed using the Tobit regression model. In this regard, Weather-related variables (average, standard deviation, and coefficient of variation for rainfall and maximum temperature) were used directly in the Tobit regression analysis. Due to multicollinearity effect that was observed among the variables, minimum temperature variable was excluded from the model, while the indexed value was used for model estimation. The Tobit model was adopted from Bierens (2004) and the selected model can be expressed as:

$$LVI_i = \alpha + \sum_{j=1}^{3} \beta_j S_{ij} + \sum_{j=1}^{4} \gamma_j C_{ij} + \sum_{j=1}^{2} \delta_j I_{ij} + \sum_{j=1}^{9} \epsilon_j E_{ij} + v_i \quad (6)$$

Where LVI_i is a latent variable denoting the vulnerability of i^{th} household to climate change. In addition, j is the number of variables in each variable category. S_i, C_i, I_i and E_i are the independent variables for social, economic, institutional, and environmental variables, respectively, with β_j, γ_j, δ_j, and ϵ_j as the estimated parameters. The model's error term (v_i) usually assumed to be the independent $N(0; \sigma^2)$ distributed conditionally on independent variables.

RESULTS AND DISCUSSION

Perceived level of Livelihoods vulnerability to climate change impacts

Table 1 below illuminates that majority of respondents perceived the level of climate change impact vulnerability as moderate level in both highland and lowland agro-ecology. However, comparatively the proportion of respondents that indicates the moderate level of vulnerability for highland agro-ecology is slightly higher (63.5%) than lowland (59%) agro-ecology, while in the case of lowland areas, the second majority (33.8%) perceived high level of community vulnerability as compared to only negligible proportion of the respondent from highland agro-ecology indicating that serious situation in the lowland farming system of the study community.

Table 1. Perceived level of vulnerability to climate change impact in the study Community

		Highland		Lowland		Total	
No	**Perceived level of Vulnerability**	Actual	%	Actual	%	Actual	%
1	Highly Vulnerable	2	1.0	71	33.8	73	17.8
2	Moderately Vulnerable	127	63.5	124	59.0	251	61.2
3	Less vulnerable	58	29.0	15	7.2	73	17.8
4	Negligibly vulnerable	13	6.5	-	-	13	3.2
	Total	**200**	**100.0**	**210**	**100**	**410**	**100.0**

In addition to perceived level of vulnerability analysis, three vulnerabilities components were separately analyzed using respective indicators where the results presented in below four Tables (Table 2, 3, 4 and 5) including the perceived level of vulnerability. Among these, Table 2 was established based on IPCC-LVI sensitivity indicators which show the aggregated score below average (45%) for highland and above average (74.1%) for lowland agro-ecology which clearly indicates highest degree of sensitivity at lowland area compared with highland farming system. Specifically, the declining trend of livestock productivity appeared the highest (78.7%) score, which followed by the decline of productive farm land used for seasonal crops that rated the next top score (78.3%) in the lowland block.

Table 2. Perceived sensitivity of farm livelihood in context of climate change impact

	Highland Agro-ecology					Lowland Agro-ecology				
Category of the effect indicators	3	2	1	Total	%	3	2	1	Total	%
Productive crop Land decrease	81	168	18	267	44.5	336	126	31	493	78.3
Crops production decrease over time	51	144	41	236	39.3	207	196	39	442	70.2
Loss of crops varieties over time	90	174	31	293	48.8	222	224	19	465	73.8
Livestock number decrease over years	66	170	60	296	49.3	234	210	24	468	74.3
Livestock productivity declined	78	92	59	229	38.2	312	156	26	496	78.7
Livestock production decrease	75	186	45	306	51.0	219	224	19	462	73.3
Declining of biodiversity	30	154	80	264	44.0	192	216	34	442	70.2
Average				**270**	**45.0**				**467**	**74.1**

On the other hand, the highest score which explains declining trend of livestock production in the highland area is about the average (51%) indicating the larger disparity between the highest score of the lowland (73.3%) and highland agro-ecology. Generally, the sensitivity indicators rating scores shows that the lowland farming business is operating within the sensitive situation which is alarming situation for policy makers and producers to look for better adaptation and mitigation strategies. Table presented below (Table 3) was established based on IPCC-LVI Exposure indicators with an intention to show the degree to what extent the community is exposed to bad climate condition and weather variability in the past ten years which ultimately leads to climate change impacts and related risks. According to analyzed and interpreted survey data, among the selected seven indicators, frequent weather variability and change found to be highest (84%) in rating score in the lowland areas, while the increased agricultural pest incidence indicator scored highest (65.8%) within the highland agro-ecology.

Table 3. Exposure indicators and rating in respective of study community

Categories of effect indicator	Highland Agro-ecology					Lowland Agro-ecology				
	3	2	1	Total	%	3	2	1	Total	%
Frequency of weather variability increased	21	262	52	335	55.8	357	162	10	529	84.0
Water availability and supply declining	171	158	54	383	63.8	234	226	19	479	76.0
shortage of irrigation water source	261	64	49	374	62.3	237	158	42	437	69.4
Drought frequency increased in10 years	15	140	45	200	33.3	330	162	18	510	81.0
Rain shortage frequency increasing	27	124	83	234	39.0	324	164	19	507	80.5
Agricultural pest incidence increased	204	138	53	395	65.8	342	146	22	510	81.0
Family Natural resource reduced	168	146	55	369	61.5	159	246	34	439	69.7
Average				**327**	**54.5**				**487**	**77.3**

An aggregated score of exposure indicators for highland agro-ecology is about average level (54.5%) when compared with the highest score (77.3%) of the lowland agro-ecology situation indicating harmful situation which necessitates new strategies and approaches to divert the negative consequence of climate changes impacts and related threats in context of lowland community and ecosystem. Furthermore, the result summarized in Table 4 was handled based on the IPCC framework vulnerability indicators with the intention to show the perceived degree to what extent a particular community is susceptible to resultant effect of climate change expressed in context of seasonal weather variability in past cropping seasons which eventually leads to social in security and disorder in the exposed community. Among the selected eleven vulnerability indicators, the danger related to agricultural production found to be the highest rated indicators (87.3%) in lowland block compared with the score of the same indicators rated relatevly low (56 %) in context of highland agro-ecology.

Table 4. Livelihoods Vulnerability indicators and rating

Vulnerability Indicators	Highland Agro-ecology					Lowland Agro-ecology				
	3	2	1	Total	%	3	2	1	Total	%
Agricultural production in danger	126	190	20	336	56.0	372	138	14	524	87.3
Lack of weather early warning	60	168	51	279	46.5	150	248	27	425	70.8
Frequent change of crops choice	144	166	51	361	60.2	96	284	30	410	68.3
Shortage of potable water	132	128	37	297	49.5	234	202	18	454	75.7
Shortage of Irrigation & facilities	261	86	53	400	66.7	270	138	40	448	74.7
Small farm landholding	39	132	73	244	40.7	153	150	49	352	58.7
Poor access to extension services	45	116	119	280	46.7	213	168	36	417	69.5
Dependency on outsider	75	120	52	247	41.2	93	154	69	316	52.7
Inadequate access to credit service	48	102	106	256	42.7	84	200	52	336	56.0
Inadequate access to CC training	24	152	101	277	46.2	96	212	58	366	61.0
Lack of private land for family	90	122	73	285	47.5	93	190	69	352	58.7
Average				**297**	**49.0**				**400**	**66.7**

The aggregated vulnerability score found to be about average (49%) from bottom line for highland agro-ecology, while the lowland score (66.7%) is relatively higher and above average when compared with highland agro-ecology score. On the other hand, climate change and weather variability do affect the social, economic and natural environments of the community which in real sense depend upon the adaptive capacity of the individuals and the community.

Practically, the adaptive capacity of the community is the function of livelihoods, resources and technical dimensions of the community and service delivery system to influence the impact of climate change in a positive manner. In context of this study, IPCC-LVI adaptive capacity indicators adopted and employed to determine the most impacting factors in context of study community and Table 5 shows the summary of the results against each indicator and disaggregated results based on the respondents' rating score which indicates overall adaptive capacity of the community and to identify the most leading determinant factors. The data presented below in Table 5, shows that for highland agro-ecology exposure to extension service is the most important variable which better scored

(63.5%) followed by Technical capacity of the community to manage the adaptation and mitigation strategies and agricultural livelihood diversification with 58.7% and 58.5%, respectively. However, the majority (62.5%) of the selected adaptive capacity components rated within the range of the average level score (about 50%) by the respondents indicating that the moderate level adaptive capacity of the community and the systems to withstand the climate change related shocks and associated risks.

Table 5. Community Level Adaptive Capacity to Manage Climate Change Impacts

	Highland Agro-ecology					**Lowland Agro-ecology**				
Category of the indicators	3	2	1	Total	%	3	2	1	Total	%
Agriculture livelihood Diversification	102	172	77	351	58.5	99	260	44	403	64.0
Non-farm income diversification	15	76	84	175	29.2	39	190	60	289	45.9
Family wealth (Assets)	63	134	95	292	48.7	30	142	71	243	38.6
Family level yearly income	54	200	69	323	53.8	30	216	75	321	51.0
Improved agricultural technology	105	152	80	337	56.2	69	170	102	341	54.1
Technical capacity to manage adaptation	99	194	59	352	58.7	54	178	84	316	50.2
Exposure to techniques and technology	108	126	91	325	54.2	60	194	85	339	53.8
Exposure to extension service	129	202	50	381	63.5	63	242	59	364	57.8
Average				**317**	**52.8**				**327**	**51.9**

Similarly, the most important component for the lowland agro-ecology found to be agricultural livelihood diversification (64%), while the exposure to extension service (57.8%) and use of improved agricultural technology (54.1%) rated 2nd and 3rd level respectively in the lowland blocks of the study community. Likewise, the majority of the adaptive capacity components (62.5%) rated average level by survey respondents which shows similar trend with highland agro-ecology indicating the moderate level adaptive capacity of the community in the lowland study area and community. In these selected two agro-ecology, the overall and aggregated rating score found to be within the average range with slightly small difference, that is about 53% and 52% for highland and lowland agro-ecology, respectively, showing moderate level adaptive capacity of the study community and overall system. Therefore, the ability of the study community to adjust to climate change including weather variability and extreme to moderate the potential damage or to cope with consequences of climate change seems to be about average which needs strategies to improve the adaptive capacity to higher level so that the system remains sustainable in all aspects against climate change impacts and associated consequences.

Livelihoods Vulnerability and Determinants

Under this sub-section, Livelihood vulnerability indexing was based on the measurement of eight major components and thirty-five sub components which ultimately contributes to the indexed value of three (exposure, Sensitivity and adaptive capacity) vulnerability components where accordingly the results summarized in the below Table 6 and presented in respective of the two study agro-ecologies. From Table 6, the aggregated LVI values were found to be 0.459 and 0.556 for highland and lowland agro-ecology, respectively, indicating a bottom level moderate vulnerability to the impact of climate change for highland areas and above moderate level vulnerability of lowland livelihoods vulnerability to the impact of climate change associated risks.

Table 6. Livelihood Vulnerability situation in the study survey community

Indicators	**Number of Major components**	**Number of sub components**	**Major component index value**	
			Highland	Lowland
Adaptive capacity	3	12	0.444	0.525
Sensitivity	3	15	0.485	0.542
Exposure	2	8	0.448	0.601
Total	8	35	1.377	1.668
Livelihood Vulnerability Index (LVI)			0.459	0.556
IPCC -LVI (e-a)*S			**0.002**	**0.041**

The three contributing factors, that are adaptive capacity, sensitivity and exposure to natural disasters contributes relatively closer impacts for highland agro-ecology vulnerability, while the variation among the contributing factors are larger for lowland agro-ecology area as compared to the highland community situation. According to the findings, lowland livelihoods are highly vulnerable to climate change as identified by high index value of exposure (0.601) and relatively low (0.525) adaptive capacity. The sensitivity index for the lowlands is 0.542, while 0.485 for the highlands. For the highlands, adaptive capacity is 0.444 indicating below average level. In this regard, Ebrahim, et.al.(2022) identified that comparatively the Kolla (lowland area) as the most vulnerable (0.18) as the result of its highest exposure (0.74) and sensitivity (0.71) and lowest adaptive capacity (0.49), while Daga (highland area) is least vulnerable (0.08) because of its lowest exposure (0.61) and sensitivity (0.42); but the majority of findings mentioned in this study indicate highest vulnerability as compared to our finding in context of livelihoods vulnerability.

Relative to our findings, Getnet et.al.(2022) identified slightly highest vulnerability score (0.64) for households residing in lowland, followed by those of households in the highland (0.61) and midland (0.54) agro-ecologies in context of the same scenario which all of these findings (0.601) are slightly higher than the result of current findings. According to these authors, these findings are explained by the average score of the exposure index value of 0.46, 0.44, and 0.38 for the lowland, highland, and midland agro-ecological zones, respectively, suggesting a greater exposure of lowland communities to climatic risk than highland and midland community. In this regard, in the study conducted in Ethiopia, Dendir and Simane (2019), asserted relatively higher overall livelihoods vulnerability score for Sodo lowland (0.39) as compared to Cheha midland (0.356) and Ezha highland (0.379), indicating relatively moderate vulnerability of the locality to climate change. Furthermore, Javeed (2022) conducted the LVI analysis in context of two study areas in India without referring the agro-ecology, where he observed the index score of 0.413 in Doda and 0.408 for Bhaderwah with overall vulnerability index score of 0.411, indicating moderate vulnerability i.e. closely similar with findings of this study.

In generalized scenario, farming in the study communities is mainly dependent on rainfall amount and distribution that fit the requirement of crops to mature and livestock production. The rainfall variability, especially deficit rainfall as well as poor distribution increase the vulnerability of the livelihoods in the study community resulting from poor productivity and low production from per-unit of land. As the result, the highest value of exposure (0.601) for lowland agro-ecology indicates the degree of burden that the local community is confronting along the process of daily livelihoods making. On the other hand, the analyzed data results related to determinants of vulnerability to climate change impact are presented in the below figure (Figure 2 presents aggregated results for highland and lowland agro-ecologies in perspective of eight components) with intention to show the most contributing, moderate and least contributing factors in respective of each indicator and ultimately to overall livelihood vulnerability.

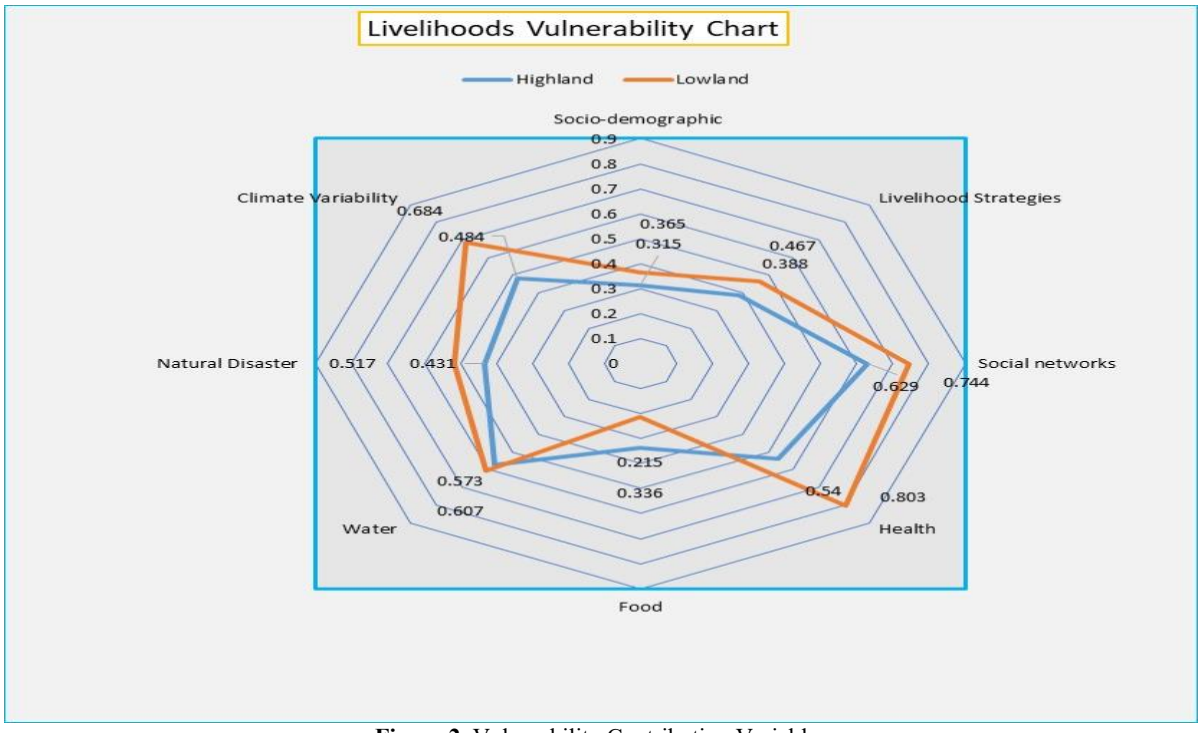


Figure 2. Vulnerability Contributing Variables

Based on extent studies and IPCC frameworks, there are eight major components and thirty-five sub-components which contribute to respective major components were selected in this study where all specified sub-components were indexed and the summarized results were presented and discussed under below section based on the Figure 2 Above which indicates the value of each component.

Socio-demographic component:- According to IPCC framework, socio-demographic component is among the components significantly contributing to adaptive capacity of the households and ultimately the community's vulnerability. In this aspect, the sub-components of this major component are dependence ratio, female headed households, head of household with no formal education, which each of them indexed based on respective measurement and major component value summarized from these indices. From Figure 2, the overall major component value for socio-demographic variable was found within the values considered as lower for both highland and lowland agro-ecology with the vulnerability score of 0.315 and 0.365, respectively, with insignificant difference between the two agro-ecologies. This study identified slightly lower vulnerability as compared to the

finding of Ebrahim, Miheretu and Alemayehu (2022), who revealed Kolla (Lowland) as most vulnerable (0.47) followed by the highland (0.39), and intermediate highland (0.38) in the context of study community. In comparison, Javeed (2022) asserted vulnerability index value of 0.365 (36.5%) in Doda (India); indicating moderate vulnerability whereas 0.363 (36.3%) in Bhaderwah which means vulnerability is just approaching moderate level. Furthermore, Dendir and Simane (2019), also asserted vulnerability value closer to Ezha with a weighted average of 0.35 point, that followed by 0.3-point score in Cheha and similar 0.3-point score in Sodo, which are representatives of the highland, midland and lowland parts of the locality, respectively; the result of which is similar with current finding.

Livelihood strategies:- In context of Livelihoods strategies, the findings of this study show that the livelihood strategies as the major component contributes moderate level (0.467) to climate change impact vulnerability for lowland area which is slightly higher than the highland contribution value (0.388) showing highly significance of this major component in the climate change impact vulnerability in the lowlands, compared with highland farming system. In this regard, Javeed (2022), further revealed the vulnerability index score of 0.290 (29%) for Doda and 0.273 (27.3%) for Bhaderwah block indicating low vulnerability level in both blocks which are by far less vulnerable as compared to the current study findings. Similar study by Dendir and Simane (2019) summarized aggregated average score for livelihood strategies indicator, where both Cheha (midland) and Sodo (lowland) districts showed greater vulnerability of 0.43 and 0.41, respectively.

Social network:- The indices and the summary of the result show extremely higher (0.744) in context of lowland farm households and also slightly higher than the value (0.629) reported for the highland. Justifiably, the contribution of this component for highland agro-ecology is the highest of all other remaining components within the same agro-ecology and the second highest when compared with the score of other components within the agro-ecology of lowland area. Generally, this finding indicated relatively stronger social network score in lowland agro-ecology, as compare to findings of Ebrahim, Miheretu and Alemayehu (2022), who noted index value score of 0.61, 0.53 and 0.63 for lowland, intermediate highland and highland respectively for social networks component. However, the findings of these authors in context of the social network was closely similar with findings reported concerning highland community in current study. Again, according to Getnet, Menberu and Linger (2022), the social capital components have the highest index value in the midland (0.35), followed by highland (0.34) and lowland (0.33) agro-ecosystems, where the lowest level of index value in terms of social network support in lowland agro-ecology is due to farmers' lack of credit access, which may impede the adoption of improved agricultural technologies and farm inputs. Furthermore, in terms of social network component, Dendir and Simane (2019), reported the more index value in Ezha highland (0.49) as compared to Sodo (0.45) and Cheha (0.42) scores which in real scenario significantly lower as compared to the findings reported in context of highland and lowland community in the current study.

Health:- The finding of this study reveals the highest contribution (0.803) at lowland, while moderate effect (0.540) at highland agro-ecology for the sensitivity indicator that manifested in context of health. Similarly, Javeed (2022) reported vulnerability index score of 0.497 (49.7%) for Doda and 0.487 (48.7%) Bhaderwah study sites without specifying the nature of agro-ecology. Furthermore, Dendir and Simane (2019) found more index score of Ezha highland with a weighted contiribution score of 0.33, while Cheha midland (0.23) showed less contribution to vulnerability; the results of which were significantly lower.

Food:- Generally, the indexed value of this major component is lower at both (highland and lowland) areas of the study. Specifically, the lowest (0.215) score for lowland agro-ecology indicates the insignificant contribution of this particular component to its vulnerability component. However, relatively higher index score (0.336) observed at highland which illustrate significant contribution of this component in context of highlanders compared with lowland situation. Overall, the lower score identified in both cases (highland and lowland) in context of this component are not a granting condition for farm households which adversely affecting the whole system of community like change of life style, migration within and outside the country deepening the instability of social system and livelihoods ultimately leading to chronic vulnerability of the community. Furthermore, Ebrahim, Miheretu and Alemayehu (2022) conducted and summarized study results which divulged Kolla (0.42) is more vulnerable while *Woina-Dega (midland)* is less vulnerable (0.34) and Daga (highland) is moderate (0.38) in terms of food major component indicators. Similarly, the study conducted by Dendir and Simane (2019) in Ethiopia, revealed that Sodo lowland was found to be more vulnerable in context of food component (0.47) as compared to the vulnerability score of Cheha (0.35) and Ezha (0.33) districts.

Water:- Water aspect as major component of sensitivity consists of five sub components, namely conflict over the water resources, sustainability of water supply for household consumption and time taken to access water sources in the community. An indices summary of this major component indicates above average score which is an indication of testing situation in highland and lowland agro-ecology. However, an indexed score of the highland (0.573) is closer to moderate relative to the lowland score (0.607), which require the adequate attention and emphasis to maintain sustainable life and livelihoods of the community. With regard to this, Getnet, Menberu and Linger (2022), reported extremely highest vulnerability index score of the water resources (0.97) for the lowland; indicating relatively more vulnerability of lowland community as compare to highland (0.91) and midland (0.70)

agro-ecology. Again, according to Dendir and Simane (2019), the LVI score showed below average score for Sodo lowland district but revealed more vulnerability of this area (0.46) than Ezha (0.37) and Cheha (0.33) districts which are representatives of midland and highland, respectively.

Natural disasters:- The overall vulnerability contribution score of highland agro-ecology seems within the bottom range of moderate (0.431) in context of water showing relatively moderate vulnerability, while aggregated score of lowland area illustrates the upper level moderate (0.517) which is closer to unsafe range when considered climate change vulnerability categories. In comparison, Javeed (2022), found 0.206 (20.6%) vulnerability index score for Doda and 0.28 (28%) in Bhaderwah study area indicating low vulnerability level in both study area as compare to current study findings in context of natural disasters scenario.

Climate change associated Risks:- The result of analysis shows that bottom range moderate (0.464) for highland agro-ecology and as expected the highest score (0.684) was obtained for lowland agro-ecology. The finding of this component is lower when compared with the findings of Ebrahim, Miheretu and Alemayehu (2022), who indicated vulnerability index score of 0.98 in Kolla (lowland), 0.98 in Woyina Daga (midland) and 0.9 in Daga (highland) which are extremely higher as compare to the score of current study findings.

Assessments of Livelihood Vulnerability Determinants using Tobit Regression

The aggregated data (merged highland and lowland agro-ecology data) were used to analyze the determinants of livelihoods vulnerability using Tobit model. The indexed value of vulnerability component indicators scores have been taken as a dependent variable, while socio-economic, technological variables and weather related parameters presented in the below Table 7, are considered as independent variables.

In this regard, vulnerability indicator components (exposure, sensitivity and adaptive capacity) scores were indexed using the Principal Component Analysis (PCA) and indexed value of those variables has been taken as dependent variables in Tobit regression analysis. Similarly, CSA practices rates of adoption and minimum temperature parameters were indexed using the same technique (PCA) in order to reduce the dimension of variables score to a single variable, where the indexed values were used as independent variables in the selected model. In this regard, rainfall and maximum temperature parameters were used directly in Tobit regression analysis, while based on the multicollinearity effect observed in the process of data analysis and management, minimum temperature parameters were indexed using PCA technique.

Table 7. Tobit Regression Analysis to determine Determinant of livelihoods Vulnerability

Explanatory variables	Inferential statistics			
	Coefficient	**Std. Err**	**t-value**	**p>/t/**
Agro-ecology	-3.830	4.662	-0.820	0.412
Age of the Respondents	-0.067	0.015	-4.460	0.000***
Education level of Households head	0.164	0.120	1.360	0.175
Family size of Households	0.175	0.056	3.140	0.002**
Crop diversification	-0.003	0.102	0.030	0.979
Alternative income source	-0.935	0.297	3.150	0.002**
Livelihood diversification	-0.330	0.129	2.570	0.011**
Drought frequency	0.419	0.106	3.970	0.000***
Farm size of Households	-0.032	0.090	-0.360	0.720
Extension Support	-0.105	0.182	0.580	0.565
Credit Support	0.882	0.255	3.450	0.001***
Rainfall mean	-0.018	0.010	-1.830	0.068*
Rainfall standard deviation	0.173	0.166	1.040	0.300
Rainfall coefficient of variation (CV)	100.380	84.628	-1.190	0.236
Maximum Temperature Mean	0.449	0.640	0.700	0.483
Max Temp Coefficient of Variation (CV)	121.207	136.916	-0.890	0.377
Minimum Temperature Index	-0.027	0.299	-0.090	0.929
CSA Practices adoption Index	-0.057	0.124	-0.460	0.647
Constant	**0.725**	**18.067**	**0.040**	**0.968**
Log Likelihood ---------- 910.078				
LR chi-square (X^2) ---------- 133.030				
Prob>chi-Square (X^2) ----------0.000				
Pseudo R^2 ----------0.068				

Among the effect of socio-economic indicators emphasized in the study, only the effect of family size and education level of households on livelihoods vulnerability was found to have positive influence indicating that as these particular variables increase in respective measurement scale, the resultant effect tends to increase households' livelihood vulnerability in context of climate change impact. In this regard, a unit increases in the scale of family size in particular household, tends to increase the vulnerability of the households by a factor of

0.175 which is statistically significant at 1% significance level. On the ather hand, the age of households heads found negative indicating that a unit increase in the measurement of households' head age tends to reduce the households' vulnerability to climate change impacts by a factor of 0.067 which is statistically significant at 1% significance level. These findings are somewhat consistent with other research findings, like Arega (2013), who asserted that an increase in family size of household heads tends to increase the odds of a household being vulnerable in the circumstance of climate change and weather variability. As presented in Table 7, among the two institutional supports included in this analysis (extension and credit), dummy credit accessibility and supports was found positive association with climate change related livelihoods vulnerability, indicating that respondents with adequate accessibility to credit had a higher likelihood of increasing the vulnerability to climate change by about factor of 0.88 point, which is statistically significant at 1% significance level, while the effect of extension support found statistically insignificant. Trustfully speaking, this result contradicts the findings of Arega (2013), who suggested that in context of drought-prone areas of Ethiopia where crop production is extremely affected by amount and sequential distribution of precipitation, access to credit enhanced seasonal food shortage of the households and assists in diversifying the livelihood options to cope with the resultant negative impacts of weather variability and climate change.

More importantly, almost all diversification approaches (crops, income sources and livelihoods diversification) found to reduce the livelihoods vulnerability related to prevailing climate change and linked impacts in the study community circumstances. Accordingly, income diversification and livelihoods diversification reduce the level of livelihoods vulnerability by a factor of 0.94 and 0.33, respectively, in the scale of livelihoods vulnerability measurement and the effect of these variables are significant at 1% and 5% level, respectively.

Regarding to weather factors, the majority of selected weather related parameters, such as rainfall mean standard deviation, rainfall coefficient of Variation (CV), maximum temperature mean and maximum temperature coefficient of variation (CV) found positive association with livelihoods vulnerability to climate change, indicating that a unit increase in particular weather parameters mentioned above tends to increases the likelihoods of the livelihoods vulnerability with the proportional amount of coefficient mentioned in respective of variables indicated in the Table 7 above, but the effect of majority found statistically insignificant except mean rainfall which found statistically significant. Specifically regarding rainfall, a unit increase in mean rainfall tends to reduce the level of livelihoods vulnerability to changing in climate parameters by a factor of 0.018 in the scale of livelihoods vulnerability and statistically significant at 10% significance level. In Similar manner, the effect of drought frequency was found positive to livelihoods vulnerability asserting an increase in the frequency of drought tends to increase the level of livelihoods vulnerability by a factor of about 0.42 point indicating the significant impact of drought on community livelihoods vulnerability and ultimately on the national economy, where the effect is significant at 1% significance level.

CONCLUSION AND RECOMMENDATION

The findings from primary data analysis and literatures review indicate significant impact of climate change on the livelihood of community and apparent in terms of economic burden and social instability which leads to food insecurity, where this was found to be more pronounced in lowland drought prone areas of the study. Specifically, about 95% of respondent perceived climate change associated impact as the major threat of community's livelihoods and majority of respondents' reported that natural resource which mainly farmland is suffering very severe problem as the result of climate change leading to low productivity.

Accordingly, the Livelihoods vulnerability diagnostics conducted indicate the average level of vulnerability, where comparatively lowland area livelihoods are highly vulnerable to climate change impacts and associated risks. More importantly, of total respondents, the majority perceived negative impact of climate change on community livelihoods as compare to positive influence and revealed that most practiced livelihoods are vulnerable to climate change associated risks. On the other hand, it was asserted by majority of respondents and secondary data, the most frequent natural disasters (drought and over flooding) are already identified as the major source of crisis leading to incompetence of the individuals in respective of livelihoods performance. Specifically, of total respondents in the lowland community, relatively the majority (about 53%) revealed drought occurrence more than 3 times in the past six cropping years, where about 95% of households reported serious crop related livelihood damage as the result of drought disaster.

From the total of highland respondent, 52% are severely dependent on natural resource exploitation during food shortage which practically leads to natural resource degradation leading to environmental calamity. On the other hand, the ability of the community to moderate the potential risks (adaptive capacity) found to be about average (overall about 53 % and 52% for highland and lowland, respectively) which need adequate effort to improve to higher level so that the system can sustainably manage the climate change impacts and related consequences. Additionally, the empirical evidences of research findings, suggest that global warming resulting from climate change is already affecting and will continue to affect the agro-ecological suitability required for the development of agricultural sectors which requires the adoption and sustainability of the adaptation and mitigation strategies in the farming community.

Commonly, adaptation strategy adoption only can be realized through adequate adaptive capacity of the system which resulted from the compounded effect of interacting sub-components along the production value chain in context of agriculture. However, the results of the study indicate unpromising system level adaptive capacity resulting from in adequate technical capacity, limited technological and institutional support to the farmers which commonly resulted from inadequacy of the right policy direction and strategies. Accordingly, policy level managers and technocrats need to revisit and/or redesign existing policies with its strategies to promote economically and environmentally sustainable development approaches emphasizing on the adaptive capacity improvement. Additionally, it is pick time for local community to re-emphasize on endogenous knowledge and traditional practices promotion to manage and control currently worsening environmental degradation, especially deforestation to maintain environmentally sustainable community development.

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Declaration of Competing Interests

The author declares that there are no recognized competing interests that appeared to influence the work described in this study paper.

Data Availability

Data will be made available on the request.

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Evaluation of surface water quality in the Southern Marmara Agricultural Basin

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Abstract

The Southern Marmara Agricultural Basin (SMAB) is a strategic region located in the northwest of Turkey, covering a significant portion of the Marmara Region. Rich in agriculture, industry, tourism, and water resources, this basin is one of the critical areas that must be protected and managed in line with Turkey's sustainable development goals. Due to its strategic importance, the basin is exposed to multiple diffuse pollution sources, which pose significant environmental challenges.Therefore, the protection and implementation of sustainable management strategies for the basin are of great importance. In this study, the current water quality was evaluated according to the Surface Water Quality Regulation using samples taken from points where diffuse pollutant sources are dense in the basin. Additionally, correlation matrices were created to examine the relationships between water quality parameters. The study results revealed significantly high concentrations of total nitrogen (TN) and total phosphorus (TP) at the sampling points, indicating substantial nutrient loading. Moreover, the high correlation coefficients between pollutants increase the likelihood that pollution is reaching the receiving environment from non-point (diffuse) sources. These findings indicate that TN and TP loads significantly impact water quality in the SMAB.

Keywords: Southern Marmara Agricultural Basin, Surface Water Quality, Diffuse Pollutant, Basin-Based Water Management

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INTRODUCTION

Considering the demands of the growing global population, the sustainable management of water resources has become an inevitable necessity (Sdiri et al., 2018; Loucks, 2000). In this context, the preservation of both the quantity and quality of water is of vital importance, especially in regions where industrialization is accelerating. To mitigate the pressure of population growth and industrial activities on water resources, it is essential to adopt comprehensive and effective strategies for their protection and management (Rezaee et al., 2021; Dawadi et al., 2013). Additionally, the increasing global threats such as climate change further complicate the management of water resources, necessitating the development of management strategies to protect these resources effectively (Wang et al., 2016; Elgendy et al., 2024; Sivakumar, 2011). Therefore, sustainable water management practices are critically important to meet the needs of current generations and to leave healthy ecosystems for future generations. In this regard, policymakers, environmental scientists, and all relevant stakeholders must collaborate to develop effective solutions.

In this context, basin-based sustainable water management approaches are of strategic importance for the integrated management and protection of water resources. Basin-based management allows for the assessment of water resources according to various usage scenarios and enables the implementation of necessary measures to minimize potential adverse effects on water quality (Pitt and Clark, 2008; Santhi et al., 2006, Randhir et al., 2001). Additionally, this method supports the natural structure and hydrological cycle of basins, contributing to the healthy functioning of ecosystems.

Pollution in basins primarily consists of two categories: point source pollution and non-point source (diffuse) pollution (Huang et al., 2015). Point source pollution is typically caused by municipal discharges or factories,

making it easier to identify and manage the pollutant at its source (Xueyong and Chaohai, 2010; Zessner and Lindtner, 2005). Non-point source pollution, on the other hand, originates from livestock, agriculture, unregulated dumping sites, or septic system effluents (Wang et al., 2021). This type of pollution can lead to the accumulation of essential nutrients such as phosphorus and nitrogen in water resources, which can have significant ecological impacts. Agricultural activities, particularly those involving fertilization and pesticide use, play a crucial role in the formation of non-point source pollution (Basnyat et al., 2000). The transport of diffuse pollutants depends on climate, meteorological conditions, geographic, and geological factors, and reaches receiving environments through transformation reactions and complex transport processes (Haksevenler Gürsoy and Ayaz, 2021).

Unlike point source pollution, identifying and controlling non-point source pollution is considerably more challenging due to its widespread and diffuse nature. This is because such pollution sources are spread over a large geographical area and originate from numerous sources that are difficult to detect rather than a specific point (Lai et al., 2011). Therefore, combating non-point source pollution is a complex process that requires integrated basin management and multidisciplinary strategies. Within the framework of sustainable water management, methods such as comprehensive monitoring networks, advanced treatment technologies, and effective land use planning should be implemented to mitigate the effects of these pollutants. These approaches are vital for the protection of water resources and for meeting the needs of future generations in a sustainable manner.

Especially in the Southern Marmara Agricultural Basin (SMAB), where industrial and livestock activities are high, identifying pollutants reaching the receiving environment will enable the implementation of necessary measures. From this perspective, the importance of basin-based water quality studies becomes evident. In this study, water quality in the basin was evaluated according to the Surface Water Quality Regulation using samples taken from points where non-point source pollutants are predominant. Additionally, water quality parameters were analyzed by creating correlation matrices, and the relationships between these parameters were examined to identify sources of water pollution.

MATERIALS AND METHODS

Study Area

The SMAB is a large area located in the northwest region of Turkiye, south of the Sea of Marmara, encompassing specific districts within the provinces of Balıkesir, Bilecik, Bursa, Çanakkale, Istanbul, Kocaeli, Sakarya, and Yalova. This geographical region holds a strategic position and includes some of Turkiye's significant agricultural, industrial, and commercial centers (MBIH, 2019).

The SMAB has a complex topography, characterized by coastal plains, fertile valleys, high mountains, and significant water resources. The region is also an area of interaction between various climate types; while the coastal areas experience a typical Mediterranean climate, inland areas are dominated by continental climate characteristics. This geographical and climatic diversity of the study area allows for a wide range of agricultural products. Consequently, the region plays a crucial role in agricultural production and export both within Turkiye and internationally. Due to its location, the basin is highly sensitive to various diffuse and point source pollution sources (Figure 1). Additionally, the basin encompasses 8 provinces and 54 districts in Turkiye, where population density and agricultural and livestock activities are highly prevalent (T.R. MoFA, 2024) (Table 1).

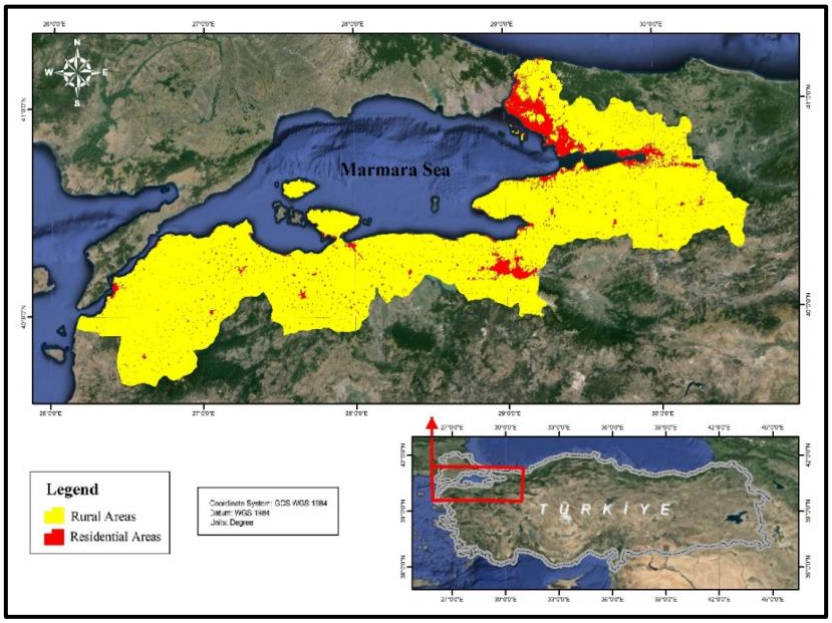


Figure 1. Boundaries of the SMAB

Table 1. Provinces and Districts within the Boundaries of the SMAB (T.R. MoFA, 2024)

Province	District
Balıkesir	Bandırma, Erdek, Gönen, Manyas, Marmara
Bilecik	Osmaneli
Bursa	Gemlik, Gürsu, Iznik, Karacabey, Mudanya, Nilüfer, Orhangazi, Osmangazi, Yıldırım
Çanakkale	Bayramiç, Biga, Çan, Lâpseki, Merkez
Istanbul	Adalar, Ataşehir, Beykoz, Çekmeköy, Kadıköy, Kartal, Maltepe, Pendik, Sancaktepe, Sultanbeyli, Tuzla, Ümraniye, Üsküdar
Kocaeli	Başiskele, Çayırova, Darıca, Derince, Dilovası, Gebze, Gölcük, Izmit, Karamürsel, Kartepe, Körfez
Sakarya	Geyve, Pamukova, Sapanca, Taraklı
Yalova	Altınova, Armutlu, Çınarcık, Çiftlikköy, Merkez, Termal

Sampling and Field Studies

In this study, to determine the surface water characteristics of the SMAB, water samples were collected from selected surface water sources. The aim was to assess the pollution level of the basin in terms of water quality parameters. Samples were taken from points where diffuse pollution sources are predominant. The coordinates of the sampling points are provided in Table 2, and satellite images of the sampling locations are shown in Figure 2.

Table 2. Coordinates of the Sampling Points

Sampling Point	Coordinates	
	X	Y
S1	40.29617	28.45861
S2	40.3709	28.48789
S3	39.89744	28.16010
S4	40.730183	29.988439
S5	40.697117	29.990495
S6	40.734987	29.9557

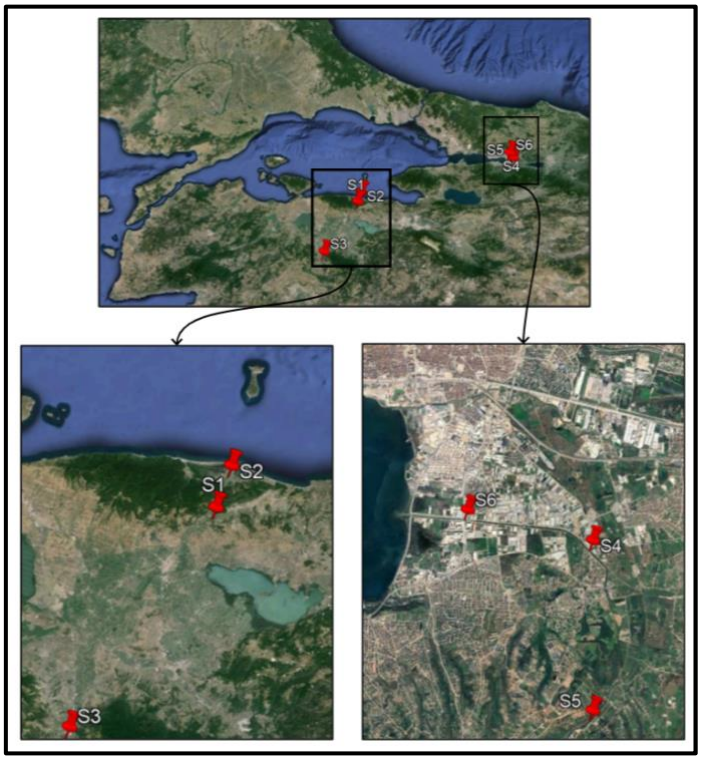


Figure 2. Satellite Images of Sampling Points

The coordinates of S1 represent the Nilüfer Stream. The Nilüfer Stream is exposed to various diffuse pollutants due to anthropogenic factors such as industrialization, intensive agricultural activities, and urban growth. In this region, particularly known for its automotive and textile industries, the stream is polluted by industrial wastes containing heavy metals, organic pollutants, and industrial solvents, agricultural runoff loaded with nutrients like NO_3-N and PO_4-P, and domestic wastewater with high organic matter content. These pollutants degrade aquatic habitats, leading to eutrophication, reducing water quality, and creating toxic effects on aquatic organisms.

The Susurluk Stream (S3) is one of the significant rivers in the Marmara Region. Similar to the Nilüfer Stream, the Susurluk Stream is exposed to various anthropogenic and natural pollution sources due to its location. Intensive agricultural activities in the region lead to the introduction of pesticides into the water, which can cause eutrophication in aquatic ecosystems and threaten biodiversity. Industrial activities also pose a significant pollution threat; industrial wastes from nearby factories and businesses contain chemical pollutants that degrade the stream's water quality. Urban development and growth are additional pollution sources for the Susurluk Stream; urban wastewater and surface runoff carry organic and inorganic substances into the stream. The coordinates of S2 represent the confluence of the Susurluk and Nilüfer Streams before they reach the Marmara Sea. Here, the pollution loads carried by both streams to the Marmara Sea were evaluated.

The coordinates of S4, S5, and S6 represent locations on the Kiraz Stream, which are subject to more urban and industrial diffuse pollution sources compared to other points. The S4 coordinate is situated near various petroleum, food-beverage, and chemical industries. The S5 coordinate is surrounded by farms and agricultural areas. The S6 coordinate represents the point before the Kiraz Stream reaches the Gulf of İzmit. This point was selected to assess the combined pollution loads brought by the S4 and S5 coordinates into the Kiraz Stream.

Surface water samples were taken from each determined point, 30 cm below the stream surface, and the samples were analyzed according to standard methods in an accredited laboratory. The sampling was conducted at points where diffuse pollution sources in the basin are predominant. Images related to the surface water sampling process are provided in Figure 3.

Experimental Measurement

The experiments on surface water samples were conducted in an accredited laboratory in accordance with Standard Methods. Samples were analyzed for Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN), Nitrate Nitrogen (NO_3^--N), Total Phosphorus (TP), Total Coliform (TC), Fecal Coliform (FC), Potassium (K), Boron (B), Fluoride (F), Selenium (Se), Molybdenum (Mo), Zinc (Zn), Copper (Cu), Manganese (Mn), Iron (Fe), and Sulfide (H_2S-S) parameters. The methods, measurement techniques, and units used in the analysis of the collected samples are summarized in Table 3. The evaluation of the parameters obtained from the water samples was conducted within the framework of the Surface Water Quality Regulation (SWQR) (SWQR, 2021). Annex 5, Table 2 of the regulation specifies the quality criteria for Inland Surface Water Resources based on General Chemical and Physicochemical Parameters. The limit values and water quality classes specified in the regulation are shown in Table 4.



Figure 3. Surface Water Sampling

Table 3. Methods and Units Used in the Analysis of Collected Samples

Parameter	Method Number	Method	Unit
Dissolved Oxygen (DO)	SM 4500 O G	Membrane Electrode Method	mg/L
Chemical Oxygen Demand (COD)	SM 5220 B	Open Reflux-Titrimetric Method	mg/L
Biochemical Oxygen Demand (BOD_5)	SM 5210 B	5-Day BOD Test	mg/L
Total Kjeldahl Nitrogen (TKN)	SM 4500 Norg B	Calculation with Nitrite, Nitrate, and Kjeldahl Nitrogen (Calculation Method)	mg/L
Ammonium Nitrogen (NH_4^+-N)	SM 4500 NH_3 F	Spectrophotometric Method	mg/L
Nitrate Nitrogen (NO_3^-N)	SM 4110 B	Calculation with Nitrite, Nitrate, and Kjeldahl Nitrogen (Calculation Method)	mg/L
Total Nitrogen (TN)	SM 4500 NO_2^- B SM 4110 B SM 4500 Norg B	Calculation with Nitrite, Nitrate, and Kjeldahl Nitrogen (Calculation Method)	mg/L
Total Phosphorus (TP)	EPA 200.7	ICP-OES Method	mg/L
Fecal Coliform (FC)	SM 9222 D	Membrane Filtration Technique	CFU/100 mL
Total Coliform (TC)	SM 9222 B	Membrane Filtration Technique	CFU/100 mL
Potassium (K)	EPA 200.7	ICP-OES Method	mg/L
Boron (B)	EPA 200.7	ICP-OES Method	mg/L
Fluoride (F)	SM 4110 B	IC Method	mg/L
Selenium (Se)	EPA 200.7	ICP-OES Method	mg/L
Molybdenum (Mo)	EPA 200.7	ICP-OES Method	mg/L
Zinc (Zn)	EPA 200.7	ICP-OES Method	mg/L
Copper (Cu)	EPA 200.7	ICP-OES Method	mg/L
Manganese (Mn)	EPA 200.7	ICP-OES Method	mg/L
Iron (Fe)	EPA 200.7	ICP-OES Method	mg/L
Sulfur (H_2S-S)	SM 4500 S^{-2} D	Spectrophotometric Method	mg/L

Assessment for the classification of surface waters has been conducted using the data obtained from monitoring results within the scope of the Regulation on Amendments to the Regulation on Surface Water Quality Management, employing the parameters and criteria specified in Annex-5. The quality classes for the parameter groups in Tables 2 of Annex-5 of this Regulation are indicated by Roman numerals (SWQR, 2021). The classification based on color, according to the standard values in Annex-5, is presented in Table 4.

Pearson Correlation Matrix

The Pearson Correlation Matrix is a statistical method that measures the linear relationship between two variables, with values ranging from -1 to +1. This method is often preferred for analyzing linear relationships between two continuous variables. The Pearson correlation coefficient is particularly used when the data exhibit a bivariate normal distribution. The strength of the correlation increases as the absolute value of the coefficient rises, approaching ±1, indicating an almost perfect linear relationship between the two variables (Schober et al., 2018).

During the calculation of correlation coefficient, sample correlation coefficients obtained for each pair of variables in the data set are used. These coefficients indicate the strength and direction of the linear relationship between the respective variables. The correlation matrix is commonly used in multivariate data analyses; it plays a significant role in methods such as factor analysis and cluster analysis. The components of the matrix include the Pearson correlation coefficients of the respective pairs of variables, and the structure of this matrix varies depending on the characteristics of the data to be analyzed (Hills, 1969).

Table 4. Quality Criteria for Inland Surface Water Resources Based on General Chemical and Physicochemical Parameters (Regulation Amending the Surface Water Quality Regulation, Annex 5 Table 2)

Water Quality Parameter	**Unit**	**Water Quality Classes** [(a)]		
		I	**II**	**III**
Dissolved Oxygen (DO)	mg/L	>8	6	< 6
Chemical Oxygen Demand (COD)	mg/L	< 25	50	>50
Biochemical Oxygen Demand (BOD_5)	mg/L	< 4	8	>8
Total Kjeldahl Nitrogen (TKN) [(b)]	mg/L	< 0.5	1.5	>1.5
Ammonium Nitrogen (NH_4-N)	mg/L	< 0.2	1	>1
Nitrate Nitrogen (NO_3^--N)	mg/L	< 3	10	>10
Total Nitrogen (TN)	mg/L	< 3.5	11.5	>11.5
Total Phosphorus (TP)	mg/L	< 0.08	0.2	>0.2
Fecal Coliform (FC)	CFU/100 mL	-	-	-
Total Coliform (TC)	CFU/100 mL	-	-	-
Potassium (K)	mg/L	-	-	-
Boron (B)	mg/L	-	-	-
Fluoride (F)	mg/L	≤ 1	1.5	>1.5
Selenium (Se)	µg/L	≤ 10	15	>15
Molybdenum (Mo)	mg/L	-	-	-
Zinc (Zn)	µg/L	-	-	-
Copper (Cu)	mg/L	-	-	-
Manganese (Mn)	µg/L	≤ 100	500	>500
Iron (Fe)	µg/L	-	-	-
Sulfur (H_2S-S)	mg/L	≤ 0.002	0.005	>0.005

[(a)] Intended uses of waters according to quality classes:

Class I - High-quality water (Class I water quality indicates "Excellent" water condition);

1) Surface waters with high potential for drinking water,
2) Water that can be used for recreational purposes, including swimming and other body contact activities,
3) Water suitable for trout production,
4) Water suitable for livestock and farm needs,

Class II - Slightly polluted water (Class II water quality indicates "Good" water condition);

1) Surface waters with potential for drinking water,
2) Water suitable for recreational purposes,
3) Water suitable for fish production other than trout,
4) Irrigation water, provided it meets the irrigation water quality criteria determined by current legislation,

Class III - Polluted water (Class III water quality indicates "Moderate" water condition);

Water that can be used for aquaculture and industrial purposes after appropriate treatment, except for facilities requiring high-quality water such as those in the food and textile industries.

(b) TKN: NH_3-N + Organic Nitrogen

(c) TN: TKN + NO_3-N + NO_2-N

RESULTS AND DISCUSSION

Evaluation of Analysis Results

The analysis results of the samples taken from the points whose coordinates are given in Table 2 are presented in Table 5.

When examining S1, it is observed that some pollution parameters of organic origin are at quite high levels. When these pollution parameters are evaluated in terms of general chemical and physicochemical parameters of inland surface water sources, the water sample falls into the I. Class – High-Quality Water category for NO_3^--N and F parameters. However, for the Mn parameter, it is classified as II. Class – Slightly Polluted Water. In contrast,

for the parameters COD, BOD_5, TKN, NH_4-N, TN, TP, Se, and SO_4-S, it is categorized as III. Class – Polluted Water.

When evaluated according to the S2 Quality Criteria, the water sample is classified as I. Class – High-Quality Water for NO_3^--N, F, and Mn parameters. However, it is categorized as III. Class – Polluted Water for COD, BOD_5, TKN, NH_4-N, TN, TP, and Se parameters.

For S3, the sample is classified as I. Class – High-Quality Water for NO_3^--N and F parameters. It falls into the II. Class – Slightly Polluted Water category for COD, NH_4-N, TN, and Mn parameters. Nevertheless, it is classified as III. Class – Polluted Water for COD, BOD_5, TP, and Se parameters.

When the water quality parameters of S4 are examined, it is classified as I. Class – High-Quality Water for COD, NH_4-N, NO_3^--N, F, and Mn parameters. However, for the TN parameter, it falls into the II. Class – Slightly Polluted Water category. In contrast, for the COD, BOD_5, and TP parameters, it is categorized as III. Class – Polluted Water.

Table 5. Analysis Results of Water Quality Parameters

Water Quality Parameter	**Unit**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**Water Quality Classes** (a)		
								I	**II**	**III**
Dissolved Oxygen (DO)	mg/L	1.9	5	6.78	8.2	7.09	7.02	>8	6	< 6
Chemical Oxygen Demand (COD)	mg/L	382	78.4	100	123	75	214	< 25	50	>50
Biochemical Oxygen Demand (BOD_5)	mg/L	152.08	31.4	40	49	29.87	85.58	< 4	8	>8
Total Kjeldahl Nitrogen (TKN) (b)	mg/L	20.93	5.27	<5	<5	<5	9.76	< 0.5	1.5	>1.5
Ammonium Nitrogen (NH_4-N)	mg/L	7.31	2.08	0.542	0.194	0.052	4.67	< 0.2	1	>1
Nitrate Nitrogen (NO_3^--N)	mg/L	<0.23	<0.23	1.15	0.95	<0.23	<0.23	< 3	10	>10
Total Nitrogen (TN)	mg/L	23.61	6.68	5.38	<5	<5	12.77	< 3.5	11.5	>11.5
Total Phosphorus (TP)	mg/L	1.043	0.414	0.63	0.335	0.021	0.48	< 0.08	0.2	>0.2
Fecal Coliform (FC)	CFU/100 mL	$22x10^4$	$17x10^3$	$38x10^2$	$9x10^2$	$14x10^2$	$49x10^3$	-	-	-
Total Coliform (TC)	CFU/100 mL	$24x10^4$	$17x10^3$	$39x10^2$	$42x10^3$	$31x10^3$	$62x10^3$	-	-	-
Potassium (K)	mg/L	24.5	8.2	9.2	4.2	1.2	8.3	-	-	-
Boron (B)	mg/L	<1	3.05	4.6	<1	<1	<1	-	-	-
Fluoride (F)	mg/L	<1	<1	<1	<1	<1	<1	≤ 1	1.5	>1.5
Selenium (Se)	µg/L	29.59	17.5	21.4	21.72	28.47	26.96	≤ 10	15	>15
Molybdenum (Mo)	mg/L	<0.01	<0.01	0.021	<0.01	<0.01	<0.01	-	-	-
Zinc (Zn)	µg/L	14.26	<10	<10	<10	<10	<10	-	-	-
Copper (Cu)	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	0.013	-	-	-
Manganese (Mn)	µg/L	186.52	22.12	205.38	12.69	20.95	260.54	≤ 100	500	>500
Iron (Fe)	µg/L	321.47	38.49	72.24	54.79	32.63	119.22	-	-	-
Sulfur (H_2S-S)	mg/L	1.363	<0.1	<0.1	<0.1	<0.1	<0.1	≤ 0.002	0.005	>0.005

The water quality parameters of the sample taken from S5 were evaluated. The parameters NO_3^--N, TP, F, and Mn are classified as Class I – High Quality Water. In contrast, the parameters DO, NH_4-N, and TN are identified as Class II – Slightly Polluted Water. Meanwhile, the parameters COD, BOD_5, and Se are classified as Class III – Polluted Water.

Finally, when evaluated according to S6 Quality Criteria, the parameters NO_3^--N and F are categorized as Class I – High Quality Water. The parameters COD and Mn are classified as Class II – Slightly Polluted Water. However, the parameters COD, BOD_5, TKN, NH_4-N, TN, TP, and Se are classified as Class III – Polluted Water.

When the experimental results are assessed comprehensively, it is observed that at all sampling points, the parameters COD, BOD_5, and Se are categorized as Class III – Polluted Water. Consequently, the COD parameter is classified as Class I – High Quality Water at only one of the six sampling points. High concentrations of COD and BOD_5 lead to a reduction in COD levels in aquatic ecosystems (Maddah, 2022). A decrease in COD levels threatens the survival of fish and other aquatic organisms and reduces biodiversity in the ecosystem (Bhat et al., 2022). Additionally, a decline in oxygen levels in water narrows the habitat of aquatic organisms and creates stress. This situation can negatively affect the reproductive and growth processes of particularly sensitive species (Limburg and Casini, 2019). Therefore, maintaining low levels of COD and BOD_5 in water resources is crucial for the protection of aquatic ecosystems and the sustainable management of water resources. Management strategies should be effectively implemented to keep these parameters under control and prevent potential negative environmental effects.

The reason for the high Se, Cu and Mn concentrations at all six sampling points is thought to be due to agricultural runoff. Fertilizers and pesticides used excessively due to agricultural activities can leach into water sources, leading to increased concentrations of Se, Cu, and Mn. These chemicals can be transported to local water sources through agricultural runoff, leading to an increase in the concentrations of these elements.

Although Cu is an essential element for aquatic organisms, it can be toxic at high concentrations. Its adverse effects, particularly on aquatic plants, invertebrates, and fish, have been observed. Cu, can increase oxidative stress, leading to damage in biological systems (Brown et al., 1974). Mn, especially in waters with low hardness, can be toxic at high concentrations. The toxic effects of Mn can cause serious health issues in aquatic invertebrates and fish, particularly affecting organisms during the spawning period and in soft waters (Harford et al., 2015). Se is generally present in low concentrations in natural water systems and is an essential element for many organisms. However, high concentrations of Se can be toxic to aquatic life. Se can cause bioaccumulation in aquatic plants and animals and lead to reproductive disorders (Lemly, 1985). Therefore, controlling elements such as Se, Cu, and Mn in surface waters is crucial for the protection of aquatic ecosystems and the sustainability of aquatic life. Management strategies should be designed and implemented to mitigate these toxic effects.

The TP parameter is classified as Class III – Polluted Water at five of the sampling points. TP leaching from fertilizers used during agricultural activities can enter water bodies, leading to elevated TP levels. This issue is particularly observed in areas with intensive agriculture (Ascott et al., 2016). Additionally, insufficiently treated or untreated phosphorus from wastewater treatment facilities can contribute to increased phosphorus concentrations in water sources (Ruan and Gilkes, 2000). High levels of phosphorus lead to excessive nutrient loading in surface waters, resulting in eutrophication. This condition can cause a decrease in oxygen levels in aquatic ecosystems and lead to biological damage such as fish kills (Mayer et al., 2013). Moreover, it can negatively impact the recreational use of water resources and drinking water quality and promote the growth of toxin-producing cyanobacteria (blue-green algae) (Xie et al., 2014). Consequently, waters with high phosphorus concentrations can increase treatment costs and complicate sustainable water management (Kuo et al., 2009).

In addition, both TC and FC bacteria were detected at every sampling point. The contamination of water sources by human and animal waste can lead to high levels of FC and TC bacteria. Manure application and dairy runoff activities are thought to potentially increase both total and fecal coliforms in the environment. Moreover, rainwater, agricultural lands, parks, and other open areas can carry coliform bacteria into water sources through surface runoff. This process is particularly intense during rainy periods and can cause seasonal fluctuations in bacterial concentrations (Isobe et al., 2004). The presence of TC and FC bacteria poses significant risks to surface water sources. These bacteria are important indicators of waterborne diseases and can signal the presence of various pathogens, potentially leading to diarrheal disease, gastroenteritis, and other serious health issues in humans (Avigliano and Schenone, 2015). High levels of FC, in particular, can result in severe health problems, especially in recreational water sources and drinking water supplies. This can impair the chemical and aesthetic quality of water and threaten the health of aquatic ecosystems in the long term (Lalancette et al., 2014). Managing waters with high levels of TC and FC necessitates strengthening treatment and disinfection processes, as well as implementing stricter water quality monitoring protocols (Brion et al., 2000). High levels of coliform bacteria in water sources pose serious risks to both ecosystem and human health. Therefore, the protection of water sources and the control of pollution sources are of great importance for public health.

Evaluation of Correlation Matrices of Water Quality Parameters

The relationships among water quality parameters were also analyzed using a correlation matrix. The correlation matrix is a crucial method for exploring the relationships among water quality indicators. This method determines the strength and direction of linear relationships between different parameters, allowing researchers to understand how these parameters interact with each other. Specifically, high correlation coefficients indicate a strong linear relationship between two variables, revealing potential relational dynamics in water quality research. Another important aspect of the correlation matrix is its role in identifying sources of water pollution. Significant correlations can indicate common pollution sources, which facilitates the development of risk assessment and water quality management strategies. Additionally, the data obtained from this analysis method can be used in the design and development of water quality monitoring and management programs, enabling more effective and

targeted approaches in environmental management. Such analyses optimize the use of time and resources in water quality research, enhancing scientific efficiency and accuracy.

Parameters such as DO, COD, BOD_5, FC, and TC are fundamental indicators used in assessing water quality. These criteria are utilized to analyze the chemical and biological quality of water. An examination of the correlation matrix of these water quality parameters reveals strong negative correlations between DO and COD, BOD_5 and COD, FC and COD, and TC and COD, with correlation coefficients of -0.74, -0.74, -0.89, and -0.82, respectively.

Additionally, it was found that there are very strong positive correlations between COD and BOD_5, COD and FC, COD and TC, BOD_5 and FC, BOD_5 and TC, and FC and TC, with correlation coefficients of 1, 0.96, 0.96, and 0.98, respectively (Figure 4).

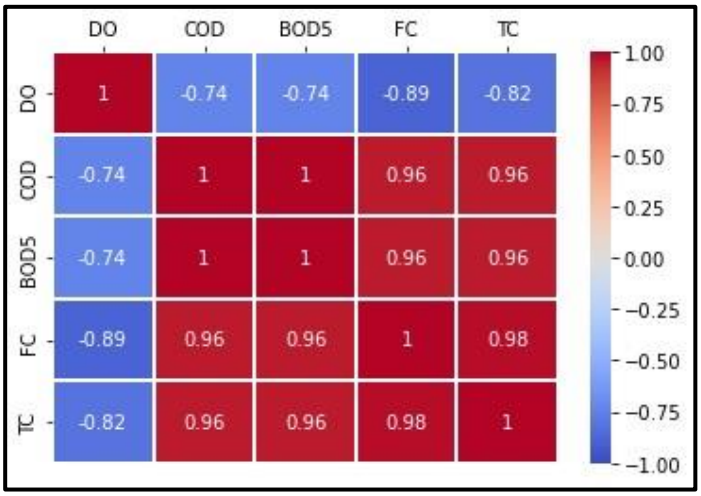


Figure 4. Correlation Matrix of Water Quality Parameters

Elements such as B, F, Fe, SO_4-S, and K play various critical roles in water quality assessments. These roles are important both in terms of the chemical composition of water and their effects on biological processes. Examination of the correlation matrix for these critical elements reveals very strong positive correlations between Fe and K, Fe and SO_4-S, and K and SO_4-S, with correlation coefficients of 0.95, 0.95, and 0.92, respectively (Figure 5). This indicates that these elements may originate from common sources or similar geochemical processes. These elements are often found in the same mineral structures and respond similarly to environmental factors. For example, atmospheric deposition can play a significant role in transporting these elements to water sources. Additionally, anthropogenic effects such as agricultural activities and industrial pollution can also increase the concentrations of these elements.

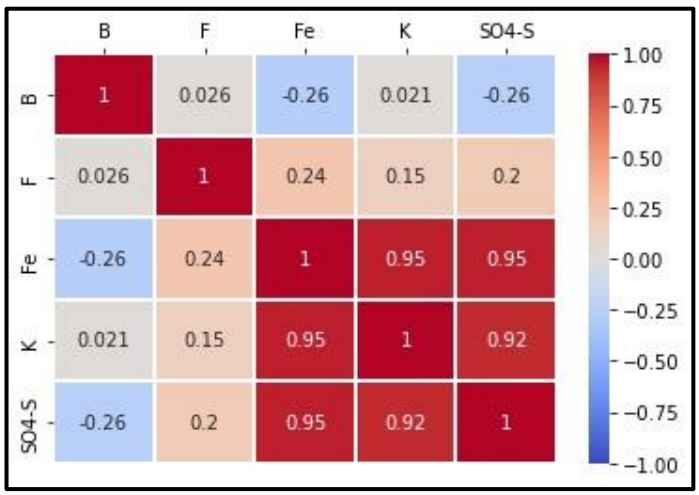


Figure 5. Correlation Matrix of Critical Water Quality Parameters

Elements such as Se, Mo, Zn, Cu, and Mn are essential trace elements required by living organisms in generally small amounts. These elements act as catalysts or coenzymes in many biological processes and are vital for health. Examination of the correlation matrix for these trace elements reveals moderate positive correlations between Se and Zn, and Mo and Zn, with correlation coefficients of 0.55 and 0.66, respectively (Figure 6).

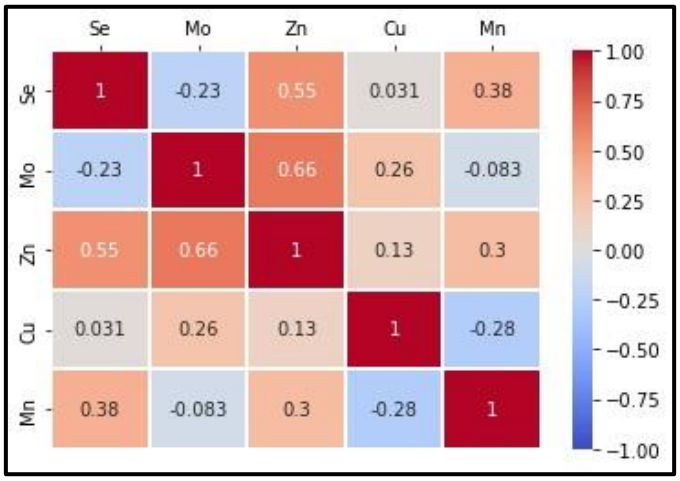


Figure 6. Correlation Matrix of Trace Water Quality Parameters

Parameters such as TKN, NH_4^+-N, TN, and TP are substances that primarily contribute to the nitrogen and phosphorus cycles in the context of water quality. They are important indicators related to nutrient loading in aquatic ecosystems. An examination of the correlation matrix for these nutrient pollution parameters reveals very strong positive correlations between TKN and NH_4^+, TKN and TN, and NH_4^+ and TN, with correlation coefficients of 0.97, 0.99, and 0.98, respectively. Additionally, strong positive correlations were found between TKN and TP, NH_4^+ and TP, and TN and TP, with correlation coefficients of 0.82, 0.78, and 0.87, respectively (Figure 7).

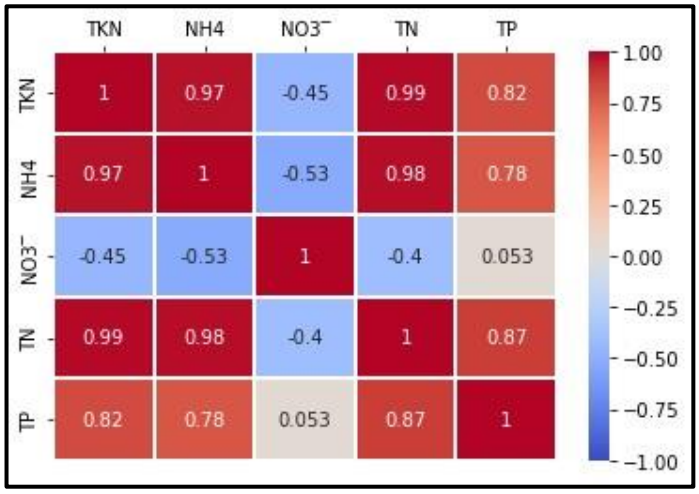


Figure 7. Correlation Matrix of Nutrient Pollution Parameters

CONCLUSION

The SMAB is a strategic region located in northwestern Turkey and encompasses a significant part of the Marmara Region. Rich in agriculture, industry, tourism, and water resources, this region is one of the critical areas that need to be protected and managed in line with Turkiye's sustainable development goals. Due to its strategic importance, the basin is exposed to numerous diffuse pollution sources. Examination of the data obtained from fieldwork reveals that high concentrations of TN and TP were detected at all six sampling points. Furthermore, the high correlation coefficients among pollutants increase the probability that pollution is reaching the receiving environment from non-point sources. These findings indicate that the TN and TP loads in the SMAB have a significant impact on water quality. Developing sustainable water management strategies is critical for controlling TN and TP loads. In this context, potential water management strategies are summarized below:

- Ensure fertilizers are applied at the correct time and in the appropriate amounts to prevent excessive use.
- Promote organic farming practices by reducing the use of chemical fertilizers and pesticides.
- Implement terracing, strip cropping, and conservation of vegetation to prevent soil erosion.
- Ensure proper collection, storage, and processing of animal wastes.
- Carefully manage the feed and nutrients used in animal husbandry.
- Establish and protect natural and artificial wetlands, aiming for these areas to naturally filter pollutants.
- Encourage the reduction of surface runoff through rainwater harvesting and green roof practices in urban areas.
- Organize training programs on sustainable agriculture and livestock practices.
- It is important to educate the public about water pollution and sustainable water use and to raise awareness.
- Ensure regular monitoring and reporting of water quality across the basin.
- Increase inspections and implement necessary legal regulations to ensure compliance with existing water quality standards.
- Establish buffer zones between agricultural areas and water sources and cover these zones with vegetation.
- Encourage the treatment of industrial and urban wastewater using advanced treatment methods.
- Facilitate the reuse of treated wastewater for agricultural irrigation or industrial processes.
- Water resources should be managed with a holistic approach, and integrated management plans should be developed with the involvement of all stakeholders.
- Update and effectively enforce legal regulations to protect water quality.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest. The paper is original unpublished work, and it is not under consideration for publication anywhere else. No data, text, or theories by others are presented, all relevant literature is cited.

Author contribution

Conceptualization, M.N.C. and H.K.Ö.; methodology, M.N.C. and H.K.Ö.; investigation, M.N.C. and H.K.Ö.; writing—original draft preparation, M.N.C. All authors have read and agreed to the published version of the manuscript.

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The interactive effects of salinity and drought stress on germination, seedling growth, and physiological parameters of pumpkin

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Abstract

This study aimed to explore the response of pumpkin to salt and drought stress during germination and early plant growth stages. Salt stress was induced with various sodium chloride concentrations (0.00%, 0.25%, 0.50%, and 0.75%) in non-drought (distilled water) and drought (15% PEG-6000) conditions. The seeds of the pumpkin cultivar T28 for snacks were germinated between filter papers using the respective solutions. In the pot experiment, the seedlings at the 2-leaf stage were exposed to these stresses for 30 days. The plant height, fresh weight, dry weight, leaf area, leaf dry matter, chlorophyll content (Chl), relative water content (RWC), and cell membrane stability (CMS) of the plants were inquired. Drought markedly reduced the germination index, plant height, fresh weight, dry weight, leaf area, RWC, and CMS. Conversely, increased mean germination time, Chl, and dry matter were determined in drought conditions. Salinity stress above 0.50% NaCl influenced these traits, with salinity's inhibitory effects surpassing those of drought. Germination percentage dropped from 100% to 46% at 0.75% NaCl under drought, whereas it remained stable under non-drought stress. Pumpkin was more sensitive to drought and salinity stress at the germination stage than at the early growth stage. The correlation between germination and seedling growth parameters indicated that the germination index and mean germination time were substantially associated with nearly all growth traits of pumpkin. The study highlights the germination index as a key indicator of stress tolerance and identifies 0.50% NaCl as a critical threshold level for pumpkin under salt and drought stress.

Keywords: *Cucurbita pepo* L., Drought, Salinity, Germination, Plant growth, Cell membrane stability

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INTRODUCTION

Today, there are five important cucurbit species - *Cucurbita pepo*, *C. moschata*, *C. maxima*, *C. ficifolia,* and *C. argyrosperma* (*C. mixta*) - are cultivated for fresh fruits, flowers, and seeds extensively worldwide (Robinson and Decker-Walters, 1997). Most pumpkins consumed as snacks belong to the *C. pepo* L. species (Gülşen et al., 2016). Its fresh fruits and flowers have been utilized as vegetables and mature seeds as snacks (Maynard and Maynard, 2000). The seeds have a high nutritional value, a high protein content ranging from 28% to 40%, and oil (35% to 50%), with 78% of the oil being unsaturated fatty acids (Turkmen et al., 2015; Dziwulska-Hunek et al., 2021; Aghbolaghi et al., 2021; Aghbolaghi et al., 2022). Moreover, it provides vitamins A, C, and E (Liang et al., 2022; Jahed et al., 2023) and is abundant in essential minerals, including K, P, Ca, Mg, and Fe (Seymen et al., 2019). Along with its nutritional values, it is a valuable alternative crop in arid and semiarid regions in Türkiye because it adapts highly to different ecological conditions (Yasar et al., 2014).

The production of pumpkins for snacks has gained popularity among farmers due to their low water requirements, especially in arid areas where rainfall is insufficient, and their high economic returns. Under these conditions, drought and salinity are common abiotic stresses for pumpkin cultivation, resulting in yield and economic losses (Seymen et al., 2019; Aghbolaghi et al., 2023). Soil salinity occurs naturally in arid regions,

resulting from low precipitation, preventing excess salt drainage from the root zone to deeper soil layers. It induces osmotic stress, inhibiting water uptake by roots, and ion toxicity due to excess Na^+ and Cl^- (Maas and Grattan, 1999; Okçu et al., 2005). According to Xu et al. (2017), pumpkin is classified as a plant with low salinity tolerance. Inhibited germination and plant growth of pumpkin under saline conditions are reported by Irik and Bikmaz (2024), with similarities to the findings of Tarchoun et al. (2022) in *C. maxima* Duchesne. In addition, less chlorophyll content (SPAD) (Taratima et al., 2022), smaller leaves (Taratima et al., 2023), and an imbalance of ions between Na, Ca, and K (Kurum et al., 2013) were identified as responses to salinity in pumpkin. Increased ion leakage and enzymatic activity (APX, SOD, CAT, and GR) were also explored (Sevengör et al., 2011; Irik and Bikmaz, 2024). Besides, drought negatively impacts pumpkin plants with similar responses to salinity, such as reduced leaf area, chlorophyll a and b content, net photosynthesis, and stomatal conductance (Aghai and Ehsanzade, 2011), as well as plant height, fruit numbers, seed and fruit yield, photosynthetic activity, and relative water content (Najafi et al., 2021), seed quantity and weight (Naeemi et al., 2012), and ultimately seed and fruit yield (Najafi et al., 2021). However, the combined effects of drought and salinity, which frequently occur together during the life cycle of pumpkin plants, have not been examined and their effects remain unclear. Therefore, this study evaluated the effects of salinity stress, specifically NaCl salt, on seed germination and the early growth stages of pumpkin growth in both drought and non-drought conditions and also examined the correlation between germination traits and plant growth parameters.

MATERIALS AND METHODS

This study assessed the responses of pumpkin (*Cucurbita pepo* L. cv. T28) to salinity and drought stress conditions by considering germination and morpho-physiological parameters. Sodium chloride (NaCl) was used to simulate salinity and polyethylene glycol (PEG-6000) induced drought stress.

Germination test

Drought stress was simulated using 15% PEG 6000, which equals about 2 bar osmotic potential according to Michel and Kaufmann (1973) and Yasar et al. (2014), and distilled water served as a non-drought control treatment. NaCl solutions of 0.00%, 0.25%, 0.50%, and 0.75% were prepared for salinity treatments. Electrical conductivity (EC) values for non-drought treatments were 0.0, 4.8, 9.1, and 13.6 dS m^{-1}; for drought, they were 0.4, 3.4, 6.5, and 9.3 dS m^{-1}, respectively.

Four replicates of 25 seeds were placed between three filter papers moistened with 7 milliliters of the respective test solutions on each paper. The papers were refreshed every two days to prevent salt and PEG accumulation. After rolling the papers with seeds, they were inserted into a zipped plastic bag to inhibit water evaporation. They were exposed to 25±1 °C in an incubator for 8 days under darkness (ISTA 2018). Daily germination scores were recorded, with a seed having a 2 mm radicle protrusion. Germination percentage and mean germination time (MGT) were calculated following the ISTA (2018) rules and the formula described by Salehzade et al. (2009) for the germination index.

Pot experiment

Seedling growth conditions

The seeds of pumpkin cultivar T28 were sown in seedling trays with a growth medium (peat, perlite, and vermiculite 6:1:1, by volume). The trays were then put in a growth chamber of 24°C day/18°C night, a photoperiod of 18/6 hours, and a relative humidity of 65-70%. Uniform and healthy seedlings at the 2-leaf stage after 14 days of planting were used in the study. The seedlings were placed in plastic pots with a volume of 0.5 L, filled with the same growing mixture used for non-drought conditions. To simulate drought stress, 150 g of PEG-6000 was added to one kilogram of the growth medium, achieving a concentration of 15% PEG-6000. Different salinity stresses were arranged using NaCl levels (0.00%, 0.25%, 0.50%, and 0.75% NaCl), as implemented in the germination test.

Plant growth conditions and stress application

The plants were irrigated with Hoagland's nutrient solution after transplantation. Climatic conditions were the same in seedling growth conditions, and they were allowed to grow under these temperature, light, and moisture conditions. After 21 days of stress incubation, the plants were cut above the soil surface, and the fresh biomass was used to determine plant fresh weight and dry weight after drying at 80°C for 24 hours.

Leaf area per plant was quantified using ImageJ software (Kaya, 2024). Dry matter was determined by calculating the percentage of dry weight relative to fresh weight. The chlorophyll content was measured using the Konica Minolta SPAD-502 (Osaka, Japan) as the SPAD index (Kulan et al., 2021). The relative water content (RWC) and cell membrane stability (CMS) of the uppermost fully expanded leaves were determined using the formulas described by Nijabat et al. (2020). To determine the leaf RWC, the second leaf from the top of the plants was pulled out, and the fresh weight was directly weighed. This leaf was then floated in distilled water in a Falcon tube for 24 h, and its turgor weight was determined after excess water on the leaf surface was gently removed with paper towels. Subsequently, the leaf was dried at 80°C for 24 h to obtain the dry weight.

$$\text{RWC (\%)} = [(\text{Fresh weight} - \text{Dry weight})/(\text{Turgor weight} - \text{Dry weight})] \times 100$$

Leaf dry matter (%)= (Dry weight / Fresh weight) × 100

Six leaf samples with a diameter of 10 mm were excised from the leaf and immersed in 25 mL of distilled water in a 50 mL glass tube. After 24 h of incubation at 20°C, the electrical conductivity of the soaking solution was measured using an EC meter (WTW 3.15i) and recorded as initial ECi. The tubes were then transferred to a water bath at 90°C for 1 h, and the final EC value (ECf) was measured after cooling to room temperature. The cell membrane stability (CMS) was calculated as:

CMS (%)= (1-[(ECi/ECf)]×100 (Farooq and Azam, 2006)

Statistical data analysis

Data were subjected to analysis of variance (ANOVA) using the JMP 13.2 software following a completely randomized design (CRD) with two factors and four replications per treatment. The significance levels between means were determined using the Least Significant Differences (LSD) test at $p < 0.05$.

RESULTS AND DISCUSSION

Drought and salinity significantly reduced germination percentage, mean germination time, and germination index of pumpkin seeds (Table 1). Furthermore, analysis of variance revealed a significant interaction between drought and salinity. Under drought stress, the mean germination percentage and the germination index decreased from 97.5% to 79.5% and from 11.5 to 5.5, respectively. However, these stresses prolonged the mean time to germination in pumpkin seeds.

Table 1. Main effects of salinity and drought on germination percentage, mean germination time, and germination index of pumpkin

Factor	Germination percentage (%)	MGT (day)	Germination index
Drought (A)			
Control	97.5[a]	2.19[b]	11.5[a]†
Drought	79.5[b]	4.50[a]	5.5[b]
Salinity (B)			
0.00%	99.0[a]	2.46[c]	10.7[a]
0.25%	97.5[a]	2.86[b]	9.45[b]
0.50%	85.0[b]	3.88[a]	7.30[c]
0.75%	72.5[c]	4.18[a]	6.44[d]
A	**	**	**
B	**	**	**
A × *B*	**	**	**

†: Letters connected with the mean in each column refer to significance level at p<0.05. **: significant at p<0.01.

In non-drought control treatment, salinity did not change the germination percentage of pumpkin seeds. However, it declined at 0.50% NaCl under drought stress (Figure 1). The lowest germination percentage of 46.0% was recorded at 0.75% NaCl under drought. Under non-drought conditions, the germination index reduced slightly from 12.3 to 10.8 with increasing salinity. However, drought significantly impacted the germination index, leading to a drop from 9.06 to 2.07. Drought delayed the mean germination time, while increased salinity significantly prolonged this period. Under drought stress, the hazardous effects of salinity on the mean germination time of pumpkin appeared.

The germination percentage began to decrease at 0.50% NaCl only under drought stress and no significant changes were observed under non-drought conditions. The germination index was also diminished by increasing salinity and a greater reduction was observed under drought stress. However, Tarchoun et al. (2022) found that rising NaCl levels decreased the germination percentage of *C. maxima*. Jahed et al. (2023), Ashraf et al. (2021) and Saadaoui et al. (2023) reported inhibited germination under drought stress. Kaya (2024) demonstrated that mean germination time and germination index were inhibited by increasing drought stress with PEG 6000. Furthermore, increased salinity retarded mean germination time, particularly under drought stress. It was obvious that the combination of drought and salt stress showed inhibitory effects on the germination performance of pumpkin. Similar findings were observed by Irik and Bikmaz (2024), who stated a prolonged germination time and a drop in germination percentage and index due to salinity stress.

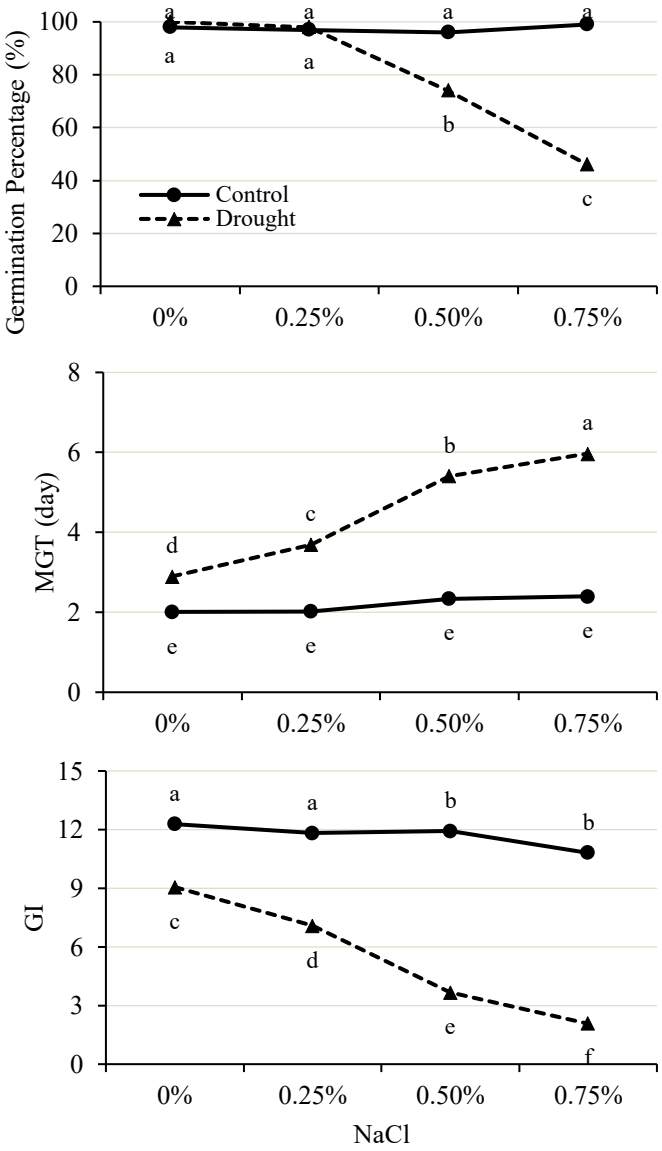


Figure 1. Changes in the germination percentage, mean germination time (MGT), and germination index (GI) of pumpkin seeds subjected to different salinity levels under drought conditions

As shown in Table 2, drought and salinity caused a significant difference in all morpho-physiological parameters of the pumpkin. The interaction between drought and salinity stress was significant, except for dry weight. Under drought stress, plant height, fresh weight, dry weight, leaf area, RWC, and CMS decreased. The interaction effects on morphological and physiological characteristics of pumpkin plants are displayed in Figure 2.

A low NaCl level of 0.25% promoted the leaf area under non-drought conditions and did not considerably decrease up to 0.75% NaCl (Figure 2). Under drought stress, a similar trend was observed, and low leaf size was obtained. However, 0.75% NaCl markedly inhibited the leaf growth of pumpkin plant. Drought also inhibited the leaf growth of pumpkin plants, resulting in a significant reduction in leaf area, which is supported by the findings of Cui et al. (2019) in cucumber. Furthermore, the leaf area was only severely affected by 0.75% NaCl under both control and drought conditions. This result demonstrates that the leaf area exhibited no sensitivity to NaCl concentrations up to 0.75%. The findings of Horuz et al. (2022) confirm the results of the present study, which indicate a reduction in pumpkin leaf area due to salinity. Moreover, the current study reports here that drought exerts a greater inhibitory effect on leaf growth compared to salinity, and the adverse effects of salinity are exacerbated under drought stress. Sucre and Suarez (2011) demonstrated that the impact of salinity on the leaf growth of *Ipomoea pes-caprae* was more detrimental under water deficit conditions.

Table 2. Analysis of variance and mean values of morpho-physiological characteristics of pumpkin plants subjected to salinity and drought stress.

Factor	Plant height (cm)	Plant fresh weight (g plant^{-1})	Plant dry weight (g plant^{-1})	Leaf dry matter (%)	Leaf area (cm^2)	Chlorophyll content (SPAD)	RWC (%)	CMS (%)
Drought (A)								
Control	17.1^a	33.3^a	3.32^a	10.76^b	576^a	32.1^b	88.1^a	63.2^a†
Drought	12.6^b	23.7^b	2.40^b	11.33^a	420^b	39.7^a	75.3^b	52.8^b
Salinity(B)								
0.00%	17.2^a	30.1^b	3.57^a	12.98^a	507^b	43.8^a	83.6^a	66.6^a
0.25%	15.8^b	32.8^a	3.25^a	11.36^b	541^a	34.9^b	86.6^b	59.3^b
0.50%	14.9^b	27.1^c	2.59^b	10.47^c	492^b	33.6^b	81.7^b	53.5^c
0.75%	11.4^c	23.2^d	2.02^c	9.36^d	453^c	31.2^c	74.9^c	52.7^c
A	**	**	**	**	**	**	**	**
B	**	**	**	**	**	**	**	**
A × *B*	**	**	*ns*	**	**	**	*	**

†: Letters connected with the mean in each column refer to significance levels at $p<0.05$. *, **: significant at $p<0.05$ and $p<0.01$, respectively.

Under non-drought stress, plant height decreased from 19.9 cm at 0.00% NaCl to 13.8 cm at 0.75% NaCl, while it dropped to 14.4 cm when plants were subjected to drought (Figure 2). Increased salinity inhibited stem elongation and caused a reduction in plant height, with a minimum reduction of 9.12 cm in plants exposed to 0.75% NaCl under drought stress. Shorter plant height under drought stress was confirmed by the findings of Wang et al. (2024), who determined plant height was significantly lower in cucumber plants exposed to 10% PEG drought. Esan et al. (2023) also reported similar results in soybean. Plant height was shorter in pumpkin plants subjected to increasing salinity levels, regardless of the availability of drought stress. However, drought promoted the severity of salinity stress to reduce the plant height, a decline from 19.9 cm to 14.5 cm. This result confirms the findings of Kurum et al. (2013) and Santos et al. (2018), who indicated that both shoot and root lengths of pumpkin were shortened by increasing salinity.

Increased NaCl concentrations under both non-drought and drought conditions resulted in lower plant fresh weight. In drought, it reduced from 36.4 g to 25.3 g, while in 0.75% NaCl, it decreased to 27.6 g. This indicates that drought exerted a greater negative effect on fresh weight than salinity and that fresh weight was more influenced by salinity under drought stress. The application of 0.25% NaCl stimulated plant fresh weight in non-drought conditions, and higher salinity levels led to a significant decrease in fresh weight. This finding is corroborated by Sevengör et al. (2011) and Kurum et al. (2013), who documented a reduction in the fresh weight of pumpkin as a consequence of salinity.

Regardless of the presence of drought, salinity consistently reduced the dry weight of pumpkin plants (Figure 2). A lower dry weight was recorded in pumpkin plants subjected to drought stress than in those not exposed to drought. Salinity caused a 40% reduction in dry weight under non-drought conditions and a 47% reduction under drought conditions. A linear decrease in dry weight due to increased salinity suggests that it is more sensitive to salinity than fresh weight. Kuşvuran et al. (2021) found a decline in root and shoot dry weight of melon seedlings exposed to salinity stress. Leaf dry matter was also reduced by drought and salinity, but this reduction was minimal compared to the other traits, implying that it was the least affected trait by drought and salinity. Similarly, the findings of Oliveira et al. (2014) and Santos et al. (2018) are consistent with this reduction in total dry matter associated with increasing salinity. A negative effect of drought on dry matter was also determined by Wang et al. (2024) in cucumber. This study indicates that the differences between non-drought and drought conditions were not prominent in dry matter, suggesting that it was not a reliable indicator of drought tolerance in pumpkin.

The chlorophyll content of pumpkin plants exposed to drought stress exceeded that of the control group. Increased salinity reduced chlorophyll content in pumpkin plants under both control and drought conditions. A NaCl level of 0.75% led to a severe depression in chlorophyll content. These results agreed with those of Sevengör et al. (2011) and Taratima et al. (2023), who found that pumpkin plants lost more total chlorophyll when they were stressed by salinity.

Pumpkin plants exposed to drought stress exhibited a lower RWC than those in non-drought conditions. Drought stress resulted in reduced RWC at all NaCl levels. This result is similar to the findings of Cui et al. (2019) in cucumbers, who reported a decrease in RWC due to drought. However, salinity levels of up to 0.50% NaCl did not considerably influence RWC under both control and drought conditions, while the lowest RWC was recorded at 0.75% NaCl. This finding indicates that drought had a more significant impact on reducing RWC than salinity. Horuz et al. (2022) reported that pumpkin plants subjected to salt stress exhibited a reduction in relative water

content (RWC) in comparison to plants that were not exposed to stress. This finding was subsequently corroborated by Rehman et al. (2024) in a study examining melon plants under conditions of drought stress.

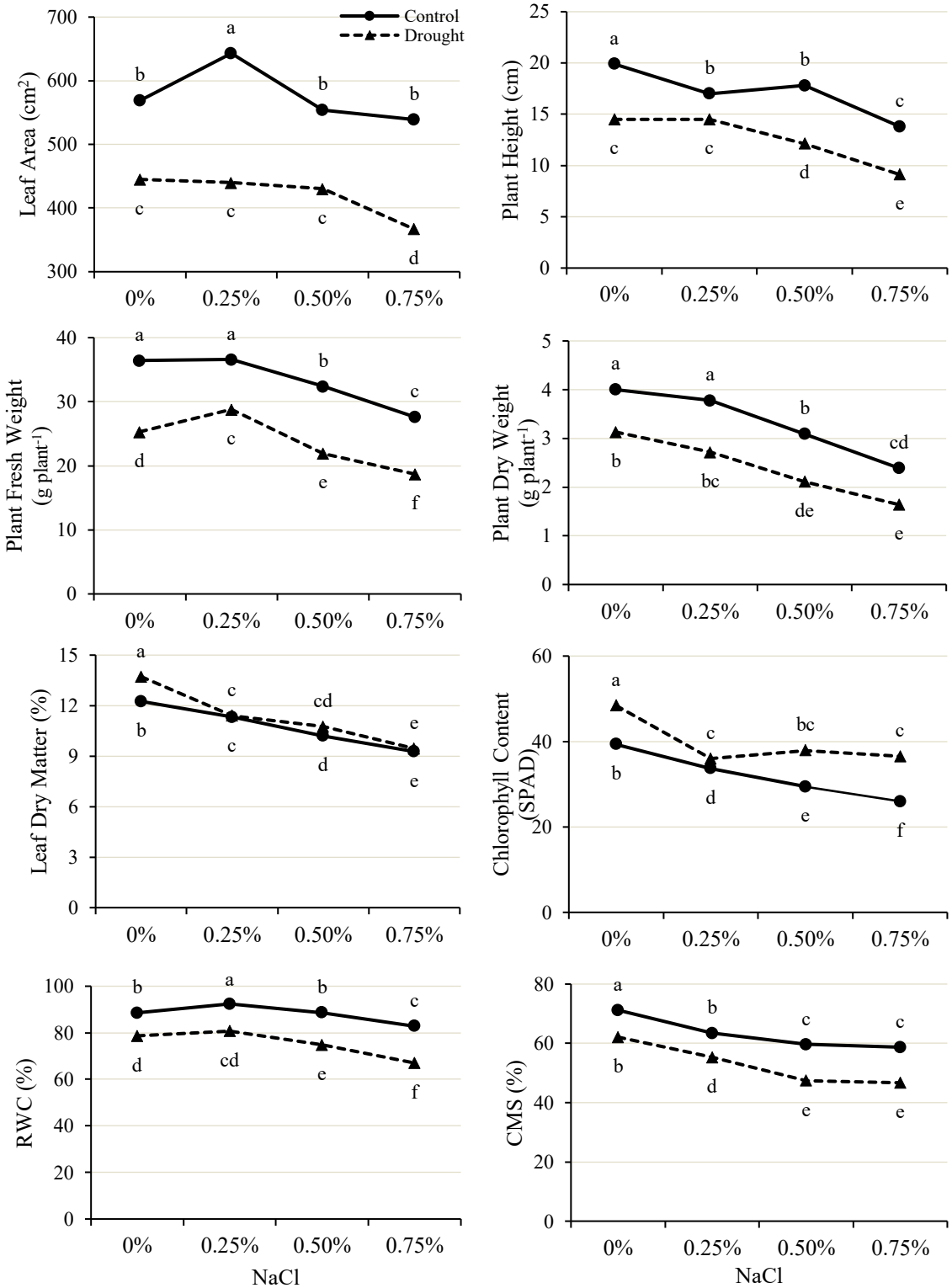


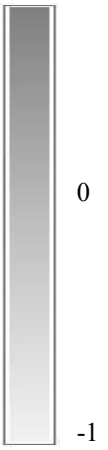
Figure 2. Changes in leaf area, plant height, plant fresh weight, plant dry weight, leaf dry matter, chlorophyll content (SPAD), relative water content (RWC), and cell membrane stability (CMS) of pumpkin plants exposed to different salinity levels under drought conditions.

The cell membrane stability was higher in pumpkin plants grown under non-drought conditions than under drought. However, it decreased with increasing salinity. Under drought conditions, the cell membrane stability decreased by 25% at a NaCl level of 0.75% but was reduced by 17.5% under non-drought conditions. This reduction due to salinity was 15.6% in control and 25.8% under drought stress, indicating that salinity exerted a detrimental effect under drought stress. Electrolyte leakage is a stress tolerance indicator showing cell membrane stability (Irik and Bikmaz, 2024). Increased electrolyte leakage or decreased cell membrane stability were declared under saline conditions by Jamil et al. (2012) in sugar beet, Farooq and Azam (2006) in wheat, Ashraf and Ali (2008) in canola, Elsheery et al. (2020) in cucumber and under drought conditions by Bajji et al. (2002) and ElBasyoni et al. (2017) in wheat and Irik and Bikmaz (2024) in pumpkin. In addition, a decreasing trend in cell membrane stability was observed with increasing salinity under both drought and non-drought conditions.

The relationship between all the characteristics examined was determined using simple correlation analysis (Table 3). All parameters showed a negative relationship with mean germination time, excluding Chl. The highest significant negative correlation coefficient (r= -0.985**) was found between the germination index and mean germination time. The weakest correlations were noted between Chl and other traits, while the strongest was obtained with plant height. The results revealed that germination traits were strongly associated with plant growth parameters under salt and drought stress. Among germination traits, the most promising trait for estimating plant growth parameters was germination index because it was significantly correlated with plant height (r= 0.857**), fresh weight (r= 0.881**), dry weight (r= 0.824**), RWC (r= 0.893**), CMS (r= 0.902**) and leaf area (r= 0.846**), suggesting that germination index should be considered as a predictor evaluating the response of pumpkin to salinity and drought stress.

Table 3. Correlation coefficients among the investigated parameters of pumpkin exposed to salinity and drought stress.

	CHL	PH	FW	DW	DM	RWC	CMS	LA	GP	MGT	GI
CHL	1.000	ns	ns	ns	**	ns	ns	*	ns	ns	ns
PH	-0.071	1.000	**	**	*	**	**	**	**	**	**
FW	-0.212	0.908	1.000	**	ns	**	**	**	**	**	**
DW	0.125	0.890	0.913	1.000	**	**	**	**	**	**	**
DM	0.782	0.410	0.272	0.581	1.000	ns	**	ns	*	ns	ns
RWC	-0.291	0.855	0.934	0.844	0.207	1.000	**	**	**	**	**
CMS	0.096	0.859	0.848	0.886	0.506	0.794	1.000	**	**	**	**
LA	-0.373	0.722	0.869	0.747	0.044	0.887	0.726	1.000	**	**	**
GP	-0.057	0.731	0.710	0.683	0.415	0.754	0.732	0.607	1.000	**	**
MGT	0.185	-0.826	-0.850	-0.801	-0.316	-0.882	-0.878	-0.799	-0.850	1.000	**
GI	-0.215	0.857	0.881	0.824	0.287	0.893	0.902	0.846	0.845	-0.985	1.000



Chl: Chlorophyll content, PH: Plant height, FW: Plant fresh weight, DW: Plant dry weight, DM: leaf dry matter, RWC: Relative water content, CMS: Cell membrane stability, LA: Leaf area, GP: Germination percentage, MGT: Mean germination time, GI: Germination index
*, ** show significance levels at p< 0.05 and p< 0.01, respectively. ns: non-significant.

CONCLUSION

Germination and morphophysiological characteristics of pumpkin were significantly affected by salt under drought stress. A NaCl concentration of 0.50% reduced and delayed germination when there was drought stress, but it did not affect germination percentage under non-drought stress. The germination index was more sensitive to drought and salinity and clearly distinguished salinity levels. This means that any increase in salinity under drought caused the germination index to drop, which could be recommended as a valuable criterion for salt and drought stress during germination. The difference between drought and non-drought conditions was evident as the salt level increased. In the pot experiment, the inhibitory effects of salinity on plant height, plant fresh and dry weight were evident. These parameters could be used as the selection criteria for salt- and drought-tolerant pumpkin genotypes. Conversely, compared to non-saline conditions, RWC decreased in the presence of 0.75% NaCl. Under drought stress, cell membrane stability decreased, and increased salinity led to a further reduction, which should therefore be considered when assessing drought tolerance in pumpkin. On the other hand, germination parameters gave significant correlations with morphological and physiological traits, and they distinguished a more precise separation between salinity levels under drought stress. The germination index was evaluated as a predictor of the response of pumpkin plants to salt and drought stress, serving as a primary selection criterion. It was evident that pumpkin showed greater tolerance to salt and drought stress during the early growth stage compared to the germination stage. In conclusion, this study demonstrated that salinity was more harmful

when there was drought stress than in non-drought conditions, with a NaCl level of 0.50% identified as a critical threshold for pumpkin growth.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest. The paper is original unpublished work, and it is not under consideration for publication anywhere else.

Author Contribution

Conceptualization, methodology, and final version were done independently by Gamze Kaya

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Investigation of the growing performance of promising almond genotypes obtained through selection breeding under *in vitro* conditions

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Abstract

Almond is one of the economically important nut species for Türkiye. There are many genotypes adapted to different regions of Türkiye. The propagation of these genotypes through plant tissue culture methods is essential. Among nut species, almonds are classified as recalcitrant plants that respond poorly to plant tissue culture techniques. In this study, the development of eight genotypes obtained through selection was examined under *in vitro* conditions in full and ½ NRM media containing different concentrations of IBA (0, 1, 2 mg/L). As a result of the study, the longest plant height was observed in genotype 48-6 (2.04 cm), while the highest number of leaves was obtained in genotype 33-B-44 (12.26 leaves). Rooting was observed in genotype 33-B-54, which was selected from Mut, Mersin, in a ½ NRM rooting medium supplemented with 2 mg/L IBA. The results are considered significant since these genotypes have been selected for the first time.

Keywords: Almond, *In Vitro*, Plant Tissue Culture, NRM

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INTRODUCTION

Almond belongs to the Rosales order and the Rosaceae family and is a nut species (Özbek, 1978). Almond, an economically important fruit species, is in increasing demand worldwide. Consequently, almond production areas have recently expanded in Türkiye and globally (Gök et al., 2020). Although production varies by year and climatic conditions, Türkiye ranks among the leading almond-producing countries. According to FAO data, the total global in-shell almond production in 2022 was 3.6 million tons. The highest almond-producing country was the United States, with 1,858,010 tons, followed by Australia, 360,328 tons, and Spain, 245,990 tons. Türkiye ranked fourth with a production volume of 190,000 tons (Anonymous, 2024a).

Almonds have been an essential food source in Asia and Europe for thousands of years. Natural selection and environmental adaptation efforts have continued, alongside challenges such as major diseases and insect pests, with farmers routinely selecting the best wild plants (Grasselly, 1972; Socias i Company, 2002; Gradziel & Martinez-Gomez, 2012). Superior genotypes have been identified and vegetatively propagated. In the Mediterranean region, Asia, and California, most modern cultivars have been developed more recently through clonal selection (Gradziel, 2003; Gradziel & Martinez-Gomez, 2012). Türkiye is known to have a rich genetic diversity of almonds, and selection studies on almonds are widespread (Şimşek, 2015).

Plant tissue culture plays an indispensable role in modern agriculture and plant breeding. Tissue culture methods enable various applications, from propagating plants at the cellular level to making genetic modifications. It is effectively used for preserving genetic diversity, developing disease-resistant plants, increasing productivity,

and supporting sustainable agricultural practices. Additionally, it plays a significant role in conserving endangered species. Plant tissue culture has great potential for increasing agricultural production and developing plants resistant to environmental conditions. Combined with genetic engineering and selection methods, it helps shape the future of agriculture (Yücel, 2022).

Numerous studies have established micropropagation protocols for *Prunus* species and hybrids by employing various nutrient media and concentrations of plant growth regulators. For example, Ünek et al. (2011) employed Murashige and Skoog (MS) medium with varying concentrations of 6-benzyleaminopurine (BAP), kinetin (Kin), 2-isopentenyladenine (2iP), and thidiazuron (TDZ); Dejampour et al. (2011) utilized a modified Driver and Kuniyaki Walnut (DKW) medium supplemented with 0.5 mg/L BAP; Sharifmoghaddam et al. (2011) used MS medium enriched with BAP and indole-3-butyric acid (IBA); Aghaye and Yadollahi (2012) experimented with MS medium containing various concentrations of BAP; Pakyürek and Hepaksoy (2019) used MS medium containing BAP, IBA, and gibberellic acid (GA_3) in different concentrations; Abbasi et al. (2019) utilized MS medium supplemented with BAP and TDZ; Ritterbusch et al. (2020) applied MS medium with different levels of BAP and Kin; and Borkheyli et al. (2021) employed MS, Woody Plant Medium (WPM), and DKW media with different BAP concentrations.

Genotypes obtained through selection studies need to be preserved and propagated. Plant tissue culture techniques provide an opportunity for propagating selected plants. This study aimed to investigate the growth performance of genotypes obtained through selection breeding under *in vitro* conditions.

MATERIALS AND METHODS

Plant Materıal

The plant materials used in this study included genotypes obtained through selection breeding from the Niğde and Mersin-Mut regions (Küden et al., 2023), as well as the previously selected local genotype 48-6 (Dokuzoğuz & Gülcan, 1973). The 48-6 genotype was obtained from the Alata Horticultural Research Institute. Eight genotypes were examined for their growth performance under *in vitro* conditions, including two from Niğde, five from the Mersin-Mut region, and the 48-6 genotype. Information regarding these genotypes is presented in Table 1.

Table 1. Names of the genotypes and the provinces where they were selected.

No	Name of genotype	Province
1	33-B-15	Mersin-Mut
2	33-B-32	Mersin-Mut
3	33-B-44	Mersin-Mut
4	33-B-54	Mersin-Mut
5	33-B-66	Mersin-Mut
6	51-B-02	Niğde
7	51-B-16	Niğde
8	48-6	Local genotype

Establishment of Experiment

Microshoots of eight genotypes were cultured in full NRM (Nas & Read, 2004) and ½ NRM media supplemented with different concentrations of IBA (0, 1, and 2 mg/L). The pH of the media was adjusted to 5.7. The media were poured into culture containers and sterilized in an autoclave at 121°C and 1.05 atm pressure. The plants were kept in darkness for the first week and then cultured under a 16-hour light/8-hour dark photoperiod. The plants remained in the culture medium for 8 weeks, and the temperature of the growth chamber was maintained at 25°C.

The experiment was designed according to a factorial arrangement in a completely randomized design (CRD) with three replications. In each treatment, 10 plants were planted. Data on plant height (cm**)** and number of leaves (count) were analyzed. The obtained data were subjected to variance analysis, and differences between significant means were determined using the LSD test. Statistical analyses were performed using JMP 5.01 software.

RESULTS AND DISCUSSION

According to the statistical analysis results, the interactions between genotypes × medium strength and genotypes × medium strength × IBA concentrations were significant regarding plant height ($P \leq 0.01$). Data on plant height (cm) and number of leaves (count) under different concentrations of IBA (0, 1, and 2 mg/L) in full NRM and ½ NRM media are presented in Table 2.

When genotype means were examined, the 48-6 genotype was identified as the most successful, with a plant height of 2.04 cm, while the 51-B-16 genotype had the shortest plant height at 0.69 cm. Regarding medium strength, plants in the ½ NRM medium reached a greater plant height (1.45 cm) than those in the full NRM medium. Differences between IBA concentrations showed that the most extended plant height was obtained in a

medium without plant growth regulators. According to the statistical analysis results, plant height ranged from 0.60 cm to 2.80 cm. The longest plant height (2.80 cm) was observed in the 48-6 genotype in ½ NRM medium supplemented with 2 mg/L IBA. In comparison, the shortest (0.60 cm) was recorded in the 51-B-16 genotype in full NRM medium supplemented with 2 mg/L IBA.

Regarding leaf number, significant differences were found between genotypes ($P \leq 0.01$). When genotype means were examined, the number of leaves was obtained in the 33-B-44 genotype (12.26 leaves), while the 33-B-54 genotype had the lowest number (6.71 leaves). Regarding medium strength, full NRM resulted in a higher number of leaves (10.20) than ½ NRM. However, differences between IBA concentrations and genotype × medium strength × IBA concentration interactions were not statistically significant. The 0, 1, and 2 mg/L IBA concentrations were in the same statistical group. According to statistical analysis results, the number of leaves ranged from 6 to 14.70. The highest number of leaves (14.70) was recorded in the 33-B-44 genotype in full NRM medium without plant growth regulators. In contrast, the lowest (6 leaves) was observed in the 33-B-54 genotype in ½ NRM medium without plant growth regulators.

Sevgin (2010) aimed to optimize the rooting stage in *in vitro* micropropagation of difficult-to-root almond cultivars **(**GF-677, Nonpareil, Ferragnes, and Tuano). As a result of the study, rooting rates in full-strength and ½-strength NRM media were similar**.** No rooting was obtained in the Ferragnes and Nonpareil almond cultivars. The best rooting was observed in the GF-677 rootstock**,** with a 64% rooting rate in the presence of 1 mg/L IBA**.** Nas et al. (2010) investigated the *in vitro* regeneration ability of *Prunus microcarpa* subsp. tortusa using various explants (root, cotyledon, and hypocotyl segments) and cytokinins (BAP), meta-topolin (mT), and TDZ). They cultured the micro shoots obtained from the proliferation medium in ½-strength NRM medium containing different concentrations of IBA. Over 70% of the microshoots were rooted within two weeks, and each shoot developed an average of three roots. In this study, rooting was observed in the 33-B-54 genotype in a ½ NRM medium supplemented with 2 mg/L IBA. However, the average root number was 0.2, which was very low. Since the rooting rate was insufficient, this data was not analyzed statistically. This result may be attributed to the genotype effect. Photographs showing the development of the 48-6 genotype in ½-strength NRM medium supplemented with 2 mg/L IBA and in full-strength NRM medium supplemented with 2 mg/L IBA are presented in Figure 1. Images of the 51-B-16 and 33-B-66 genotypes are shown in Figure 2.



2 mg/L IBA-Supplemented ½ NRM



2 mg/L IBA-Supplemented Full NRM

Figure 1. Image of the development of the 48-6 genotype.

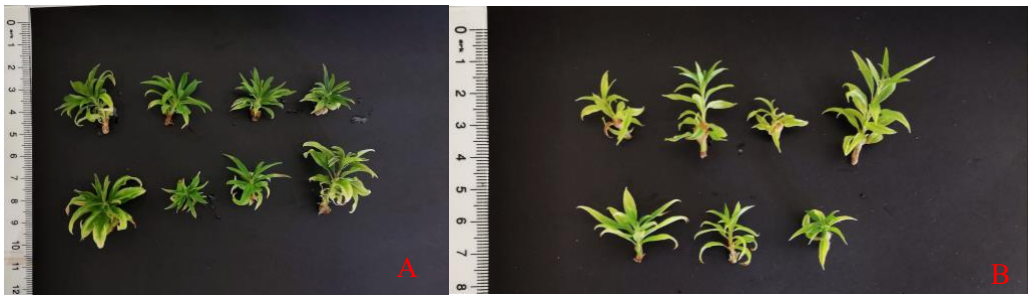


Figure 2. 51-B-16 Genotype in ½ NRM Supplemented with 2 mg/L IBA (A) and 33-B-66 Genotype in Hormone-Free Full NRM (B)

Table 2. Effects of different IBA concentrations on plant height (cm) and number of leaves in full NRM and ½ NRM media.

Genotype	Medium Strength	IBA Concentration	Plant height (cm)	Leaf Number	Plant Height Genotype Avg.	Leaf Number Genotype Avg.
33-B-15	NRM	0	1.54^{f-l}	9.80^{e-o}	1.33^{c}	
		1	1.40^{h-n}	8.80^{h-q}		
		2	0.97^{o-u}	7.90^{m-s}		
	½ NRM	0	1.35$^{ı-o}$	10.10^{e-n}		10.05^{cd}
		1	1.35$^{ı-o}$	11^{c-j}		
		2	1.38$^{ı-n}$	12.70^{a-d}		
33-B-32	NRM	0	1.12^{m-s}	10.70^{e-k}	1.42^{c}	
		1	1.16l^{m-r}	12.20^{b-e}		11^{bc}
		2	0.78^{r-u}	11.20^{e-h}		
	½ NRM	0	1.75$^{d-ı}$	10.30^{d-m}		
		1	1.75 $^{d-ı}$	10.50^{d-l}		
		2	1.98^{b-e}	11.10$^{e-ı}$		
33-B-44	NRM	0	1.82^{d-g}	14.70^{a}	1.59^{b}	
		1	1.33^{j-o}	13.10^{a-c}		
		2	1.17^{l-r}	10.80^{e-k}		12.26^{a}
	½ NRM	0	1.75$^{d-ı}$	11.70^{e-f}		
		1	1.71 $^{d-j}$	11.20^{e-h}		
		2	1.79^{d-h}	12.10^{e-e}		
33-B-54	NRM	0	0.86^{p-u}	6.90^{q-s}	0.90^{e}	
		1	0.84^{q-u}	6.90^{g-s}		
		2	0.74^{s-u}	7.70^{n-s}		
	½ NRM	0	1.35$^{ı-o}$	6^{s}		6.71^{f}
		1	0.90^{p-u}	6.60^{q-s}		
		2	0.75^{s-u}	6.20^{rs}		
33-B-66	NRM	0	2.07^{b-d}	11.70^{e-f}	1.68^{b}	
		1	1.44^{g-m}	11.90^{e-e}		
		2	1.20^{k-q}	11.30^{e-g}		
	½ NRM	0	1.85c-f	8.20^{l-s}		10.07^{cd}
		1	1.75$^{d-ı}$	8.60^{j-r}		
		2	1.81^{d-g}	8.75$^{ı-q}$		
51-B-02	NRM	0	1.25^{k-p}	14.55^{ab}	1.07^{d}	
		1	1.20^{k-q}	8.50^{k-r}		
		2	1.35$^{ı-o}$	10.70^{e-k}		9.37^{d}
	½ NRM	0	1.01^{n-t}	7.20^{p-s}		
		1	0.89^{p-u}	7.60^{o-s}		
		2	0.75^{s-u}	7.70^{n-s}		
51-B-16	NRM	0	0.67^{tu}	8.20^{l-s}	0.69^{f}	
		1	0.78^{r-u}	9^{g-q}		
		2	0.60^{u}	7.20^{p-s}		
	½ NRM	0	0.73^{s-u}	7.60^{o-s}		7.73^{e}
		1	0.68^{tu}	7.20^{p-s}		
		2	0.70^{tu}	7.20^{p-s}		
48-6	NRM	0	2.24^{bc}	11.40^{e-g}	2.04^{a}	
		1	1.58^{e-k}	10.40^{d-l}		
		2	1.50^{f-m}	9.40^{f-p}		
	½ NRM	0	1.85^{c-f}	12.20^{b-e}		11.36^{ab}
		1	2.30^{b}	12.10^{e-e}		
		2	2.80^{a}	12.70^{a-d}		
	IBA Concentration Avg.	0	1.45^{a}	10.07^{a}		
		1	1.31^{b}	9.72^{a}		
		2	1.26^{b}	9.66^{a}		
	Medium Strength Avg.	NRM	1.23^{b}	10.20^{a}		
		½ NRM	1.45^{a}	9.43^{b}		

Plant length: Lsd $_{Genotype}$:0.083 Lsd $_{Genotype * Nutrient Medium *IBA dose}$: 0.20 Lsd$_{number of leaves}$: 1.23
Number of Leaves: Lsd $_{Genotype}$:1.23 Lsd $_{Genotype * Nutrient Medium *IBA dose}$: 0.20

CONCLUSION

Almond is an economically important fruit. Since genotypes obtained through breeding are new individuals, they are valuable plant materials. Therefore, they must be preserved and propagated. In this regard, tissue culture techniques are effectively utilized. The micropropagation and rooting of selected genotypes through tissue culture techniques are particularly crucial. As these genotypes may possess characteristics of new rootstocks and cultivars, every finding obtained is of great significance.

Since almond is classified among the recalcitrant fruit species that respond poorly to tissue culture studies, the results of this study are valuable. This study investigated the development of almond genotypes selected from different regions under tissue culture conditions. As a result of the study, rooting was observed in the 33-B-54 genotype in a ½ NRM medium supplemented with 2 mg/L IBA. However, the average number of roots was 0.2, indicating insufficient rooting. Modifying nutrient media, developing new nutrient formulations, and using plant growth regulators at different concentrations and combinations to enhance growing performance under in vitro conditions is recommended.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that there is no actual, potential or perceived conflict of interest in this research article.

Author contribution

S.G, M.H.E, D.D, Y.A.K: Study data analysis, conceptualization and design of the research; S.G: Wrote the original draft; Y.A.K, D.D: Editing and preparation of the manuscript. All authors have read and approved the manuscript.

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Anti-infective effect of *Aquilaria malaccensis* L. essential oil against *Candida* strains, the leading cause of yeast infectious

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Abstract

Aquilaria malaccensis L., known as Agarwood, is widely found in India, Malaysia, Bhutan, and Indonesia. It is a pleasantly scented plant used in the production of resin. It is an interesting material in the field of health due to its resin and essential oil, which exhibit antimicrobial properties. This study aimed to evaluate the antifungal properties of *A. malaccensis* L. essential oil and determine its minimum inhibitory concentrations (MIC) against the *Candida* species tested. The inhibitory effect of *A. malaccensis* L. essential oil was tested on five *Candida* species. The broth microdilution method was used to determine the MIC values against the tested microorganisms, and the viability of microorganisms exposed to the plant essential oil was assessed using resazurin sodium salt. According to the results, the MIC of the plant essential oil against *Candida tropicalis, Candida krusei* and *Candida albicans* is 62.50 µL/mL. While the MIC against *Candida glabrata* is 31.25 µL/mL, the MIC against *Candida parapsilosis* is 7.81 µL/mL. These results show the potential of *A. malaccensis* L. as an anti-candidal agent. The continuation of this study revealed the need for optimizing and implementing more comprehensive antimicrobial tests.

Keywords: *Aquilaria malaccensis* L., Agarwood, Anti-candidal activity, Candidiasis, MIC value

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INTRODUCTION

Aquilaria malaccensis L., also known as Agarwood, is a plant from the Thymelaeaceae family with a long history. It is used in cultural rituals and medicine, especially in Asia. Agarwood is used in perfumery, cosmetics, medicines, balms, soaps and body lotions. It is known as gaharu, a precious tree, or a short tree in Indonesia. It is used in the production of perfume and incense due to the smell and structure of its resin (Naef, 2011). The composition of agar tree resin is mainly composed of mixtures of sesquiterpenes and 2-(2-phenylethyl) chromones (PECs) (Chen et al., 2012; Subasinghe et al., 2015). It has been shown that sesquiterpenoids and chromones are also present in its essential oil (Fazila et al., 2012; Jayachandran et al., 2014). Chromones are a large group of secondary metabolites with a wide range of potential therapeutic indications for immunomodulation, inflammation, cancer, diabetes, neurological conditions, and bacterial and viral infections (Tawfik et al., 2014). *A. malaccensis* L. tissues contain flavonoids, glycosides, triterpenoids, and tannins. The antimicrobial properties of these components are well-documented, and this effect has been demonstrated in studies published in the literature (Batubara et al., 2021). All these important compounds contribute to the antimicrobial activity of agarwood. It is an interesting material in the field of health because its resin and oil show antimicrobial properties (Yang et al., 2012; Gogoi et al., 2023). Its antimicrobial activity offers us the opportunity to discuss this plant and its content in terms of health. There is limited literature on its anti-candidal properties. Candidiasis is an infection caused by the overgrowth of *Candida* species in the body. It is frequently encountered in the oral mucosa and female reproductive system in babies. Due to regional sensitivities, natural ingredients are preferred in the treatment of

candidiasis. In our research, the anti-candidal property of *A. malaccensis* L. extract was tested using *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida albicans* and *Candida parapsilosis*. In previous studies, the genotoxicity, antioxidant, anti-inflammatory, anti-diabetic, acetylcholinesterase inhibitor, anti-urolytic, tyrosinase inhibitor, and antimicrobial properties of *A. malaccensis* essential oil have been investigated (Gogoi et al., 2023). There is limited data on its anti-candidal activity. Our research investigated the anti-candidal potential of *A. malaccensis* L. essential oil.

MATERIALS AND METHODS

Aquilaria malaccensis L. essential oil

In this research, Agarwood oil (Katre-i Sifa, Istanbul, Turkiye), a 100% natural, undiluted fragrance therapeutic grade essential oil for aromatherapy, was used. The essential oil was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) at a 1:1 ratio and subjected to antifungal testing.

Determination of Antifungal Activity

Microorganisms and Media

The antifungal activity of *A. malaccensis* L. essential oil was tested against *Candida albicans* and non-*albicans Candida* strains. Five *Candida* strains, the leading causes of *Candida* infections, were used in the activity tests. These strains are *Candida tropicalis* (ATCC 13803), *Candida glabrata* (ATCC 2001), *Candida krusei* (ATCC 14243), *Candida albicans* (ATCC 14053) and *Candida parapsilosis* (ATCC 22019), all purchased from the American Type Culture Collection (VA, USA). Sabouraud broth (Biolife, Milan, Italy) and sabouraud 4% glucose agar (Chemsolute, Renningen, Germany) were used for subculture and activity tests of *Candida* strains.

Determination of Minimum Inhibitory Concentration (MIC) for Yeast Strains

The minimum inhibitory concentration (MIC) of the tested oil was determined by making minor modifications to the previously applied method (Unver et al., 2024; Unver & Gurhan, 2024a and 2024b). Two dilution methods were applied for the antifungal activity determination test: agar and resazurin-based broth dilutions. In the broth dilution method, the essential oil, dissolved in DMSO, was diluted from the first to the tenth well on the microplate. Since 500 µL/mL of the oil added to the first well was diluted two-fold, it was present at 0.977 µL/mL in the tenth well. The eleventh and twelfth wells were negative and positive controls, respectively. The eleventh well represents the viability of the yeast strain used in the same row and contains only the broth medium and the yeast strain of the same row. The twelfth-row controller indicates no contamination when the experimental protocol was implemented and contains only broth medium. After diluting the essential oil, the standard inoculum of each strain was prepared. Each strain was suspended in distilled water, and the turbidity was adjusted to 0.5 McFarland (1-1.5 x 10^6 cfu/mL). Subsequently, 1 µL of each *Candida* suspension was inoculated into all wells except the negative control wells. *C. tropicalis, C. glabrata, C. krusei, C. albicans* and *C. parapsilosis* were inoculated into the wells in rows A, B, C, D and E, respectively. Then, the microplate was incubated at 35 °C for 24 hours. The next day, 15 µL of resazurin (0.15% v/v) (Sigma, Germany) was added to each well, and the microplate was incubated at 35 °C for 4-6 hours to observe the color change.

In the agar dilution method, essential oil was added to the first plate at a concentration of 500 µL/mL, and a two-fold dilution was made, starting from the first plate and proceeding to the eleventh plate. Therefore, the oil concentration varied between 500 and 0.488 µL/mL from the first plate to the eleventh plate. Afterward, as applied in the broth dilution method, a standard inoculum was prepared and inoculated onto the agar plates divided into five regions *C. tropicalis, C. glabrata, C. krusei, C. albicans* and *C. parapsilosis* were inoculated in the regions A, B, C, D and E on the plates, respectively. Subsequently, plates were left in an incubator and waited at 35 °C for 24 hours. The next day, MIC values were determined by observing the growth of colonies on the plates..

RESULTS AND DISCUSSION

In the broth dilution method, the color change on the microplate, from blue-purple to pink-salmon, indicates the active reproduction of the microorganism in the wells (Elshikh et al., 2016). Accordingly, *C. tropicalis, C. krusei,* and *C. albicans* inoculated in rows A, C, and D grew from the fifth well. For these reasons, the value in the well with the lowest oil concentration in which no growth was observed (62.5 µL/mL) was determined as the MIC value. *C. glabrata* inoculated into row B showed growth starting from the sixth well. Therefore, the MIC value for *C. glabrata* was determined to be 31.25 µg/mL. Finally, *C. parapsilosis* inoculated in row E grew from the eighth well. Thus, the MIC value for this microorganism was found to be 7.81 µL/mL (Figure 1).

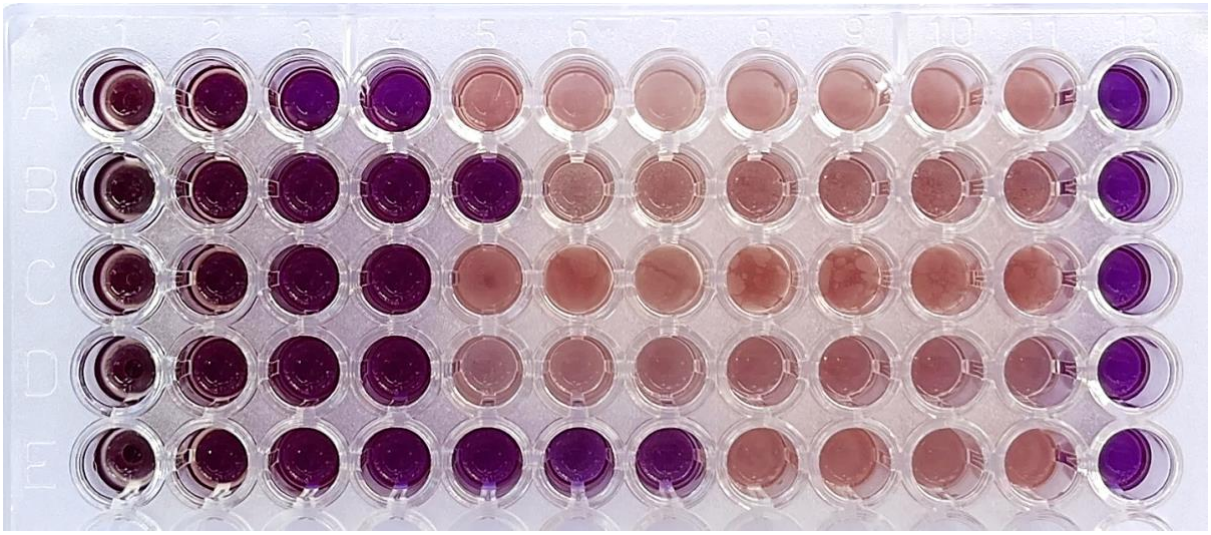


Figure 1. The antifungal activity of *Aquilaria malaccensis* L. essential oil against *Candida tropicalis* (A), *Candida glabrata* (B), *Candida krusei* (C), *Candida albicans* (D) and *Candida parapsilosis* (E) using the resazurin-based broth dilution method. The essential oil concentration ranged between 500 and 0.977 µL/mL from the first to the tenth wells. The eleventh and twelfth wells are negative and positive controls, respectively.

The agar dilution results were the same as the resazurin-based broth dilution results, and the MIC values were the same (Figures 2 and 3). As a result of the agar dilution method, *C. tropicalis, C. krusei,* and *C. albicans* inoculated into the first, third, and fourth zones on the agar plates started to grow from the E plate. Therefore, the concentration of oil in plate D (62.5 µL/mL) was determined as the MIC value. Since the colonies of *C. glabrata* inoculated to the second area on the plate started to appear from the F plate, the MIC value was determined as 31.25 µL/mL, which is the value in the plate with the lowest oil concentration in which no growth was observed. Finally, *C. parapsilosis* inoculated into the fifth zone showed growth starting from the H plate. Therefore, the MIC value of the oil against *C. parapsilosis* was determined to be 7.81 µg/mL (Figure 2).

Agarwood is used in Chinese medicine as a natural sedative, pain reliever, digestive aid, and carminative (Ye et al., 2016; Ma et al., 2021). *A. malaccensis* L. is a quality, fragrant, and medicinally significant plant-based essential oil with pharmacological potential and genotoxicity (Samadi et al., 2016). According to the results of our research, the anti-candidal activity of *A. malaccensis* L. was observed. The MIC value, which represents the minimum concentration of the essential oil required to inhibit the growth of the fungi, of *A. malaccensis* L. against *C. tropicalis, C. krusei* and *C. albicans* is 62.50 µL/mL. The MIC value against *C. glabrata* is 31.25 µL/mL, while the MIC value against *C. parapsilosis* is 7.81 µL/mL. These results support the anti-candidal activity of *A. malaccensis* L.

The dominant compounds in plant essential oil directly affect the medicinal and pharmaceutical use of the plant (Gogoi et al., 2023; Unver & Gurhan, 2024). In a study in the literature, the three most abundant compounds in the plant essential oil content were determined to be cubanol (22.26%), agarospirol (14.35%), and aristolene (13.22%) (Gogoi et al., 2023). Although antibacterial activity was tested in the same study, the disk diffusion method was employed, and the inhibition zones obtained were evaluated to draw inferences about the antimicrobial activity. Therefore, these results did not provide reliable MIC values (Gogoi et al., 2023). With their potential, these compounds are thought to contribute to the essential oil's antifungal activity, offering hope for future treatments.

It is crucial to develop this subject further for future research, particularly by applying different analyses at varying concentrations. The use of herbal agents in treating *Candida* species, which cause complex and persistent infections, is particularly necessary for women, mothers, and babies. *A. malaccensis* L. could serve as a potent alternative in this regard. While our research was not conducted under *in vivo* conditions, the preliminary nature of this study should be seen as an advantage in revealing the potential of *A. malaccensis* L. as a natural anti-candidal agent. This underscores the importance of continued research in this area.

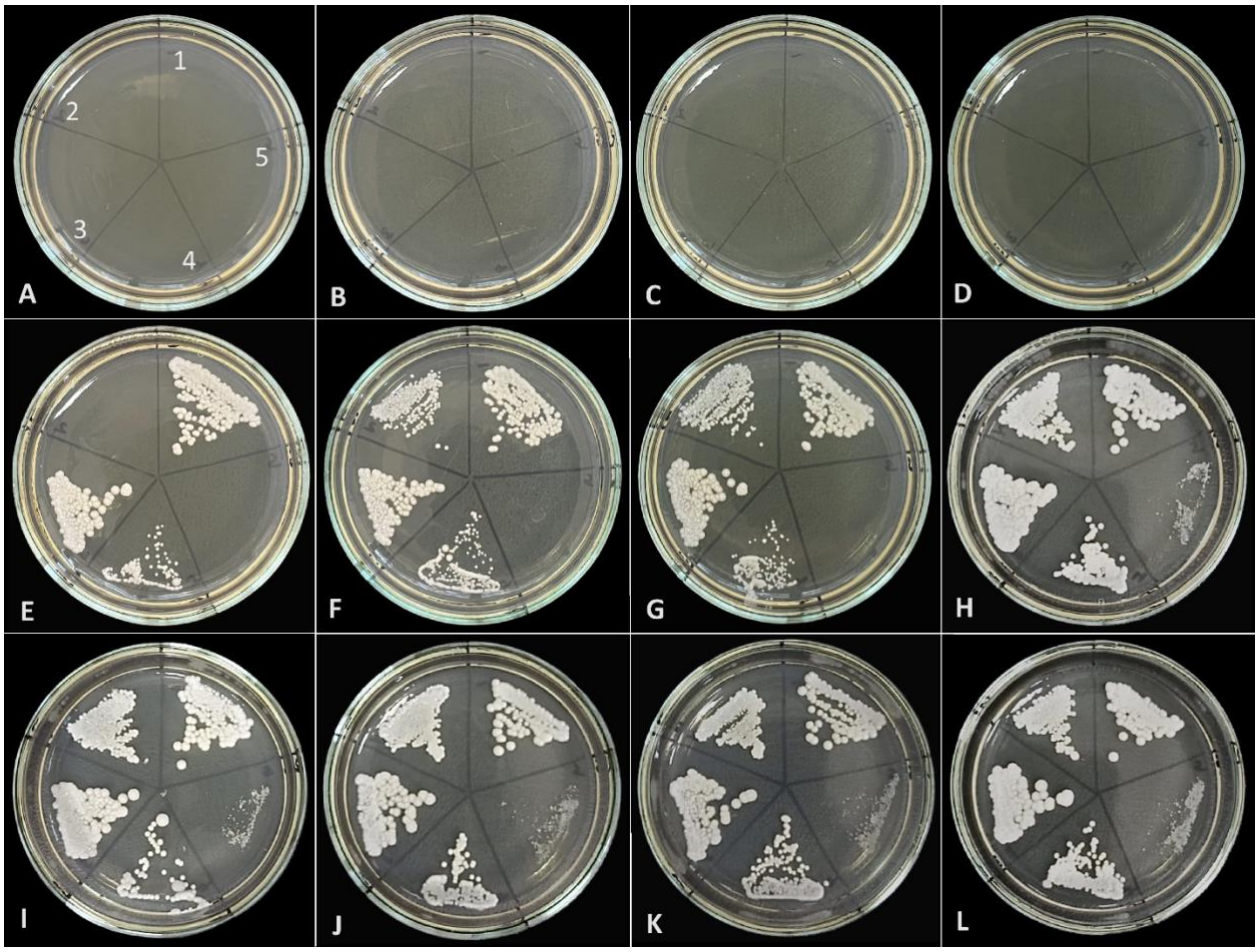


Figure 2. The antifungal activity of *Aquilaria malaccensis* L. essential oil against *Candida tropicalis* (1), *Candida glabrata* (2), *Candida krusei* (3), *Candida albicans* (4) and *Candida parapsilosis* (5) using agar dilution method. The essential oil concentration ranged from 500 to 0.488 µL/mL across plates A to K. The twelfth plate (L) is the control.

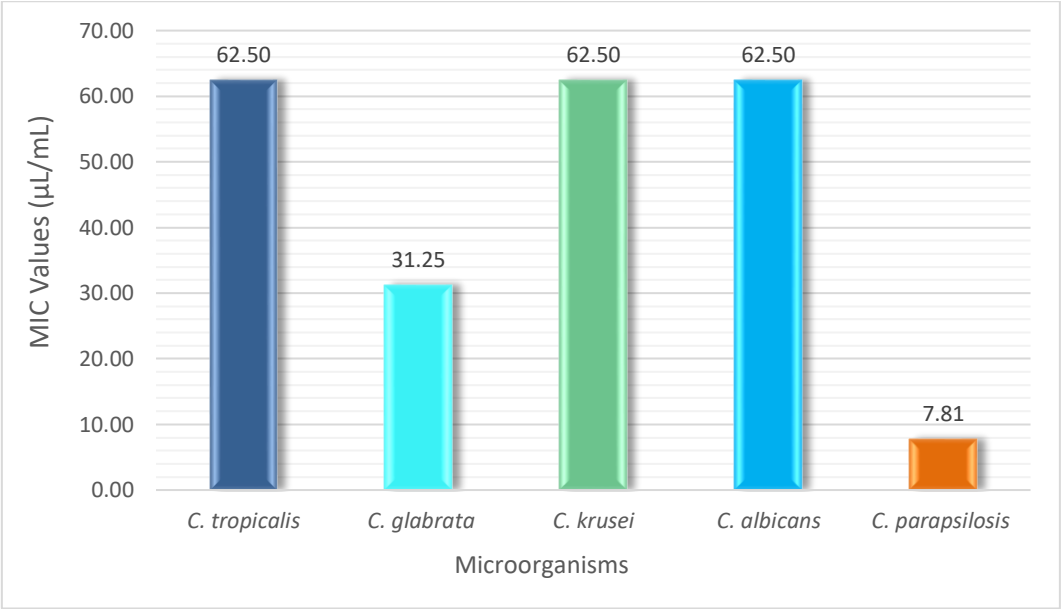


Figure 3. Comparative MIC values of *Aquilaria malaccensis* L. essential oil against tested yeast species

CONCLUSION

A. malaccensis L. is a natural and reliable therapeutic agent with antimicrobial properties. Studies conducted with Agarwood essential oil in the literature to date have indicated the existence of important pharmacological properties of this oil (Zhang et al., 2025; Ma et al., 2025; Jayaprakash et al., 2025). Therefore, scientists have always been interested in studies on Agarwood. Its potential for use in infections that are both dominant and difficult to treat, such as *Candida* infections, has been studied to a limited extent in the literature. This preliminary study has raised the possibility that *A. malaccensis* L. essential oil could be used for *Candida* infections. In subsequent studies, the necessity of interspecies comparisons and different analyses was revealed.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflicts of interest.

Author contribution

All authors declare that they have participated in the design, execution, and analysis of the study and have approved the final version.

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Sensitivity analysis of uranium reduction and its reoxidation by Fe(III)-(hydr)oxides biogeochemical reaction dynamics

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Abstract

Sensitivity analysis is a useful tool in modeling environmental systems to identify how variations in model parameters would impact model outputs. In modeling environmental processes in biological systems, the rates and processes of biodegradation reactions are described using biokinetic parameters. Reducing the parameter uncertainty in modeling efforts would be important for reliable and accurate model results. Understanding the impact of variations in the biokinetic parameters would be highly critical to help to reduce the uncertainity in model predictions. This study presents a sensitivity analysis of the biokinetic parameters affecting the biogeochemical reaction network for uranium biotransformation dynamics. The main reactions included in the network are sulfate bioreduction, Fe(III) bioreduction, U(VI) reduction to U(IV), Fe(III) reduction by sulfide, U(IV) reoxidation to U(VI) and sulfur precipitation-dissolution reactions. The sensitivity analysis results revealed that changes in the biokinetic parameters to the sulfate bioreduction reaction had the most significant impact to the model outputs. Among the parameters, maximum substrate utilization rate and yield coefficient had the most significant impact, whereas half-saturation constants had slightly less impact on model results. U(VI) concentration predictions were the most sensitive towards variations in biokinetics parameters among the species monitored within the biogeochemical reaction network. Fe(III) bioreduction, Fe(III) reduction by sulfide and sulfur precipitation/dissolution reactions were not shown to be sensitive to any changes in the biokinetic parameters.

Keywords: Sensitivity analysis, Biokinetic parameters, Uranium, Reoxidation, Biogeochemical processes

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INTRODUCTION

Modeling and simulations are important components of biodegradation processes in terms of efficient process design and evaluation of system performance. Simulations of contaminant biodegradation processes can be used both to understand the system dynamics as well as to predict contaminant concentrations under different environmental factors that would affect the operational performance. Due to the complex nature of the environmental systems and variability in environmental conditions which might change over space and time, some level of uncertainty might be introduced into the models. Depending on the choice of the model structure, uncertainty in the model parameters would need to be considered for accurate predictions and overall reliability of the model outcomes. The parameters used in the models developed for the biotreatment processes are commonly referred to as biokinetic parameters, which are constants used to describe the rates and processes of the biological degradation reaction of concern. An accurate and reliable estimation of these biokinetic parameters are highly important for accurate and reliable simulation results that can efficiently represent the system (Zhou et al., 2012).

Sensitivity analysis is a useful tool to investigate the uncertainty of biokinetic parameters in model predictions and also determine which parameter would have the most significant impact on the model outputs (Neumann 2012). Various studies have been conducted to investigate the effect of kinetic parameters in modeling a diverse range of pollutant degradation processes by means of sensitivity analysis (Seyfi et al., 2021; Baalbaki et al., 2017; Vázquez-Rodríguez et al., 2006; Lin and Wu 2011; Malaguerra et al., 2011; China and Kumar, 2004; Pontes et al.,

2010). However, studies related to sensitivity analysis for biological transformations of uranium in environmental systems are highly limited.

Uranium has been one of the most important contaminants that has been reported in water, soil and sediments; and has been considered to be a significant threat due to its mobility, solubility and toxicity (Renshaw et al., 2005; Tokunaga et al., 2008; Wilkins et al., 2006). The oxidized form of U(VI), which is commonly found as the uranyl ion (OU_2^{+2}) is more toxic, soluble and mobile; and can be microbially reduced to the tetravalent form, U(IV), which is less toxic, less mobile and insoluble in the form of biogenic uraninite mineral ($UO_2(s)$) under reducing conditions (Gu et al., 2005; Renshaw et al., 2005; Wufuer et al., 2017). This microbially mediated remediation strategy for soluble U(VI) to insoluble U(IV) has therefore been considered to be the most cost-effective and promising approach among other removal mechanisms for uranium. Metal-reducing bacteria, particularly iron reducing and sulfate-reducing bacteria (SRB) can facilitate the reduction of U(VI) to U(IV) in the presence of suitable electron donors (Anderson et al., 2003; Gihring et al., 2011; Wufuer et al., 2017; Yabusaki et al., 2011). However, experiments conducted by Sani et al. (2004) reported that the insoluble uraninite might be reoxidized back to the soluble U(VI) in the presence of Fe(III)-hydr(oxides) when the electron donor in the medium (e.g., lactate) is consumed, highlighting the challenge for uranium bioremediation efforts. Spycher et al. (2011) have described the biogeochemical reaction network of the experiments conducted by Sani et al. (2004) capturing the important thermodynamic constraints effecting the uranium transformations. In their study, the competing rates of reactions for U(IV) reoxidation, Fe(II) and sulfide generation were presented and biokinetic parameters for the biogeochemical reactions were calibrated to capture the experiment data. Particular emphasis has been provided for the relative rates of U(IV) reoxidation versus Fe(III) oxidation by sulfide, where sulfide is generated by sulfate reduction reactions in the system. Based on the sensitivity of the competing reaction systems, it would be important to investigate the impact of uncertainty of the calibrated biokinetic parameters on model outcomes, as the variations in these parameters would reflect the local changes in the factors affecting the relative rates of the reactions. Variations on the calibrated biokinetic parameters would be highly likely in natural systems, which would have significant influence on contaminant predictions, especially for similar biogeochemical reaction networks consisting of a delicate balance between competing reactions. Although Spycher et al. (2011) have described the biogeochemical reaction network, the sensitivity of the biokinetic parameters on model outcomes related to uranium transformations has not been examined before. Therefore, the present study provides a sensitivity analysis of the biokinetic parameters affecting the biogeochemical reaction network dynamics. The extent of the effect of each parameter on the overall biogeochemical processes and competing reactions are demonstrated here.

MATERIALS AND METHODS

Description of the Biogeochemical Reaction Network

The biogeochemical reaction network is composed of the biotic and abiotic reactions outlined by Spycher et al. (2011), which include sulfate bioreduction, Fe(III) bioreduction, U(VI) reduction, Fe(III) reduction by HS^-, U(IV) reoxidation and sulfur precipitation-dissolution reactions as described in detail below.

Sulfate bioreduction: microbial reduction of sulfate to HS^-, where lactate is oxidized to acetate is expressed as follows:

$$2C_3H_5O_3^- + SO_4^{-2} \rightarrow 2CH_3COO^- + 2CO_3^{-2} + HS^- + 3H^+ \quad (1)$$

Fe(III) bioreduction: microbial reduction of Fe(III) to Fe^{+2} coupled to lactate oxidation to acetate is expressed as follows:

$$C_3H_5O_3^- + 4Fe^{+3} + 2H_2O \rightarrow CH_3COO^- + CO_3^{-2} + 4Fe^{+2} + 6H^+ \quad (2)$$

These microbially-mediated reactions are modeled using dual-Monod biomass growth kinetics, where the reaction rate, R is described as (Spycher et al., 2011; Şengör et al., 2015):

$$R = qC_B \frac{C_D}{k_D + C_D} \frac{C_A}{k_A + C_A} f_G \quad (3)$$

with

$$R_{rxn} = YR - bC_B \quad (4)$$

$$f_G = (1 - Q/K) \quad (5)$$

where C_B, C_D, and C_A, are the concentrations of biomass, electron donor and electron acceptor, respectively (in units of moles/L). k_D and k_A are the half saturation constants for electron donor and acceptor, respectively (in units of moles/L). q is the rate of maximum substrate utilization (in units of moles per time, per biomass), Y is the microbial yield coefficient (in units of biomass per substrate), b is the cell decay rate (in units of per time). f_G is the term for affinity which indicates whether the reaction is at equilibrium (being equal to 0) or away from equilibrium (being equal to 1), where Q is the ion activity product and K is the equilibrium constant of the corresponding reaction. For the Fe(III) bioreduction reaction, the rate is considered to be only limited by the

electron donor, lactate, as unlimited supply of the electron acceptor, (Fe(III) or hematite solids) are assumed; therefore, the Monod term consisting of $C_A/(k_A+C_A)$ in Eqn. (3) is taken to be equal to 1 (Spycher et al., 2011).

U(VI) reduction: The combined biotic and abiotic U(VI) reduction reaction to U^{+4} is expressed as follows:

$$4UO_2^{+2} + HS^- + 7H^+ \rightarrow 4U^{+4} + SO_4^{-2} + 4H_2O \quad (6)$$

The rate of this reaction was limited by lactate, as expressed by the following Monod kinetics:

$$R = r\frac{C_D}{k_D + C_D} f_G \quad (7)$$

where r and k_D are the reaction rate constant (in units of mol/time) and half saturation constant for lactate (in units of moles/L), respectively, and these parameters were adjusted to reproduce the observed experimental results by Sani et al. (2004). As discussed by Spycher et al. (2011), implicitly limiting this reaction by lactate was the simplest approach tested while also the number of adjustable model parameters were minimized.

Fe(III) reduction by HS^-: The fully abiotic Fe(III) reduction to Fe^{+2} reaction coupled by HS^- oxidation to sulfate is describes as:

$$8Fe^{+3} + HS^- + 4H_2O \rightarrow 8Fe^{+2} + SO_4^{-2} + 9H^+ \quad (8)$$

U(IV) reoxidation: The fully abiotic U(IV) oxidation to U(VI) coupled to Fe(III) reduction to Fe^{+2} reaction is describes as:

$$U^{+4} + 2Fe^{+3} + 2H_2O \rightarrow UO_2^{+2} + 2Fe^{+2} + 4H^+ \quad (9)$$

Sulfur precipitation/dissolution: The fully abiotic Sulfur precipitation/dissolution reaction is also expressed kinetically to yield consistent results with experimental data, and expressed as:

$$2Fe^{+3} + HS^- \rightarrow 2Fe^{+2} + S_{(s)} + H^+ \quad (10)$$

The rates of the abiotic reactions were considered to be limited only by thermodynamic constraints and therefore expressed as:

$$R = r f_G \quad (11)$$

where r (in units of moles per time) is the reaction rate constant.

The reaction rate parameters discussed above were calibrated by fitting the predicted concentrations of lactate, acetate, sulfate and U(VI) with the measured experimental data, where the calibrated parameters are provided in Table 1, as reported by Spycher et al. (2011).

An illustration of the conceptual framework of biogeochemical reactions is provided in Figure 1. Sulfate bioreduction reaction to biogenic HS^- and the Fe(III) bioreduction reaction to Fe^{+2}, coupled to the oxidation of lactate, providing the electrons from the organic substrate source, are illustrated on the left-hand-side of the Figure. The abiotic Fe(III) reduction by the available HS^- producing Fe^{+2} and sulfate; as well as the abiotic sulfur precipitation/dissolution reaction oxidizing the available HS^- to produce sulfur minerals as $S_{(s)}$ are also depicted. Right-hand-side of the Figure shows the U(VI) reduction, where aqueous U(VI) is reduced to U(IV) minerals; as well as the reoxidation of U(IV) back to soluble U(VI) into the medium.

Sensitivity Analysis

Sensitivity analysis relies on a robust statistical analysis, which provides the essential tools for quantifying the variability and uncertainty in model parameters. Sensitivity analysis to quantify the impact of each variable on the simulation results has been conducted using the least-square method, where the value of each variable is increased and decreased by 50 % one at a time from the baseline value, while keeping the other parameters constant. The least-square method is a commonly used statistical approach to minimize the sum of the squares of the differences between baseline and simulated values. The baseline values are the previously calibrated model parameters as described by Spycher et al. (2011) for the concentration data obtained for sulfate, lactate, acetate and U(VI) with regards to the U(VI) bioreduction followed by U(IV) reoxidation batch experiments originally conducted by Sani et al. (2004). The least-square value (LSV) which provides a quantitative comparison of simulated and baseline simulation results is described as (Lin and Wu, 2011):

$$LSV = \frac{1}{N}\sum_{i=1}^{N}\sqrt{\frac{\left(S_{simulated,i} - S_{baseline,i}\right)^2}{\left(S_{baseline,i}\right)^2}} \quad (12)$$

where N refers to the number simulation data points, $S_{simulated,i}$ and $S_{baseline,i}$ are the simulation and baseline simulation points, respectively. In the least-square method, a decrease in the LSV results indicates a strong agreement between the model simulation and baseline simulation results (Lin and Wu, 2011).

In order to quantify the model simulation results with the measured experimental data points, two statistical parameters are used: coefficient of determination, R2, and Mean Relative Error (MRE). Coefficient of determination, R2 can be expressed as (Walpole & Myers, 1989):

$$R2 = 1-\left\{\frac{\sum(X-Ssimulated,i)^2}{[\sum X^2]-\frac{[\sum X]^2}{N_{data}}}\right\} \quad (13)$$

Mean Relative Error (MRE) can be described as (Scarlat et al., 2014):

$$MRE = \frac{1}{N_{data}}\sum_{i=1}^{N_{data}}\frac{|S_{simulated,i}-X_i|}{X_i} \quad (14)$$

where N_{data} is the number of experimental data points, X is the experimental data and $S_{simulated,I}$ is the simulation data points. Ideally, the R2 value of a perfectly fitted model should be close to 1 and the residual errors should be close to zero, where MRE would approach zero (Moriasi et al., 2015).

The simulations presented in this study are carried out by the speciation and geochemical calculation program code PHREEQC (Parkhurst and Appelo, 2013). PHREEQC is a widely used computer program that is designed to perform a wide variety of aqueous geochemical calculations. The program implements various geochemical speciation reactions including batch-reactions, one-dimensional transport, and inverse geochemical calculations with reversible and irreversible reactions (Parkhurst and Appelo, 2013).

Table 1. Kinetic rate expresions and calibrated parameters for the U(VI) bioreduction reaction network (from Spycher et al., 2011)

Reaction	**Kinetic Rate Expresssion**	**Calibrated Parameters Considered as Baseline Values**				
		q (mol/s/mg$_{cells}$) or r (mol/s)	**k_D (mol/L)**	**k_A (mol/L)**	**Y (mg$_{cells}$/mol)**	**b (1/s)**
Sulfate bioreduction	$R = qC_B \frac{C_D}{k_D + C_D}\frac{C_A}{k_A + C_A} f_G$	10^{-8}	$2x10^{-2}$	$2x10^{-2}$	1600	10^{-8}
Fe(III) bioreduction	$R = qC_B \frac{C_D}{k_D + C_D}\frac{C_A}{k_A + C_A} f_G$	10^{-11}	$2x10^{-2}$	-	1600	10^{-8}
U(VI) reduction	$R = r\frac{C_D}{k_D + C_D} f_G$	$8x10^{-11}$	$4x10^{-2}$			
Fe(III) reduction by HS-	$R = r f_G$	$0.45x10^{-11}$				
U(IV) reoxidation	$R = r f_G$	$2x10^{-11}$				
Sulfur ppt/ dissolution	$R = r f_G$	$1.4x10^{-11}$				

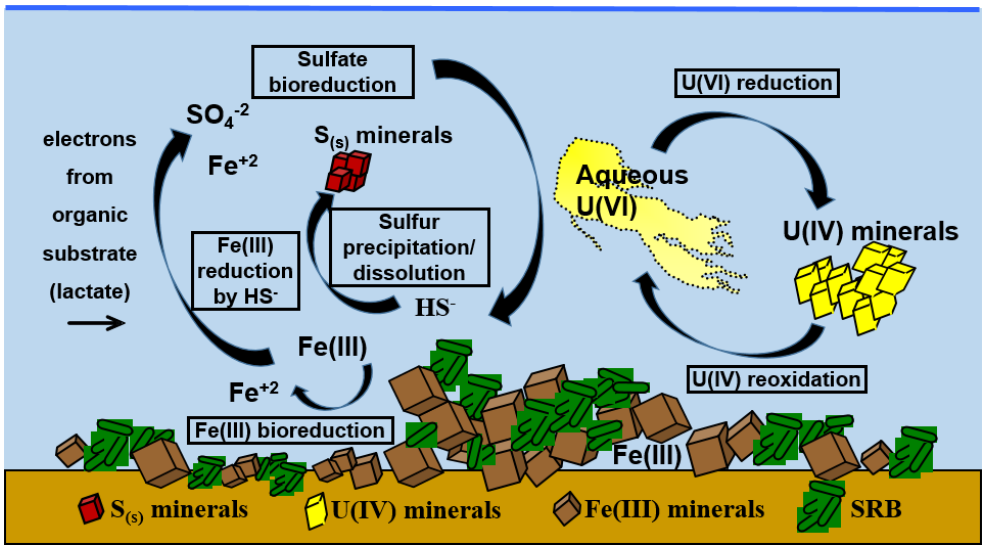


Figure 1. Illustration of the conceptual framework for biogeochemical reactions

RESULTS AND DISCUSSION

In this section, the results of the sensitivity analysis are presented along with the details of the impact of each biokinetic parameter on each of the biogeochemical reactions: sulfate bioreduction, Fe(III) bioreduction, U(VI) reduction, Fe(III) reduction by HS-, U(IV) reoxidation and Sulfur precipitation/dissolution reactions. The sensitivity analysis is conducted where the value of the selected parameter is varied ± 50% while all other parameters remained constant at base-line values, as reported in Table 2. Table 2a presents the results of average LSV of each biokinetic parameter variation by 50% increase on U(VI) concentration, acetate concentration and sulfate concentration predictions; and Table 2b presents the results of average LSV variation by 50% decrease on the concentration predictions. The impact of ± 50% biokinetic parameter variations on the discrepancies of model results with the measured data is also demonstrated with R2 and Mean Relative Error Analysis results for each reaction, as seen in Table 2a and 2b. The biokinetic parameters tested in this study include maximum substrate utilization rate (q), half saturation constant for electron donor (k_D) and acceptor (k_A) for sulfate bioreduction reaction; maximum substrate utilization rate (q) and half saturation constant for electron donor (k_D) for Fe(III) bioreduction reaction; microbial yield coefficient (Y) and cell decay rate coefficient (b) for sulfate bioreduction & Fe(III) bioreduction reactions; rate constant (r) for U(VI) reduction reaction; rate constant (r) for Fe(III) reduction by HS^- reaction; rate constant (r) for U(IV) reoxidation reaction and rate constant (r) for Sulfur precipitation/dissolution reaction. The results for each reaction is discussed in detail below.

Sulfate bioreduction

Figure 2 shows the impact of maximum substrate utilization rate (q) on (a) sulfate, (b) acetate, (c) U(VI) concentration; impact of half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration; and the impact of half saturation constant for electron acceptor (k_A) on (g) sulfate, (h) acetate, (i) U(VI) concentration with regards to the sulfate bioreduction reaction. As seen from Figure 2 (a-e) and Table 2, a 50 % increase in the maximum substrate utilization rate (q) has a slight decreasing effect in sulfate concentrations and corresponding slight increasing effect in acetate concentrations with average LSV of 0.0483 and 0.0792, respectively; following the reaction kinetics as shown in Eqn (3) and the stoichiometry of reaction (1). A 50 % decrease in q, however, results in a more significant increase in sulfate and decrease in acetate concentrations with LSV of 0.2358 and 0.1648, respectively. The U(VI) concentration predictions are more significantly affected as can be seen from Figure 2c and higher average LSV values, especially with 0.6459 LSV for 50% decrease in q is observed. As seen from the reaction (1), when sulfate reduction reaction is increased, this causes a further decrease in lactate concentrations, which affects the rate of U(VI) reduction reaction, where the rate of this reaction is limited by lactate as by Eqn (7). The decreased lactate in the system results in a decrease in U(VI) reduction reaction with higher U(VI) concentrations; and reverse effects are observed with the decrease in sulfate reduction reaction, resulting in U(VI) concentration trends as depicted in Figure 2c.

50 % increase and decrease in the half saturation constant for electron donor (k_D) and acceptor (k_A) has slight impact on sulfate and acetate concentration trends as can be seen from Figure 2d & 1e, and Figure 2g & 1h, respectively, as well as from the relatively lower LSV results (ranging from 0.0475-0.0706) as seen in Table 2. An increase in either k_D or k_A values results in a decrease in the overall rate of sulfate reduction reaction as seen in Eqn. (3) which ends up with higher sulfate and lower acetate concentrations based on reaction (1). This has relatively more significant impact again on the U(VI) concentrations, as can be seen from Figure 2(f) and 2(i) and

relatively higher LSV results (ranging from 0.3243-0.3879) in Table 2. Again, based on the available lactate in the environment, a decrease in the overall rate of sulfate reduction reaction results in higher lactate availability, which causes an increase in the U(VI) reduction reaction (Reaction 6) ending up in lower U(VI) (and again with the reverse effect resulting in higher U(VI)) concentrations, as seen in Figures 2(f) and 2(i).

The comparison of model predictions with the measured data is also assessed by R2 and MRE values for uranium, acetate and sulfate concentrations, as can be seen from Table 2. As can be seen from the analysis results, an increase in the sulfate bioreduction reaction by either 50% increase in q or decrease in k_D or k_A values results in a further improved fit for sulfate and acetate concentrations, as seen from Figure 2 comparative simulation runs with the measured data points. This can also be confirmed with high R2 values (ranging from 0.9788-0.9854) and very low MRE values (ranging from 0.0313-0.1299) as seen in Table 2, indicating a very good fit between measured and predicted values for sulfate and acetate. However, this increase in the sulfate bioreduction reaction did not improve the fits for U(VI) concentrations, confirmed with relatively lower R2 and MRE values as seen in Table 2. When the sulfate bioreduction reaction rate is decreased on the other hand, either by 50% decrease in q or increase in k_D or k_A values, deviations from the measured data are observed for all concentration predictions, including sulfate, acetate and U(VI), as can be seen from Figure 2 and statistical analysis results in Table 2 (relatively lower R2 values ranging from 0.6414-0.9429, relatively higher MRE values ranging from 0.1378-0.5163) as seen from Table 2.

Sulfate bioreduction reaction has important environmental implications, where it can be used to treat sulfate-rich wastewaters, which can come from various industrial sources. With the production of biogenic sulfide, HS^- becomes available to be used in the proceeding chain of reactions to reduce Fe(III) and/or U(VI) in the reducing environment. The sulfides can also precipitate to remove other heavy metals, such as copper, zinc, cadmium, lead, nickel if present in the medium. These metal sulfides are insoluble and can be recovered for reuse purposes (Lens et al., 2008).

Fe(III) bioreduction

Figure 3 shows the impact of maximum substrate utilization rate (q) on (a) sulfate, (b) acetate, (c) U(VI) concentration; and the impact of half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration with regards to the Fe(III) bioreduction reaction. As seen from Figure 3 and relatively very low average LSV results (ranging between 0.0002-0.0037) in Table 2, the sulfate, acetate and U(VI) concentrations do not result in any significant deviations when the biokinetic parameters for Fe(III) bioreduction reaction is varied ±50 % from the baseline value. The lack of sensitivity of the q and k_D parameters revealed that the Fe(III) bioreduction reaction did not have much controlling factor among the biogeochemical reaction network. Model prediction comparisons with the measured data are therefore ultimately aligned with the baseline simulation outcomes, where a reasonably good fit of the original model predictions can be seen from the R2 (ranging between 0.9537-0.9721) and MRE (ranging between 0.0911-0.2330) values for uranium, acetate and sulfate concentrations as seen in Table 2.

The impact of microbial yield coefficient (Y) on (a) sulfate, (b) acetate, (c) U(VI) concentration; and the impact of cell decay rate coefficient (b) on (d) sulfate, (e) acetate, (f) U(VI) concentration for the sulfate bioreduction & Fe(III) bioreduction reactions are shown in Figure 4. The microbially mediated sulfate bioreduction and Fe(III) bioreduction reactions share the common growth kinetic parameters of Y and b, as seen from Eqn. (4). As seen from Figure 4(a-c) and Table 2 sensitivity analysis results, the ±50 % variation of Y from the baseline value results in a similar sensitivity in sulfate, acetate and U(VI) concentrations, compared to the effect with ±50 % variation in q for sulfate bioreduction (see Figure 2(a-c)), where 50% reduction in Y had again slightly more impact. Based on the reaction rates modeled with dual-Monod biomass growth kinetics (Eqn. 3), the similar impact of Y and q on the reaction rates would be expected. On the other hand, ±50 % variation of cell decay rate coefficient (b) did not have any significant deviations on the sulfate, acetate and U(VI) concentrations, indicating that cell decay rate coefficient was not sensitive on the microbially-mediated reaction network considered in the system (see Figures 4(d-f)). The comparison of model predictions with the measured data can be seen in Table 2 by means of the R2 and MRE values for uranium, acetate and sulfate concentrations pertaining to the variations in Y and b parameters.

This reaction has important implications from environmental perspective, where the reduction of Fe(III) to Fe(II) can degrade organic contaminants. The produced Fe(II) can participate in other redox reactions, potentially reducing other contaminants. The reaction can also lead to the formation of reactive minerals such as magnetite (Fe_3O_4) and green rust, where these minerals can adsorb and degrade contaminants, providing a long-term remediation solution (Chaudhuri et al., 2001).

U(VI) reduction

The impact of rate constant (r) on (a) sulfate, (b) acetate, (c) U(VI) concentration; and the impact of half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration for the U(VI) reduction reaction is provided in Figure 5. As can be seen from Figure 5(a-b) and Figure 5(d-e), sulfate and acetate concentration predictions are not sensitive to the variations in either r or k_D. However, U(VI) concentrations

resulted in significant deviations from the baseline outcome results, when the biokinetic parameters, r or k_D, are varied ±50%. The resulting sensitivity analysis can be seen from the relatively higher LSV results in Table 2, ranging between 0.3470-0.5982. As seen from Reaction (6), when the rate of U(VI) reduction reaction is increased either by an increase in the rate constant, r, or a decrease in the half saturation constant, k_D, further depletion in U(VI) results in the decrease of U(VI) concentrations, as expected from the reaction stoichiometry. Similarly, the reverse effect is seen when the rate is decreased by decreasing the rate constant, r, or increasing the half saturation constant, k_D. Significant deviations from the measured data points for U(VI) concentration trends can also be seen from the R2 (ranging between 0.6788-0.8533) and MRE (ranging between 0.4162-0.6682) values in Table 2. The greater sensitivity of U(VI) might be attributed to the relatively lower concentration values compared to sulfate and acetate, where variations in lower concentration amounts might show more visible effect in general.

U(VI) reduction reaction is the main reaction mediating the conversion of aqueous U(VI) into insoluble U(IV) form; which is considered as a promising strategy for in-situ remediation of subsurface uranium. This reaction has significant environmental implications, where the engineered biostimulation of indigenous microorganisms to mediate the conversion of U(VI) to immobile U(IV) form has been demonstrated in various uranium contaminated sites (Anderson et al., 2003; Yabusaki et al., 2011).

Fe(III) reduction by HS^- reaction

Figure 6 shows the impact of rate constant (r) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the Fe(III) reduction by HS^- reaction. As can be seen from Figure 6, sulfate, acetate and U(VI) concentration trends did not result in any significant deviations when the rate of Fe(III) reduction by HS^- is varied ±50 % from the baseline value. The relatively low average LSV results, ranging between 7.55E-6-0.0191, can also be seen in Table 2. The lack of sensitivity of the rate constant, r, on concentration prediction outcomes demonstrated that the Fe(III) reduction by HS^- reaction did not have much influence over the biogeochemical reaction network considered in this study.

Similar to the Fe(III) bioreduction, Fe(III) reduction by HS^- reaction has impacts on water quality based on the release of soluble Fe(II) into the subsurface pore water environment, which can enhance the mobility and bioavailability of iron in solution. This would facilitate the transport of iron and other nutrients, supporting microbial activity and bioremediation processes. Again, the formation of magnetite and/or green rust as secondary reactive minerals can also take place, which can also serve as adsorbents for removing various pollutants in the media (Chaudhuri et al., 2001).

U(IV) reoxidation

The impact of rate constant (r) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the U(IV) reoxidation reaction is depicted in Figure 7. As seen from Figure 7, the results of the sensitivity analysis show the sensitivity of U(VI) concentration predictions to the ±50% variations in r. The relatively higher LSV results for U(VI) ranging between 0.2569-0.3282 can also be seen in Table 2. When the rate of U(IV) reoxidation reaction (see Reaction 9) is increased by increasing the rate constant, r, further increase in U(VI) results based on the reaction stoichiometry; and the reverse effect causes a decrease in the U(VI) concentration trends when the rate constant, r, is decreased. The observed deviations from the measured data points for U(VI) can also be seen from the R2 (ranging between 0.8727-0.8854) and MRE (ranging between 0.3242-0.3595) values in Table 2. The sulfate and acetate concentrations on the other hand, were not sensitive to the variations in r, as can also be seen from the relatively low LSV results (ranging between 1.33E-6-3.18E-6) in Table 2. The greater sensitivity of U(VI) results might again be due to the relatively lower concentration values of U(VI), when compared to sulfate and acetate concentrations.

From an environmental point of view, this reaction has significant impact in terms of the stability of bioreduced uranium. U(IV) has been shown to be susceptible to re-oxidation and remobilization under electron-donor limiting and sulfate reducing conditions (Sani et al., 2004). Subsurface porous medium is known to contain Fe(III)-oxide minerals, where the available Fe(III) can serve as the electron acceptor to reoxidize U(IV) back to U(VI), thereby significantly affecting uranium biodegradation efforts (Sani et al., 2004; Spycher et al, 2011).

Sulfur precipitation/dissolution reaction

The impact of rate constant (r) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the sulfur precipitation/dissolution reaction is illustrated in Figure 8. As can be seen from Figure 8, sulfate, acetate and U(VI) concentration trends did not result in any significant fluctuations with the ±50% variations in the sulfur precipitation/dissolution reaction rate constant. The relatively low average LSV results, ranging between 2.4E-7-0.0156, can also be seen in Table 2. The sensitivity analysis results showed that the sulfur precipitation/dissolution reaction rate was also insensitive to the overall concentration predictions, and therefore this reaction did not have much control towards the biogeochemical process dynamics.

Considering the environmental impact of this reaction, sulfur precipitation can influence the pH and redox conditions of the water, which can have further influence on the solubility and mobility of other contaminants in

the medium. Elemental sulfur also plays an important role in biogeochemical cycling of heavy metals, where its dissolution to form hydrogen sulfide, HS-. would facilitate the formation of metal sulfide precipitates to remove metal contaminants from aqueous environment.

Overall Evaluation

The results show that variation in the kinetic parameters pertaining to the sulfate bioreduction reaction had the most influence over the biogeochemical reaction dynamics considered in this study. For sulfate bioreduction, the biokinetic parameters, k_D, and k_A had similar sensitivity towards sulfate and acetate concentration predictions, whereas 50 % decrease in q as well as Y had the most impact. Changes in the biokinetic parameters for U(VI) reduction and U(IV) reoxidation reactions did not show any sensitivity towards the model outcomes, except for U(VI) concentration predictions. Among the concentrations tested, U(VI) concentration was observed to be the most sensitive parameter for the changes in biokinetics parameters among the competing biogeochemical reaction network, which might be due to its lower concentration values compared to other species concentrations in the system. Fe(III) bioreduction reaction kinetic parameters, cell decay rate coefficient, Fe(III) reduction by HS^- and sulfur precipitation/dissolution reaction's kinetic parameters also did not significantly affect the biogeochemical process dynamics.

Sensitivity analysis studies reported in literature have also demonstrated the impact of biokinetic parameters in modeling various contaminant biodegradation processes, highlighting the importance of accurate monitoring and prediction of the specific contaminant of concern. Sensitivity analyses for bioactive granular activated carbon adsorbers for alachlor removal was studied by Badriyha et al. (2003), where their results for thin biofilm model showed the significant sensitivity of the Monod coefficients, maximum substrate utilization rate and Monod half-velocity constant, and moderate sensitivity of maximum biofilm thickness on process performance. Den and Pirbazari (2002) conducted a study to investigate the biokinetic and adsorption parameters for TCE using a series of minibiofilter and miniadsorber column experiments, where model sensitivity was examined to understand the effect of adsorption equilibrium, transport and biological parameters on biofilter dynamics. Sensitivity analysis results indicated that the Monod constants (maximum substrate utilization rate constant and half saturation constant for Monod kinetics) as well as the biofilm thickness had a significant control on the biofilter performance. Sensitivity analysis study conducted by Dastidar and Wang (2009) for biological oxidation of As (III) in batch reactors for various As concentration ranges revealed that their yield coefficient and substrate inhibition coefficient were the most sensitive parameters in their model predictions, whereas endogenous decay coefficient was the least sensitive. In their another fixed-film bioreactor modeling study for biological oxidation of As (III) under different operating conditions, maximum specific utilization rate constant was observed to be the most sensitive parameter under steady-state conditions compared to half-saturation constant (Dastidar and Wang, 2012). A study reported by Zhou et al. (2012), which quantified the growth biokinetic parameters of biofilm in wastewater treatment using oxygen microelectrodes showed that their maximum specific oxygen uptake rate was more sensitive in their Monod kinetic model, compared to their Monod half saturation constant and maintenance decay coefficient for oxygen. The determination of biokinetic parameters of biofilms by measuring oxygen uptake profiles was also investigated by Riefler et al. (1998) for a completely mixed attached growth bioreactor, where their sensitivity analysis demonstrated that their maximum specific growth rate coefficient as well as half-saturation coefficient were the most sensitive parameters in their transient biofilm model.

The results demonstrated in the current study might have important consequences in modeling uranium contaminated environments for U(VI) removal strategies, where similar competing reaction process dynamics would be of concern. The higher sensitivity of U(VI) to changes in biokinetics parameters would imply the importance of sensitivity analysis in this context, as U(VI) might the primary monitored contaminant in the system. Due to the nature of local variabilities in biokinetic parameters especially in the natural environment, uncertainity in the calibrated parameters in modeling efforts is of paramount importance, especially for modeling complex biogechemical reaction networks. Understanding how the variations in the calibrated parameters would affect the model output would help to reduce model uncertainity in model predictions. Also, the sensitivity of biokinetic parameters over a range various competing reactions in the system would provide useful information to understand the system dynamics and behavior under different conditions. Furthermore, sensitivity analysis of model input parameters would help to identify the key parameters that control the system performance, guiding model refinement and improvement in efforts to increase process efficiency and system design.

Table 2a. Sensitivity analysis results of the increased 50 % variation of biokinetic parameters on each reaction.

Reaction	**Parameter**	**Average least-square value**			
	Baseline value	Increased 50 %	Uranium concentration	Acetate concentration	Sulfate concentration
Sulfate bioreduction	q (10^{-8} mol/s/mg_{cells})	$1.5x10^{-8}$	LSV = 0.3628 R2 = 0.8696 MRE=0.3798	LSV = 0.0792 R2 = 0.9824 MRE=0.0313	LSV = 0.0483 R2 = 0.9788 MRE=0.1328
	k_D ($2x10^{-2}$ mol/L)	0.03	LSV = 0.3243 R2 = 0.8762 MRE=0.3472	LSV = 0.0495 R2 = 0.9429 MRE=0.1378	LSV = 0.0570 R2 = 0.9415 MRE=0.2412
	k_A ($2x10^{-2}$ mol/L)	0.03	LSV = 0.3879 R2 = 0.8449 MRE=0.3799	LSV = 0.0604 R2 = 0.9347 MRE=0.1487	LSV = 0.0667 R2 = 0.9317 MRE=0.2567
Fe(III) bioreduction	q (10^{-11} mol/s/mg_{cells})	$1.5x10^{-11}$	LSV = 0.0019 R2 = 0.9541 MRE=0.2318	LSV = 0.0004 R2 = 0.9702 MRE=0.0911	LSV = 0.0037 R2 = 0.9721 MRE=0.1754
	k_D ($2x10^{-2}$ mol/L)	0.03	LSV = 0.0009 R2 = 0.9538 MRE=0.2330	LSV = 0.0002 R2 = 0.9700 MRE=0.0916	LSV = 0.0018 R2 = 0.9717 MRE=0.1779
Sulfate bioreduction & Fe(III) bioreduction	Y (1600 mgc_{ells}/mol)	2400	LSV = 0.1960 R2 = 0.9327 MRE=0.2609	LSV = 0.0303 R2 = 0.9828 MRE=0.0550	LSV = 0.0274 R2 = 0.9840 MRE=0.1325
	b (10^{-8} 1/s)	$1.5x10^{-8}$	LSV = 0.0012 R2 = 0.9539 MRE=0.2329	LSV = 0.0002 R2 = 0.9699 MRE=0.0916	LSV = 0.0002 R2 = 0.9718 MRE=0.1774
U(VI) reduction	r ($8x10^{-11}$ mol/s)	$1.2x10^{-10}$	LSV = 0.4985 R2 = 0.7911 MRE=0.4418	LSV = 2.58E-5 R2 = 0.9700 MRE=0.0915	LSV = 0.0011 R2 = 0.9718 MRE=0.1769
	k_D ($4x10^{-2}$ mol/L)	0.06	LSV = 0.3470 R2 = 0.8533 MRE=0.4162	LSV = 1.38E-5 R2 = 0.9700 MRE=0.0915	LSV = 0.0008 R2 = 0.9719 MRE=0.1774
Fe(III) red. by HS-	r ($0.45x10^{-11}$ mol/s)	$6.75x10^{-12}$	LSV = 0.0150 R2 = 0.9578 MRE=0.2240	LSV = 7.8E-6 R2 = 0.9700 MRE=0.0915	LSV = 0.0010 R2 = 0.9719 MRE=0.1767
U(IV) reoxidation	r ($2x10^{-11}$ mol/s)	$3x10^{-11}$	LSV = 0.2569 R2 = 0.8854 MRE=0.3242	LSV = 1.55E-6 R2 = 0.9700 MRE=0.0915	LSV = 3.1E-6 R2 = 0.9719 MRE=0.1771
Sulfur ppt/dissolution	r ($1.4x10^{-11}$ mol/s)	$2.1x10^{-11}$	LSV = 0.0156 R2 = 0.9508 MRE=0.2373	LSV = 2.4E-7 R2 = 0.9700 MRE=0.0915	LSV = 2.1E-7 R2 = 0.9719 MRE=0.1771

Table 2b. Sensitivity analysis results of the decreased 50 % variation of biokinetic parameters on each reaction.

Reaction	**Parameter**	**Average least-square value**			
	Baseline value	Decreased 50 %	Uranium concentration	Acetate concentration	Sulfate concentration
Sulfate bioreduction	q (10^{-8} mol/s/mg_{cells})	$1.5x10^{-8}$	LSV= 0.6459 R2 = 0.6414 MRE=0.5163	LSV = 0.1648 R2 = 0.8514 MRE=0.2344	LSV = 0.2358 R2 = 0.8296 MRE=0.3904
	k_D ($2x10^{-2}$ mol/L)	0.03	LSV= 0.3325 R2 = 0.8891 MRE=0.3452	LSV = 0.0632 R2 = 0.9854 MRE=0.0283	LSV = 0.0475 R2 = 0.9835 MRE=0.1281
	k_A ($2x10^{-2}$ mol/L)	0.03	LSV= 0.3474 R2 = 0.8794 MRE=0.3622	LSV = 0.0706 R2 = 0.9842 MRE=0.0295	LSV = 0.0476 R2 = 0.9815 MRE=0.1299
Fe(III) bioreduction	q (10^{-11} mol/s/mg_{cells})	$1.5x10^{-11}$	LSV= 0.0010 R2 = 0.9537 MRE=0.2334	LSV = 0.0004 R2 = 0.9699 MRE=0.0918	LSV = 0.0037 R2 = 0.9716 MRE=0.1788
	k_D ($2x10^{-2}$ mol/L)	0.03	LSV= 0.0018 R2 = 0.9542 MRE=0.2318	LSV = 0.0003 R2 = 0.9701 MRE=0.0913	LSV = 0.0037 R2 = 0.9721 MRE=0.1754
Sulfate bioreduction & Fe(III) bioreduction	Y (1600 mgc_{ells}/mol)	2400	LSV= 0.5270 R2 = 0.7435 MRE=0.4706	LSV = 0.0704 R2 = 0.9234 MRE=0.1616	LSV = 0.1049 R2 = 0.9190 MRE=0.2805
	b (10^{-8} 1/s)	$1.5x10^{-8}$	LSV= 0.0012 R2 = 0.9540 MRE=0.2323	LSV = 0.0002 R2 = 0.9701 MRE=0.0913	LSV = 0.0002 R2 = 0.9719 MRE=0.1768
U(VI) reduction	r ($8x10^{-11}$ mol/s)	$1.2x10^{-10}$	LSV = 0.5982 R2 = 0.6788 MRE=0.6683	LSV = 2.8E-5 R2 = 0.9700 MRE=0.0915	LSV = 0.0016 R2 = 0.9718 MRE=0.1776
	k_D ($4x10^{-2}$ mol/L)	0.06	LSV = 0.5062 R2 = 0.7874 MRE=0.4349	LSV = 2.39E-5 R2 = 0.9700 MRE=0.0915	LSV = 0.0011 R2 = 0.9719 MRE=0.1768
Fe(III) red. by HS-	r ($0.45x10^{-11}$ mol/s)	$6.75x10^{-12}$	LSV= 0.0191 R2 = 0.9484 MRE=0.2406	LSV = 7.55E-6 R2 = 0.9701 MRE=0.0915	LSV = 0.0010 R2 = 0.9718 MRE=0.1775
U(IV) reoxidation	r ($2x10^{-11}$ mol/s)	$3x10^{-11}$	LSV= 0.3282 R2 = 0.8727 MRE=0.3595	LSV = 1.33E-6 R2 = 0.9700 MRE=0.0915	LSV = 3.18E-6 R2 = 0.9719 MRE=0.1771
Sulfur ppt/dissolution	r ($1.4x10^{-11}$ mol/s)	$2.1x10^{-11}$	LSV= 0.0076 R2 = 0.9536 MRE=0.2331	LSV = 5.0E-7 R2 = 0.9700 MRE=0.0915	LSV = 5.3E-7 R2 = 0.9719 MRE=0.1771

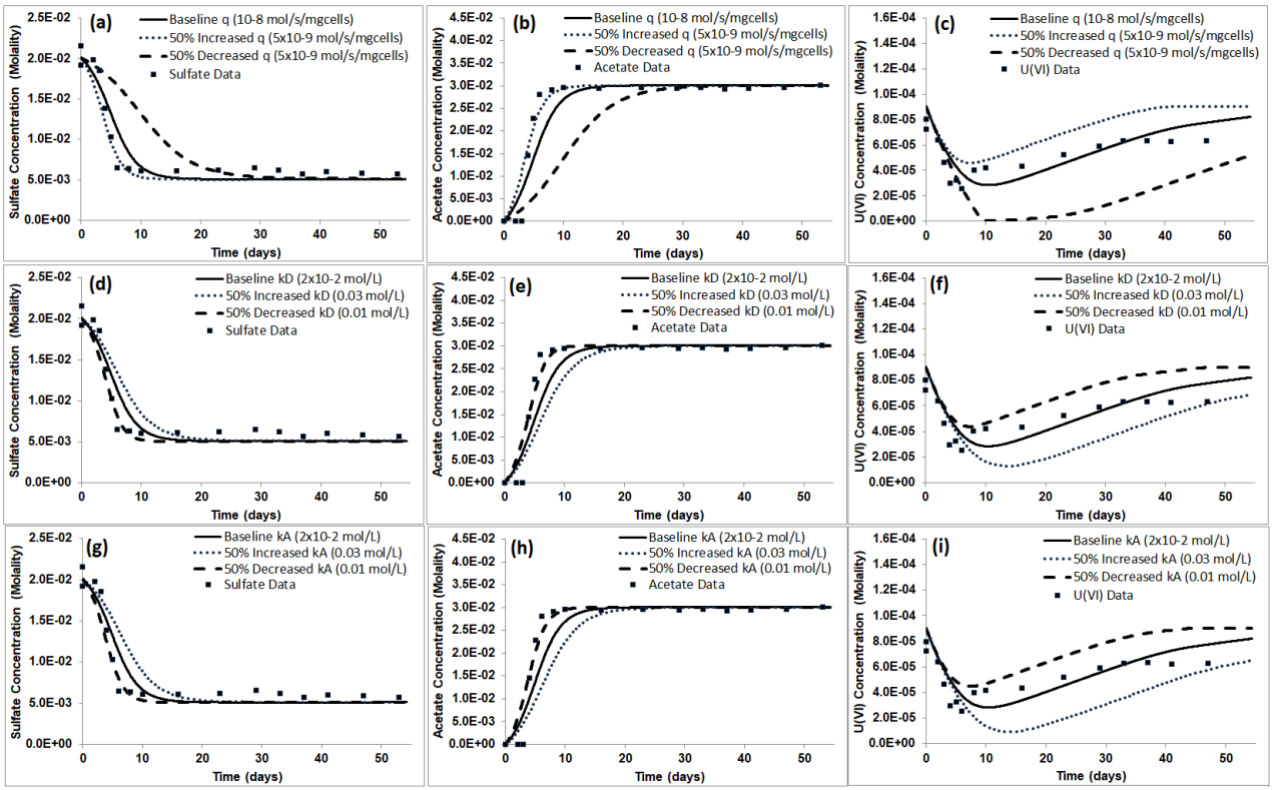


Figure 2. Impact of (a-c) maximum substrate utilization rate (q) on (a) sulfate, (b) acetate, (c) U(VI) concentration; impact of (d-f) half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration; impact of (g-i) half saturation constant for electron acceptor (k_A) on (g) sulfate, (h) acetate, (i) U(VI) concentration for the sulfate bioreduction reaction.

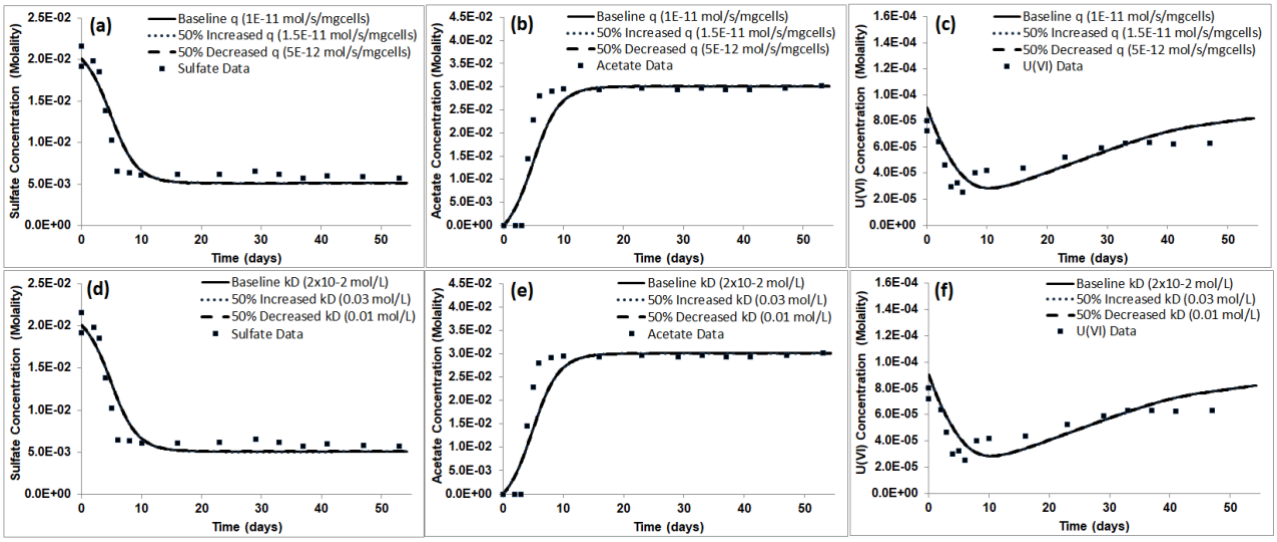


Figure 3. Impact of (a-c) maximum substrate utilization rate (q) on (a) sulfate, (b) acetate, (c) U(VI) concentration; impact of (d-f) half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration for the Fe(III) bioreduction reaction.

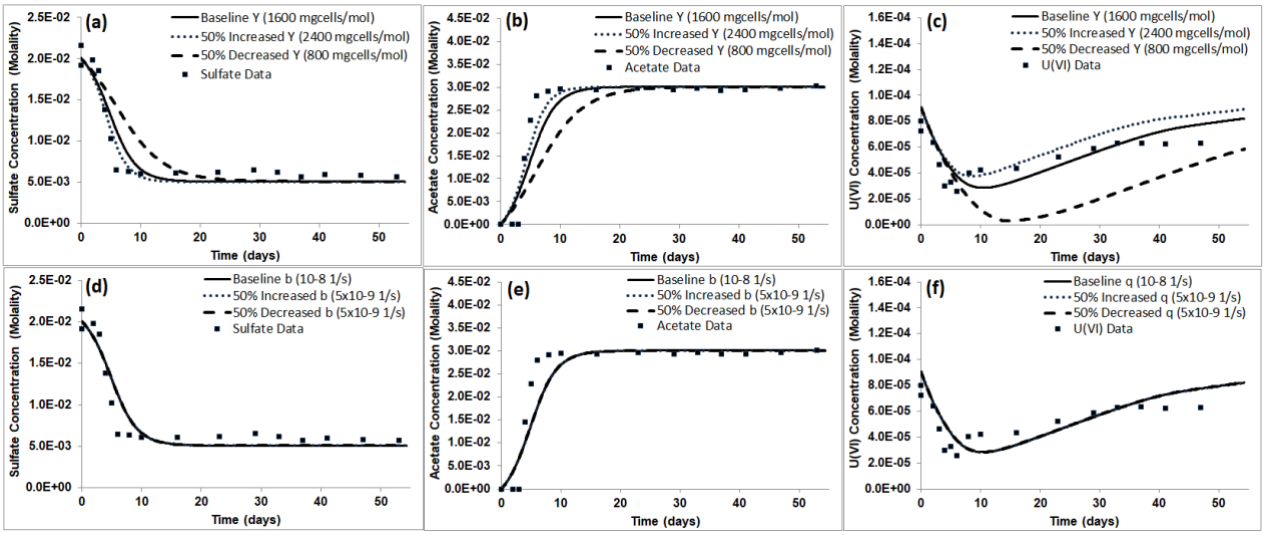


Figure 4. Impact of (a-c) microbial yield coefficient (*Y*) on (a) sulfate, (b) acetate, (c) U(VI) concentration; impact of (d-f) cell decay rate coefficient (*b*) on (d) sulfate, (e) acetate, (f) U(VI) concentration for the sulfate bioreduction & Fe(III) bioreduction reactions.

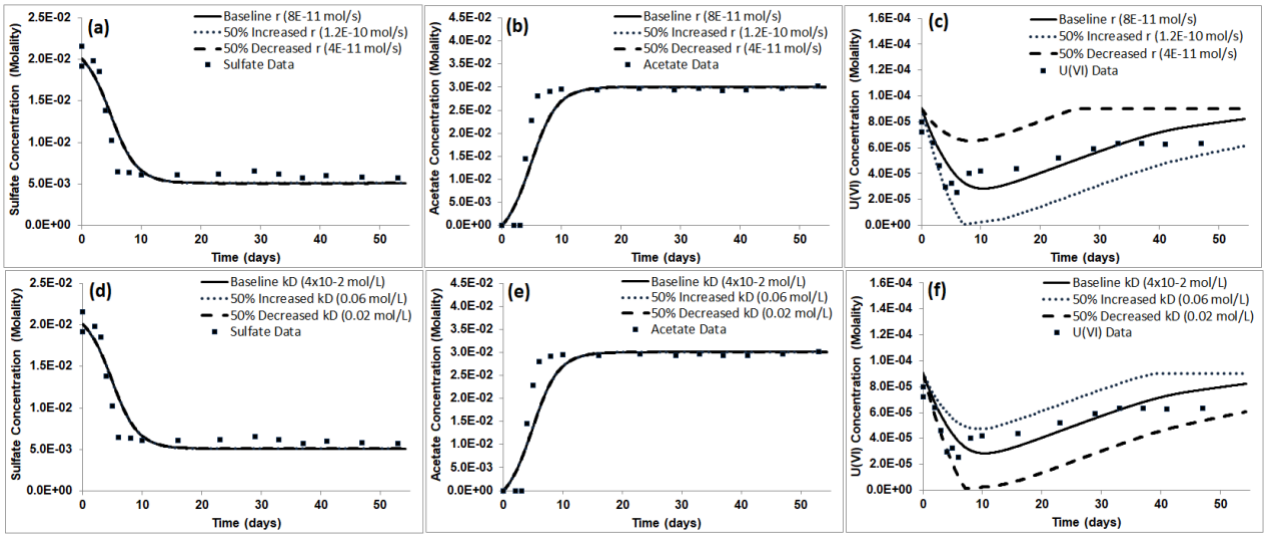


Figure 5. Impact of (a-c) rate constant (*r*) on (a) sulfate, (b) acetate, (c) U(VI) concentration; impact of (d-f) half saturation constant for electron donor (k_D) on (d) sulfate, (e) acetate, (f) U(VI) concentration for the U(VI) reduction reaction.

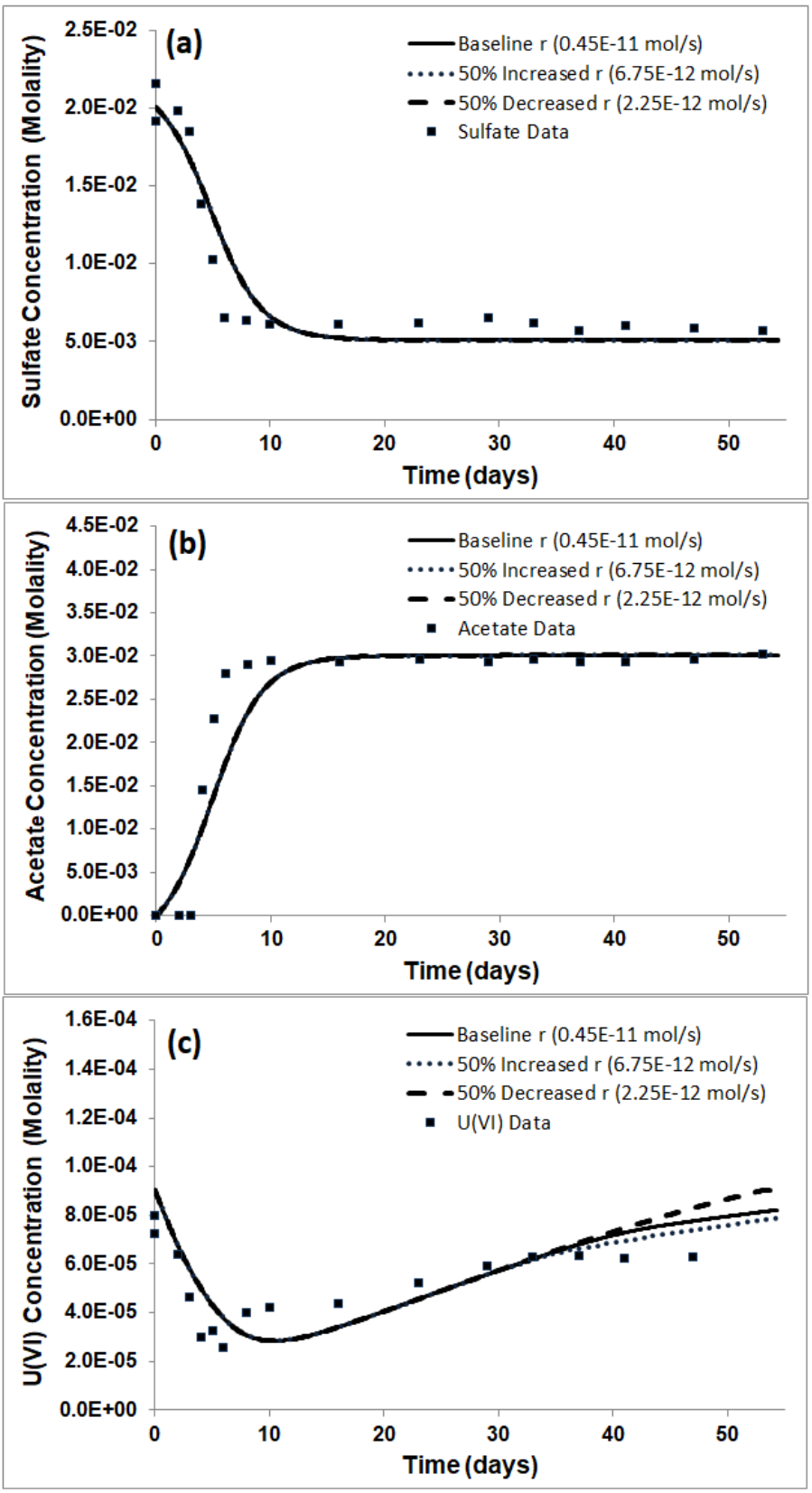


Figure 6. Impact of (a-c) rate constant (*r*) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the Fe(III) reduction by HS^- reaction.

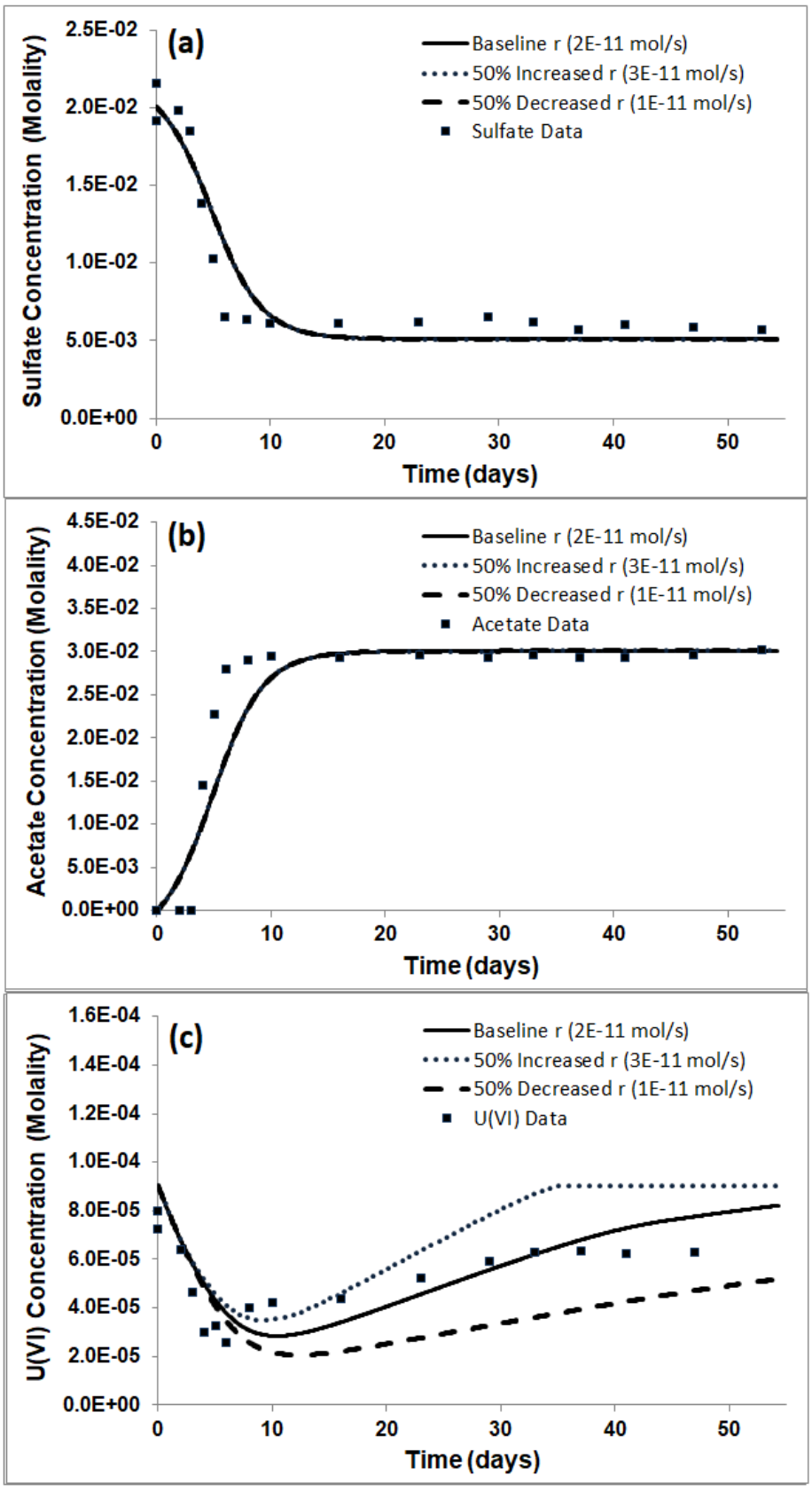


Figure 7. Impact of (a-c) rate constant (*r*) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the U(IV) reoxidation reaction.

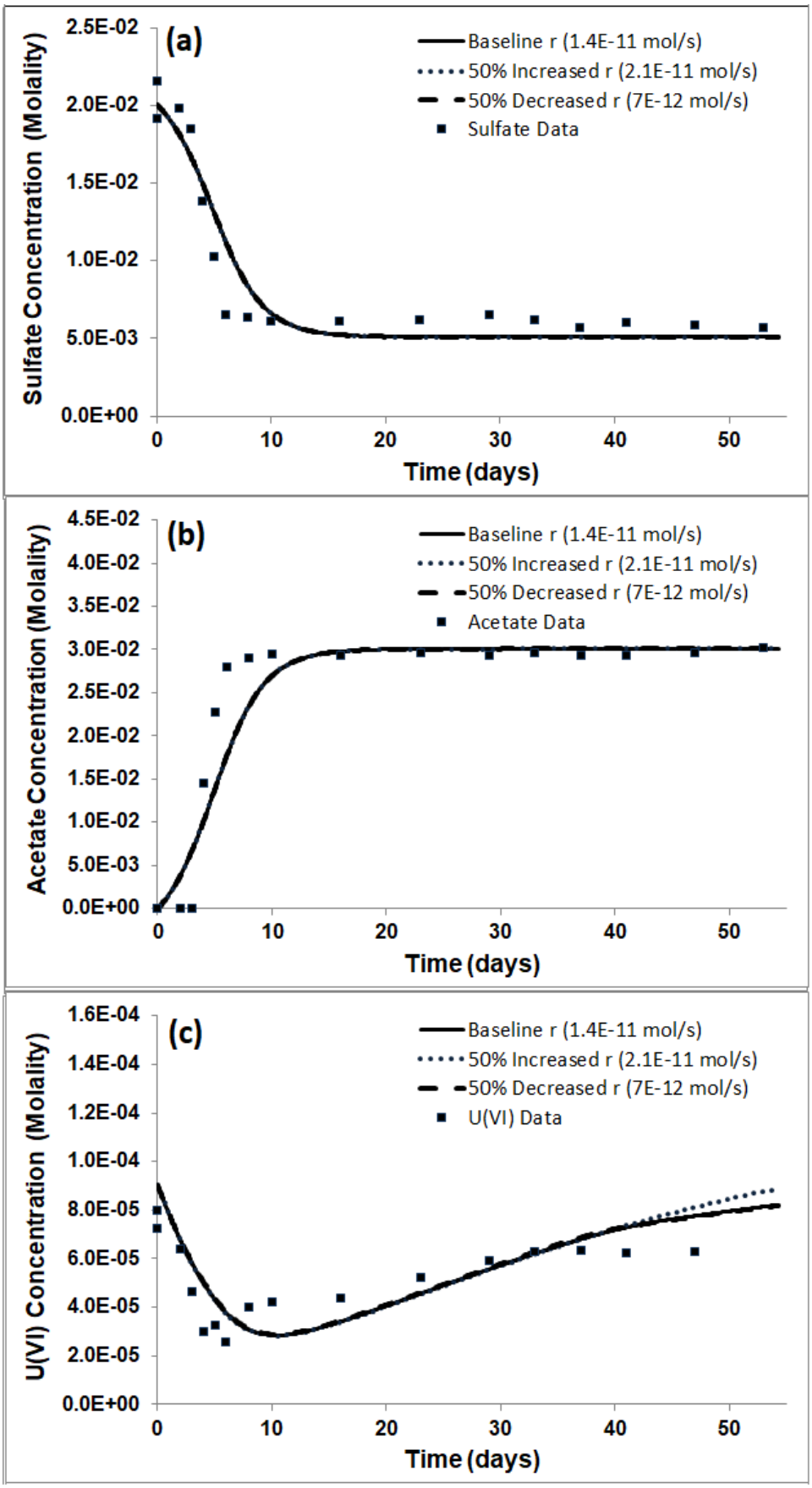


Figure 8. Impact of (a-c) rate constant (*r*) on (a) sulfate, (b) acetate, (c) U(VI) concentration for the Sulfur precipitation/dissolution reaction.

CONCLUSION

In this study, the impact of biokinetic parameters in modeling biological transformations of uranium are presented, based on the biogeochemical reaction network presented by Spycher et al. (2011) to model uranium bioreduction followed by reoxidation by Fe(III) (hydr)oxides. The key biogeochemical reactions include sulfate bioreduction and Fe(III) bioreduction reactions by lactate, U(VI) reduction to U(IV), Fe(III) reduction by HS^-, U(IV) reoxidation reaction to U(VI) and sulfur precipitation-dissolution reaction. The results of the sensitivity analysis show that the sulfate bioreduction was the most sensitive reaction to changes in the kinetic parameters; where the rate of maximum substrate utilization and yield coefficient had the most significant impact, and half-saturation constants had slightly more mild impact on model outcomes. U(VI) reduction and U(IV) reoxidation showed sensitivity only to the U(VI) concentration predictions with the changes in biokinetic parameters in these reactions. U(VI) concentrations were observed to be the most sensitive for the changes in biokinetics parameters among the simulated concentration predictions for all biogeochemical reactions. Although a delicate balance occurs in the system for competing rates of reactions for U(IV) reoxidation versus sulfide (HS^-) oxidation by Fe(III), Fe(III) reduction by HS^- and sulfur precipitation/dissolution reactions as well as Fe(III) bioreduction reactions did not show any sensitivity towards the changes in biokinetic parameters. The results presented in this study can help to improve process design conditions and removal efficiencies for uranium contaminated environments to be used for prediction purposes or for biodegradation models for uranium remediation.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

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Analysis of physicochemical and mechanical quality characteristics of early-maturing table grape varieties

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Abstract

In table grape production, the economic value, which depends on ecological conditions, is closely associated with the ripening period, yield, and the physical and chemical properties of the clusters and berries of the selected varieties. The selection of varieties with superior physicochemical and textural characteristics can mitigate yield losses and prevent price reductions during ripening, harvest, and marketing. In this study, Early Sweet, Prima, and Trakya İlkeren varieties were found to ripen in the first half of June in the Mediterranean region, while Black Magic, Yalova İncisi, Efem, and Spil Karası ripened during the second half, and Victoria ripened in the first half of July. The total soluble solids (TSS) content of all varieties was determined to be 13-14%, with a maturity index of >20 at harvest. The largest cluster and berry sizes were observed in the Victoria and Efem varieties, while the smallest were recorded for Early Sweet. Regarding mechanical and physical properties, berry cracking tolerance was highest in Victoria and Spil Karası (4236 g and 3082 g, respectively) and lowest in Trakya İlkeren and Yalova İncisi (1657 g and 1842 g, respectively). Berry elasticity was highest in Early Sweet and Spil Karası (342.7 g mm^{-1} and 335.8 g mm^{-1}, respectively) and lowest in Trakya İlkeren and Yalova İncisi (271.3 g mm^{-1} and 259.6 g mm^{-1}, respectively). The berry removal force was also comparatively high in Victoria and Spil Karası (847.6 g and 782.1 g, respectively). Based on these findings, it is recommended that grape breeding programs prioritize varieties with large clusters, firm and seedless berries, and high TSS levels. Additionally, the Prima and Early Sweet varieties, characterized by their firmer berries, may be particularly suitable for transportation, extended shelf life, and enhanced competitiveness in both local and international markets.

Keywords: Viticulture, Early ripening, Grape quality, Berry texture

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INTRODUCTION

Grape harvesting, a critical stage in viticulture and a key component of the Turkish agricultural sector, typically begins in the Mediterranean region. The cultivation of early grape varieties in this region plays a significant role in contributing to sustainable viticulture both ecologically and economically. Early ripening of grape varieties at the start of the summer season minimizes the adverse effects of drought stress and allows early-season products to command higher market prices.

Grapes are highly perishable fruits, and their post-harvest shelf life varies significantly depending on the variety (Zoffoli and Latorre, 2011). Consequently, for fresh market consumption, post-harvest handling practices, including packaging, transportation, storage, and market compliance, must be carefully managed. Berry quality at harvest is determined by crucial physiological and biochemical changes that occur in the grape berry during the ripening process (Kunter et al., 2013; Peña-Neira et al., 2023). Texture analyses, which assess the mechanical and physical properties of grape berries, are key agronomic indicators and are typically evaluated through instrumental measurements of the skin and flesh tissue (Crespan et al., 2021).

In table grape production, selecting varieties with superior ripening time, yield, and cluster quality, along with desirable physicochemical and textural properties, offers a distinct advantage in minimizing yield and price losses during the ripening, harvest, and marketing phases.

In terms of ripening time, varieties such as Yalova İncisi and Trakya İlkeren are widely cultivated in the Mediterranean region due to their early maturity. Additionally, varieties developed through crossbreeding in Türkiye, such as Efem and Spil Karası, along with globally popular early-ripening varieties like Early Sweet, Black Magic, Prima, and Victoria, are expected to contribute to an expansion of vineyard areas in the region.

This study aims to evaluate the berry texture and quality characteristics of early grape varieties cultivated in the Mediterranean region, including newly developed varieties that ripen during the same period.

MATERIALS AND METHODS

Material

This study was conducted in 2024 under the climatic conditions of the Eastern Mediterranean region. Grape samples were collected from the Experimental Vineyard of the Department of Horticulture, Çukurova University, located at an altitude of 70 meters, with coordinates 37° 01'50" N and 35° 22'46" E. The study utilized 8-year-old Black Magic, Early Sweet, Efem, Prima, Spil Karası, Trakya İlkeren, Yalova İncisi, and Victoria grape varieties (*Vitis vinifera* L.), all grafted onto 1103 P rootstock.

The experimental plot consisted of vines trained in a bilateral cordon system, planted in a North-South orientation with a row spacing of 3.5 x 2.0 meters. During winter pruning, an average of 20 buds per vine were retained. The vineyard soil was classified as Haplic Calcisol (Loamic, Hypercalcic) according to the International Soil Classification System (IUSS-WRB Working Group, 2015). The soil has a clay-loamy texture, a sub-alkaline pH (7.5–8.0), high calcium carbonate content (50–55%), and low organic matter (<1%) (Tangolar et al., 2024).

The climate of the study site is classified as a hot-summer Mediterranean climate (Csa) subclass, according to the Köppen climate classification system. During the vegetation period (April to October), the site recorded a mean annual temperature of 21 °C, mean relative humidity of 70%, and total precipitation of 141 mm (MGM, 2024).

Additional characteristics and the harvest times of the grape varieties examined in the study are presented in Table 1 and Figure 1.

Table 1. Some characteristics of grape varieties and harvest dates

Variety	Origins	Berry skin color	Seedness	Date of harvest
Efem	Türkiye	Blue-Black	Seeded	June 26, 2024
Spil Karası	Türkiye	Blue-Black	Seeded	June 26, 2024
Trakya İlkeren	Türkiye	Blue-Black	Seeded	June 13, 2024
Yalova İncisi	Türkiye	White	Seeded	June 25, 2024
Black Magic	Moldova	Blue-Black	Seeded	June 25, 2024
Early Sweet	Israel	White	Seedless	June 12, 2024
Prima	France	Black	Seeded	June13, 2024
Victoria	Romania	White	Seeded	July 02, 2024

Method

A total of 30 clusters from each grape variety were used for the analyses, conducted in three replicates. The width (cm), length (cm), and weight (g) of the clusters were measured in the laboratory. Subsequently, from the middle one-third of each cluster (2 berries per cluster), a total of 60 grape berries, including their pedicels, were sampled—20 berries per replicate—for further measurements.

The following parameters were assessed in the berry samples: berry length (mm), berry diameter (mm), berry weight (g), berry removal force (g), berry flesh firmness (g), berry cracking tolerance (g), berry elasticity (g mm^{-1}), and seed and trace number (n/berry). Additionally, 60 berries were squeezed using cheesecloth, and analyses were conducted on the must to determine total soluble solids (TSS) (%), pH, and titratable acidity (%), followed by the calculation of the maturity index.

For the determination of moisture and dry matter content, the fresh weights of 20 berries per replicate were recorded. The berries were then dried in a drying oven at 70°C for approximately one week until a constant weight was achieved, after which the dry weights were measured using a precision balance. Berry moisture content and berry dry matter content were expressed as percentages (%) following the method described by Al-Obeed et al. (2010).

$$\text{Berry moisture (\%)} = \frac{\text{Average fresh weight} - \text{Average dry weight}}{\text{Average fresh weight}} x\ 100$$

$$\text{Berry dry matter (\%)} = \frac{\text{Average dry weight}}{\text{Average fresh weight}} x\ 100$$

Berry shape index, berry volume, and berry surface were obtained using the berry diameter and length measurements. The berry shape index (berry length/berry diameter) was calculated according to Wang et al. (2020). Berry volume (cm^3) and berry surface area (cm^2) were calculated according to the following formula reported by Crespan et al. (2021):

$$\text{Volume } (cm^3) = \frac{4\ \pi\ \text{a.b.c}}{3}$$

$$Surface\ area\ (cm^2)\ = 4\pi\left(\frac{a^p.b^p\ +\ a^p.c^p\ +\ b^p.c^p}{3}\right)^{1/\text{p}}$$

where a = b = I/2; c = L/2; for surface assessment, p= 1.6075
I: berry diameter, L: berry length

The cluster width and length were measured using a ruler, while berry diameter and length were measured with a caliper. A precision scale with 0.1 mg sensitivity was used to determine both cluster and berry weight. The total soluble solids (TSS) content of the must (%) was measured using a digital refractometer (Hanna H196801), and pH was determined with a pH meter (pH330-WTW). Acidity was calculated as tartaric acid via titration with 0.1 N NaOH for 100 mL of must (Tangolar et al., 2024). The maturity index was subsequently calculated as the ratio of TSS to acidity.

Berry elasticity, cracking tolerance, flesh firmness, and removal force were measured using a SUNDOO SH-100 Model Digital Force Gauge. The instrument was mounted on a mobile stand and equipped with additional components modified according to Kamiloğlu (2013). For the measurement of berry elasticity, the berry was placed horizontally between two parallel glass surfaces, and the instrument was zeroed with minimal compression. The elasticity force was then measured as the force required to compress the berry by 1 mm between the surfaces.

Berry cracking tolerance was determined as the force (g) applied horizontally by the glass surface at the moment the berry cracked. Berry flesh firmness was measured as the force (g) required to insert a 5.8 mm thick cylindrical metal probe with teeth 5.5–6.0 mm deep into the equatorial region of the berry without removing the skin. Berry removal force (g) was expressed as the force necessary to detach the berry from its pedicel.

All texture measurements were performed on the harvest day to minimize any potential changes due to post-harvest handling or storage.

Experimental design and statistical analysis

The experiment was designed following a randomized plot design with three replicates, each consisting of 10 clusters and 20 berries. Variance analysis of the data was conducted using the SAS-based JMP 13.0 statistical software, and the least significant difference (LSD) test was applied at a 5% significance level to identify statistically different groups.

Further statistical analysis was performed using Python 3.12.7, employing the following libraries: *numpy* (version 1.26.4) and *scikit-learn* (version 1.5.1) for Principal Component Analysis (PCA), and *seaborn* (version 0.13.2) and *matplotlib* (version 3.9.2) for correlation analysis and data visualization.

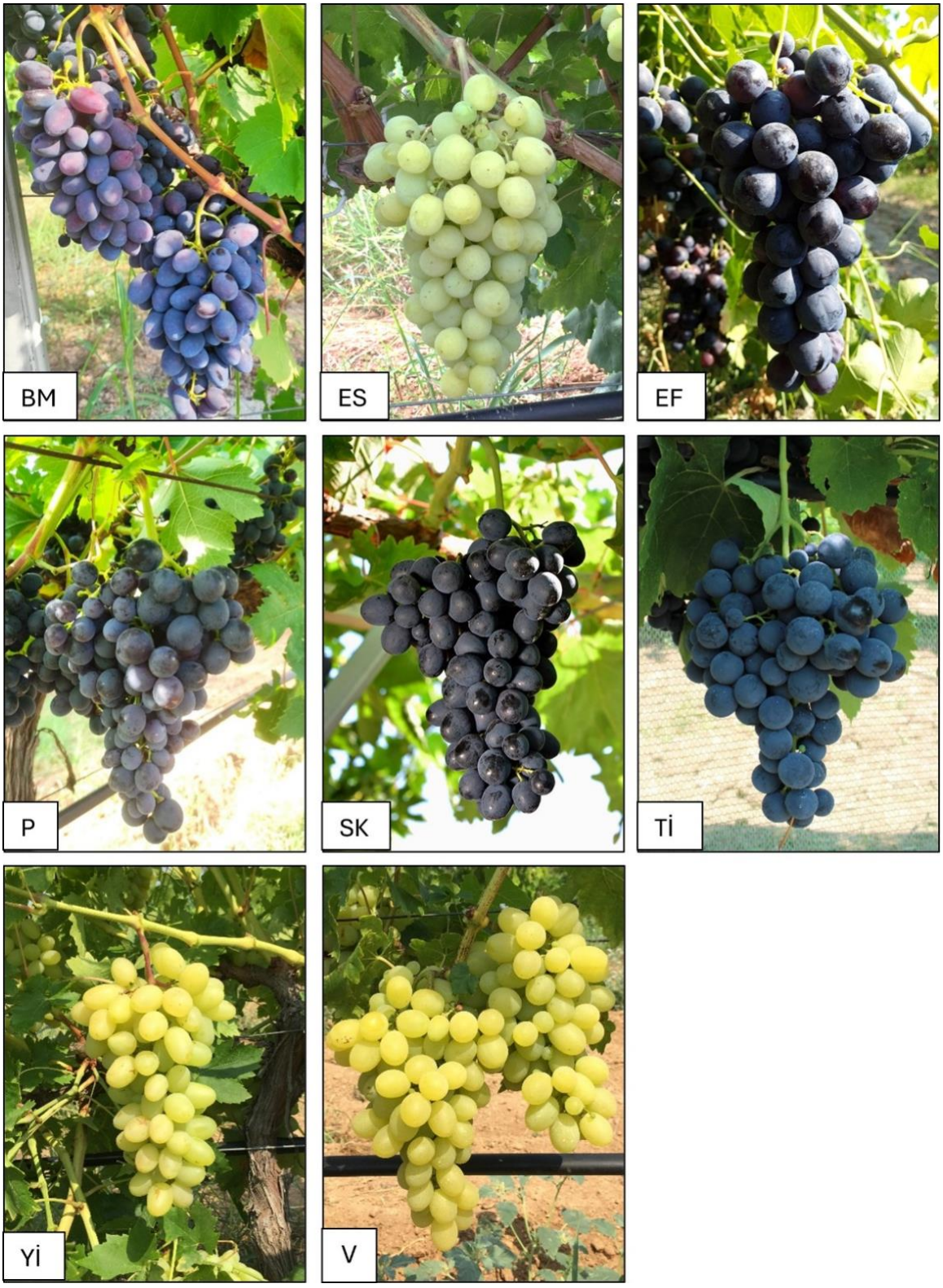


Figure 1. The grape varieties used in the study: BM: Black Magic, ES: Early Sweet, EF: Efem, P: Prima, SK: Spil Karası, Tİ: Trakya İlkeren, Yİ: Yalova İncisi, V: Victoria (Original photographes: Sevil Cantürk)

RESULTS AND DISCUSSION

Preferences for fresh grape consumption are influenced by the specific characteristics of grape varieties (Tóth et al., 2022). In this study, the highest cluster weight and size were observed in the Victoria variety, while the longest cluster length and greatest cluster width were recorded in the Spil Karası and Efem varieties, respectively. The seedless Early Sweet variety, which exhibited the lowest cluster weight among the studied varieties, fell into the same statistical group as Black Magic and Prima in terms of cluster length and cluster size (Table 2).

Consistent with our findings, a study by Kamiloğlu and Demirkeser (2019) reported that Early Sweet had the lowest cluster weight (287.91 g) among eight grape varieties. Similarly, previous studies determined cluster weights of 366.02 g for Trakya İlkeren and 278.54 g for Yalova İncisi under Hassa-Hatay conditions (Kamiloğlu et al., 2011), 324.90 g for Efem, and 247.30 g for Spil Karası (Gönen, 2021). Additionally, cluster weights of 390.5 g for Prima (Control) (Tangolar et al., 2021), 362.10 g for Black Magic (Demir and Tangolar, 2021) under Adana conditions, and 455.82 g for Victoria (Control) under semi-arid Mediterranean conditions (Al-Fadheel et al., 2018) have been reported. These findings align with our results for Efem, Spil Karası, Trakya İlkeren, Early Sweet, and Victoria.

However, the cluster weight of Yalova İncisi in our study was higher than that reported by Kamiloğlu et al. (2011), likely due to ecological variations. Conversely, the cluster weights for Black Magic and Prima in this study were lower than those observed in previous research (Demir and Tangolar, 2021; Tangolar et al., 2021), potentially due to differences in cultural practices and climatic conditions over different growing seasons. Notably, the study by Özdemir and Bayhan (2018), conducted under Eğil-Diyarbakır conditions, reported significantly lower cluster weights for Yalova İncisi (166.67 g) and Trakya İlkeren (122.22 g) compared to our findings, further highlighting the influence of regional ecological factors.

Victoria and Efem were identified as the varieties with the highest berry weight, while Early Sweet exhibited the lowest berry weight. In terms of berry dry weight, Victoria was followed by Efem, while the remaining varieties, except Black Magic, recorded lower values and fell within the same statistical group (Table 3). Compared to the fresh and dry weights of the varieties, the differences in berry moisture (%) and dry matter (%) were significant. Specifically, Yalova İncisi, which had the highest moisture content, was identified as the variety with the lowest berry dry matter content, whereas most other varieties, except Efem, exhibited an inverse relationship (Table 3).

In a study examining 13 grape varieties, including Red Globe, Perlette, Flame Seedless, Crimson, Superior, Thompson Seedless, King Ruby, and six local varieties, berry dry matter contents ranged from 10–33% in the first year and 13–26% in the second year (Al-Obeed et al., 2010). Similarly, Korkutal et al. (2023) reported berry dry weight values between 11.93% and 15.85% in the Michele Palieri variety (control). Additionally, Hamie et al. (2023) found dry matter contents at harvest to range from 17.64% to 20.54% in the Black Pearl variety, 15.85% to 19.20% in the Superior Seedless variety, 14.33% to 24.06% in the Crimson Seedless variety, and 16.19% to 20.67% in Red Globe at different altitudes.

Table 2. Cluster weight and size of the grape varieties.

Variety	Cluster weight (g)	Cluster length (cm)	Cluster width (cm)	Cluster size (cm^2)
Efem	338.3 bc[x]	17.56 bc	12.09 a	211.2 bc
Spil Karası	299.6 cd	20.14 a	11.51 ab	232.3 ab
Trakya İlkeren	316.0 c	18.51 ab	10.00 c	185.1 cd
Yalova İncisi	365.9 b	16.79 bc	10.62 bc	178.4 d
Black Magic	266.0 de	16.25 c	10.00 c	162.5 d
Early Sweet	244.8 e	16.28 c	10.24 bc	167.1 d
Prima	296.9 cd	16.08 c	9.87 c	158.7 d
Victoria	451.0 a	19.89 a	12.87 a	256.1 a
p-value	<0.0001	0.0007	0.0016	<0.0001
LSD (0.05)	46.4	1.88	1.39	31.7

[x] Different letters within the columns indicate significant differences among means ($p < 0.05$)

In this study, the berry dry weight of the varieties, which ranged from 13.10% to 16.11% (excluding Yalova İncisi), was consistent with the second-year results of Korkutal et al. (2023) and Al-Obeed et al. (2010) and was somewhat lower than the findings reported by Hamie et al. (2023). Additionally, the berry moisture values in this study were within the range of 75.43% to 92.34%, as observed in the findings of Ullah et al. (2019).

Table 3. Fresh and dry berry weight, berry moisture, and dry matter percentage of the grape varieties.

Variety	Berry fresh weight (g)	Berry dry weight (mg)	Berry moisture (%)	Berry dry matter (%)
Efem	8.23 a[x]	1.077 ab	86.90 (68.91) ab	13.10 (21.09) ab
Spil Karası	4.13 cd	0.666c	83.89 (66.34) b	16.11 (23.66) a
Trakya İlkeren	4.18 cd	0.660 c	84.21 (66.59) b	15.79 (23.41) a
Yalova İncisi	6.81 b	0.609 c	91.07 (72.66) a	8.93 (17.34) b
Black Magic	5.21 c	0.739 bc	85.50 (67.94) b	14.50 (22.06) a
Early Sweet	3.33 d	0.536 c	83.92 (66.36) b	16.08 (23.64) a
Prima	4.18 cd	0.667 c	84.03 (66.45) b	15.97 (23.55) a
Victoria	8.34 a	1.233 a	85.31 (67.49) b	14.69 (22.51) a
p-value	<0.0001	0.0047	0.0460	0.0460
LSD (0.05)	1.29	0.34	3.91	3.91

[x] Different letters within the columns indicate significant differences among means ($p < 0.05$)

The marketability of table grapes is primarily determined by berry size, color, taste, and texture (Tóth et al., 2022). Due to cell division and subsequent growth, berries exhibit variations in length and width (Zhang et al., 2021). In the present study, measurements on berry samples taken from the middle portion of the clusters revealed that Efem had the largest berry width, while Victoria recorded the greatest berry length. Conversely, Early Sweet exhibited the lowest values for both parameters (Table 4).

Fruit shape, an essential defining characteristic in horticultural plants, has been the subject of relatively few studies in grape research (Zhang et al., 2021). The fruit shape index offers a scientific quantification of fruit shape (Wang et al., 2020). Al-Obeed et al. (2010) reported a two-year average berry shape index ranging from 0.9 to 1.7 in 13 grape varieties, including Red Globe, Perlette, Flame Seedless, Crimson, Superior, Thompson Seedless, and King Ruby. In the present study, the berry shape index ranged from 0.973 in Trakya İlkeren to 1.443 in Black Magic and Victoria. According to the classification of Zhang et al. (2021), Victoria and Black Magic fall under the long elliptical category, Trakya İlkeren is oblate spherical, and Efem is spherical.

Additionally, the berry surface area and volume were found to be the highest in the Victoria and Efem varieties. However, similar to the results observed for berry weight, Early Sweet, a seedless variety, exhibited the lowest values for these two berry traits (Table 4). This finding aligns with previous reports indicating that seed development and seed-produced growth regulators stimulate fruit growth in seeded varieties, thereby increasing berry weight, whereas seedlessness is associated with reduced berry size (Crespan et al., 2021).

Moreover, Barbagallo et al. (2011) and Melo et al. (2015) observed that in the Syrah variety, the skin surface area (cm^2/kg) increases as berry fresh weight decreases. Unlike table grapes, this reduction in berry size is a desirable characteristic for enhancing wine grape quality.

Table 4. Berry physical characteristics of the grape varieties.

Variety	Berry diameter (mm)	Berry length (mm)	Berry shape index	Berry surface area (cm^2)	Berry volume (cm^3)
Efem	23.97 a[x]	24.07 c	1.003 d	18.23 ab	7.39 a
Spil Karası	18.45 c	20.75 d	1.123 c	11.64 d	3.74 cd
Trakya İlkeren	19.20 c	18.62 e	0.973 d	11.39 de	3.63 cd
Yalova İncisi	20.77 b	26.73 b	1.287 b	16.30 b	6.14 b
Black Magic	18.18 c	26.27 b	1.443 a	13.64 c	4.61 c
Early Sweet	16.67 d	18.56 e	1.117 c	9.44 e	2.74 d
Prima	18.25 c	21.15 d	1.160 c	11.66 d	3.76 cd
Victoria	21.38 b	30.82 a	1.443 a	18.88 a	7.55 a
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD (0.05)	1.36	1.57	0.054	1.98	1.14

[x] Different letters within the columns indicate significant differences among means ($p < 0.05$)

In terms of grape quality, texture encompasses all physical and tactile properties that can be objectively measured in terms of force, distance, and time under an applied force (Letaief et al., 2008). In this study, the findings obtained from instrumental measurements of the mechanical and physical properties of grape berries, along with the average number of seeds (both fully developed and rudimentary) per berry, are presented in Table 5.

Previous studies have shown that the berry removal force ranges between 370.2 and 847.6 g in early grape varieties. Specifically, Kamiloğlu (2013) determined it to be between 333 and 576 g in six early grape varieties grown in Hatay, while Ergönül et al. (2018) reported values ranging from 115 to 453 g in seven mid-late and late grape varieties grown in Tekirdağ. In this study, Black Magic (370.2 g) and Trakya İlkeren (455.3 g) exhibited the lowest berry removal forces, while Victoria and Spil Karası demonstrated a very strong berry-pedicel connection, consistent with the findings of Kamiloğlu (2013) and Ergönül et al. (2018).

Berry elasticity, an important textural attribute, was found to be lowest in the Trakya İlkeren (271.3 g mm^{-1}) and Yalova İncisi (259.6 g mm^{-1}) varieties. In contrast, Early Sweet (342.7 g mm^{-1}) and Spil Karası (335.8 g mm^{-1}) recorded the highest values. Regarding berry cracking tolerance, Victoria (4236 g) ranked the highest, followed by Spil Karası (3082 g). The remaining varieties exhibited berry cracking tolerance values between 1922 and 2428 g, except for Trakya İlkeren (1657 g) and Yalova İncisi (1842 g), which had the lowest values. These findings align with Aydın's (2009) study, where berry cracking tolerance among 12 grape varieties ranged from 581.45 to 1972.74 g, with Trakya İlkeren and Yalova İncisi recording 1661.50 g and 1657.59 g, respectively. In another study, Kamiloğlu (2013) found that berry cracking tolerance varied between 1007 and 2672 g in Ora, Prima, Trakya İlkeren, Flame Seedless, Superior Seedless, and Ergin Çekirdeksizi varieties.

Berry flesh firmness is a critical quality parameter for table grapes from a consumer perspective (Balic et al., 2014; Correa et al., 2016; Gross et al., 2016). Fruit firmness is also indicative of physical and physiological disorders, including berry cracking, wilting, sunburn, and insect damage (Hamie et al., 2023). Previous studies have demonstrated that postharvest softening in berries, classified according to their initial hardness at harvest, is influenced by their initial firmness (Moggia et al., 2022). Furthermore, Takahashi et al. (2010) noted that seededness does not significantly impact berry flesh firmness. In the present study, the highest berry flesh firmness was observed in the seeded Prima and the seedless Early Sweet varieties, which exhibited similar values (1378 g and 1357 g, respectively). The lowest values were recorded in Yalova İncisi (786 g) and Black Magic (840 g).

Hamie et al. (2023) found that berry flesh firmness in grape varieties grown at different altitudes ranged from 1.8 to 2.2 KgF in Black Pearl, 2.4 to 2.6 KgF in Crimson Seedless, 1.6 to 2.1 KgF in Superior Seedless, and 1.7 to 2.1 KgF in Red Globe. Cantürk and Kunter (2021) reported that berry hardness varied between 3.96 and 4.34 N in the Trakya İlkeren variety grown under Ankara conditions.

In terms of seed count, the number of seeds and/or traces per berry was less than 2.0 in Black Magic, Early Sweet, and Spil Karası varieties. The highest seed counts were recorded in the Efem (3 seeds/berry) and Prima (2.87 seeds/berry) varieties (Table 5). Kamiloğlu (2013) similarly reported seed counts ranging from 1.83 to 3.07 per berry.

Table 5. Berry texture characteristics and seed numbers of the grape varieties.

Variety	Berry removal force (g)	Berry elasticity (g mm^{-1})	Berry cracking tolerance (g)	Berry flesh firmness (g)	Seed and trace number (n $berry^{-1}$)
Efem	571.3 b[x]	319.8 abc	2428 c	1218 c	3.00 a
Spil Karası	782.1 a	335.8 ab	3082 b	1299 b	1.95 cd
Trakya İlkeren	455.3 de	271.3 de	1657 f	1066 d	2.63 ab
Yalova İncisi	476.9 cd	259.6 e	1842 ef	786 e	2.37 bc
Black Magic	370.2 e	320.5 abc	2244 cd	840 e	1.33 e
Early Sweet	519.2 bcd	342.7 a	2054 de	1357 ab	1.60 de
Prima	566.3 bc	306.9 bc	1922 def	1378 a	2.87 a
Victoria	847.6 a	293.7 cd	4236 a	1213 c	2.53 abc
p-value	<0.0001	0.0002	<0.0001	<0.0001	0.0001
LSD (0.05)	90.5	29.9	364.3	65.7	0.59

[x] Different letters within the columns indicate significant differences among means ($p < 0.05$)

Most researchers (Muñoz-Robredo et al., 2011; Garrido et al., 2014; Hamie et al., 2023) agree that the main ripeness indicators for table grapes are TSS, pH, titratable acidity, and the maturity index (TSS/Acidity ratio). The results for these characteristics of the studied varieties at harvest time are presented in Table 6. In this study, the Prima, Spil Karası, and Efem varieties were grouped with Black Magic as those exhibiting the highest TSS content. The lowest TSS value was recorded in Victoria, followed by Early Sweet. The sugar content, which is commonly used as a ripeness indicator in grapes, is measured as TSS in the must and is associated with the technological maturity of table grapes. According to European Union regulations, the maturity limit for Vitis vinifera L. varieties is set at 13 °Brix for seeded varieties and 14 °Brix for seedless varieties (Maante et al., 2015).

In this experiment, all varieties exceeded these minimum thresholds. In California, grapes are typically harvested with TSS values ranging from 14.0 to 17.5%, depending on the variety and production area (Crisosto and Smilanick, 2007). The highest acidity levels were observed in Prima, Trakya İlkeren, and Efem varieties, while the lowest were found in Victoria and Yalova İncisi. Despite Victoria having the lowest TSS content, it exhibited

the highest maturity index, along with Yalova İncisi, due to its relatively higher acidity. The maturity index was lowest in Trakya İlkeren, Prima, and Efem varieties.

In general, the fact that the maturity index for most varieties was above 20 suggests their suitability for harvest at optimal maturity. Indeed, in viticultural regions where early ripening is prioritized, a TSS/acid ratio of ≥20 is typically desired for varieties that exceed the minimum TSS threshold (Crisosto and Smilanick, 2007). This advantage suggests that the harvest times for Victoria, Yalova İncisi, and Black Magic varieties could potentially be advanced.

Table 6. Must characteristics of the grape varieties.

Variety	TSS (%)	pH	Titratable acidity (%)	Maturity index
Efem	16.03 ab[x]	3.56 e	0.572 a	28.02 d
Spil Karası	16.13 ab	3.68 c	0.439 b	36.75 c
Trakya İlkeren	15.80 bc	3.36 g	0.613 a	25.80 d
Yalova İncisi	15.57 bc	3.95 a	0.290 d	54.02 a
Black Magic	16.87 a	3.64 cd	0.371 c	45.50 b
Early Sweet	14.97 cd	3.58 de	0.444 b	33.71 c
Prima	16.30 ab	3.44 f	0.616 a	26.62 d
Victoria	14.57 d	3.87 b	0.273 d	53.26 a
p-value	0.0010	<0.0001	<0.0001	<0.0001
LSD (0.05)	0.87	0.08	0.047	3.90

[x] Different letters within rows indicate significant differences among means ($p < 0.05$)

According to principal component analysis showing the relationship between varieties and the traits examined, Component 1 (47.3%) and Component 2 (22.1%) together explain 69.4% of the total variability in grape variety characteristics (Figure 2). This means that most of the variation among the grape varieties can be understood by analyzing these two components. Component 1 primarily separates grape varieties based on size-related attributes such as berry diameter, volume, and cluster size. Grapes with higher values in Component 1 tend to be larger, heavier, and have bigger clusters, while those with lower values tend to be smaller but have higher dry matter, elasticity, and acidity. Component 2 differentiates grape varieties based on quality-related traits. Attributes such as berry elasticity, acidity, and dry matter align more with Component 2, suggesting that these traits play a significant role in distinguishing grape varieties along this axis. Some grape varieties cluster together, indicating they share similar characteristics. For instance, Early Sweet, Prima, and Trakya İlkeren are positioned close to each other on the left side of the plot, suggesting they have smaller sizes and higher acidity. Efem and Spil Karası are positioned near attributes like seed and trace number, elasticity, and firmness, implying these varieties may have firmer berries with more seeds. Black Magic and Yalova İncisi are located on the lower right, associated with higher moisture content. Regarding specific grape varieties, Victoria is positioned far on the positive side of PC1, indicating it has large clusters, big berries, and high weight. Yalova İncisi is negatively positioned along PC2, suggesting it has a higher moisture content and a softer texture. This PCA analysis provides valuable insights for grape breeding, cultivation, and commercialization by identifying which grape varieties possess desirable traits based on berry size, firmness, moisture content, and other key characteristics.

The correlation analysis of grape variety characteristics reveals significant relationships among size-related, quality-related, and structural traits (Figure 3). Strong positive correlations exist among berry size attributes such as fresh weight, volume, diameter, and surface area, indicating that larger berries generally exhibit greater mass and dimensions. Similarly, cluster-related traits, including cluster weight, length, and width, show strong interdependence. In contrast, a clear trade-off is observed between moisture content and firmness (-0.70), elasticity (-0.59), and dry matter (-1.00), suggesting that juicier berries tend to be softer and less elastic. Additionally, acidity and pH (-0.93), as well as acidity and maturity index (-0.98), demonstrate an inverse relationship, meaning that as grapes mature, their acidity decreases while pH increases. Furthermore, larger berries tend to have lower acidity and slightly higher seed counts, as indicated by the positive correlation between berry diameter and seed number (0.66). These findings are crucial for grape breeding and selection strategies, as they highlight how size and quality attributes interact, allowing for targeted improvements in texture, acidity balance, and overall berry composition to meet market and consumer preferences.

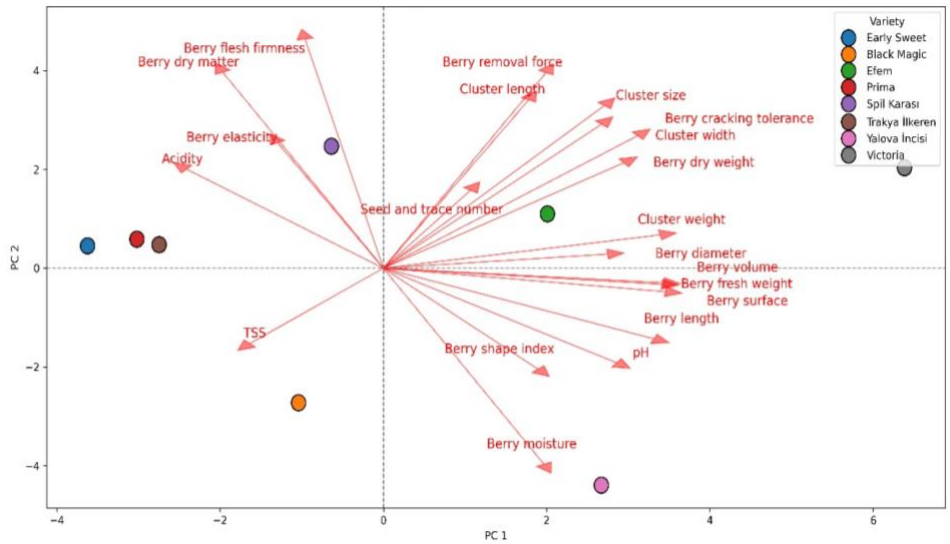


Figure 2. Principal component analysis of the grape varieties and the parameters examined

CONCLUSION

In viticulture, significant differences in growth, development, and product quality can occur within the same grape variety, depending on factors such as vineyard region and environmental conditions (climate, temperature, humidity, and altitude). These variations directly influence the economic value and commercial yield of the grape. This study evaluated various physicochemical and mechanical quality properties critical for fresh consumption and marketability in early table grape varieties. The varieties studied ripen early (June-July) under Mediterranean climatic conditions. The physical, chemical, and textural properties of the varieties at harvest were found to be significant for marketing strategies.

The results showed that the grape varieties could be grouped according to their quality parameters. During harvest, Victoria and Efem varieties exhibited the largest cluster and berry sizes, while Early Sweet displayed the smallest. Similarly, berry width, length, surface area, volume, and fresh weight were highest in Victoria and Efem varieties. In terms of berry moisture and dry matter ratio, Yalova İncisi had the highest moisture content and the lowest dry matter content, while Efem and other varieties showed higher dry matter percentages.

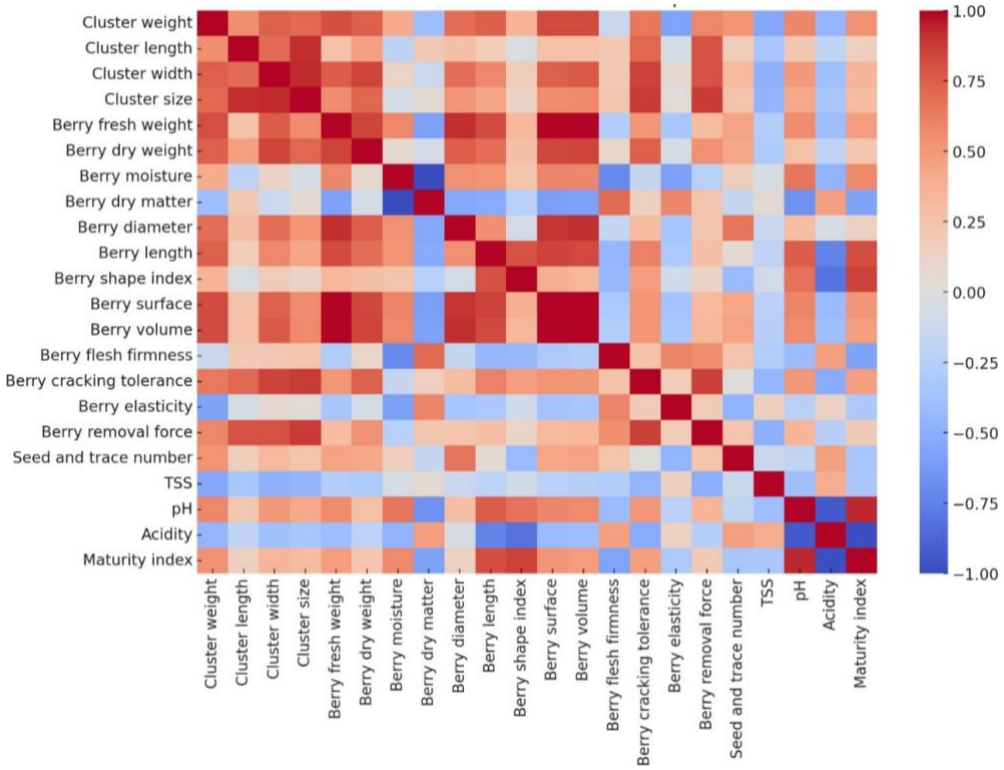


Figure 3. The correlation analysis of the grape variety characteristics

Regarding mechanical and physical properties, berry cracking tolerance was highest in Victoria and Spil Karası varieties, and lowest in Trakya İlkeren and Yalova İncisi. The highest berry elasticity was measured in Early Sweet and Spil Karası, and the lowest in Trakya İlkeren and Yalova İncisi. In terms of TSS values, Black Magic had the highest value, while Early Sweet and Victoria exhibited the lowest. Nevertheless, all varieties met the European Union table grape standards for TSS.

Based on these findings, Victoria and Efem varieties are distinguished by their large berries, making them particularly attractive for consumers preferring large-berried table grapes. Early Sweet, being seedless, is suitable for consumers who prefer seedless varieties. Additionally, varieties such as Prima and Early Sweet, with their firm berries, are better suited for transportation and extended shelf life, making them important for marketing strategies.

In conclusion, it is recommended that grape breeding and hybridization programs focus on increasing varieties with large, firm berries, seedlessness, and high TSS levels. Furthermore, future studies should explore changes in the quality characteristics of grape varieties over time.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Author contribution

Conceptualization: Serpil Tangolar, Önder Kamiloğlu; Methodology: Önder Kamiloğlu, Serpil Tangolar; Writing - original draft preparation: Önder Kamiloğlu, Serpil Tangolar; Writing - review and editing: Sevil Cantürk, Semih Tangolar; Supervision: Serpil Tangolar; Monitoring of trial vines, Cultural practices, Sampling: Önder Kamiloğlu, Serpil Tangolar, Semih Tangolar

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Soil chemistry in apricot cultivation: evaluation of C and N dynamics by PCA and correlation analysis

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Abstract

This study uses statistical methods to analyze soil carbon (C) and nitrogen (N) dynamics in four different apricot growing regions (Battalgazi, Akçadağ, Doğanşehir and Hekimhan) in Malatya. Correlation analysis, hierarchical clustering (HCA) and Principal Component Analysis (PCA) were applied to examine the relationships between soil organic matter (OM), soluble carbon (Soluble C), biomass nitrogen (Biomass N), total nitrogen (N) and other soil chemical parameters. According to the results obtained, a strong positive correlation (r = 0.67) was found between organic matter (OM) and total nitrogen (N), indicating that the organic matter content in the soil contributes directly to the nitrogen mineralization process. Positive correlations were found between soluble carbon (Soluble C) and biomass carbon (Biomass C) and N, indicating that microbial activity and carbon cycling in soil are directly related to nitrogen dynamics. In PCA analysis, the first two principal components (PC1 and PC2) explained 82.1% of the total variance. The PC1 axis is related to soil macronutrients (C, N, K, P) and organic matter content, while the PC2 axis is related to lime ($CaCO_3$), pH and micronutrients (Fe, Mn, Zn). When the regional distribution is analyzed, Battalgazi and Doğanşehir show similar chemical characteristics, while Hekimhan is differentiated by high pH and lime content. Hierarchical cluster analysis (HCA) shows that Battalgazi and Doğanşehir have similar soil characteristics, but Akçadağ and especially Hekimhan have different soil composition. Hekimhan's high $CaCO_3$ content and alkaline pH level may negatively affect productivity by suppressing organic matter and nitrogen levels. The results of this study can contribute to the development of soil management strategies in apricot farming in Malatya. In particular, organic matter management, pH regulation strategies and optimization of macro/micro nutrient balance are needed. A detailed study of C and N cycles is a critical step for sustainable soil management and improving the productivity of apricot production.

Keywords: Apricot, Carbon, Nitrogen, PCA, Correlation Soil

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INTRODUCTION

Apricot (Prunus armeniaca L.) cultivation is one of the most important agricultural activities in Turkey, covering a wide area from the southeast to the western regions of the country. In apricot cultivation, balanced management of soil carbon (C) and nitrogen (N) cycles directly affects plant nutrition and soil fertility. Apricot has a great economic, cultural and social importance especially in Malatya province. Malatya is the largest apricot producer in Turkey and accounts for 85% of Turkey's total dried apricot production (Öztürk and Karakaş, 2017; Ercisli and Güleryüz, 2001). Apricot is one of the key elements of agricultural development in the region and also stands out as an important agricultural product that strengthens the local economy (Duru et al., 2022).

The optimal growth and productivity of apricot trees are closely influenced by soil characteristics. Specifically, well-drained soils with high organic matter content promote robust root development and enhance nutrient uptake

efficiency in apricot plants. Studies indicate that maintaining soil pH within the range of 6.0 to 7.5 is essential for achieving maximum yield potential in apricot cultivation (Eriş, 1995). Furthermore, key factors such as soil fertility, organic matter dynamics, and the management of soil carbon processes play a pivotal role in ensuring sustainable and efficient agricultural production systems (Montanaro et al., 2012; Gerke, 2022).

In addition, soil nutrients also play a decisive role in the quality of apricot. Potassium improves fruit quality, while nitrogen promotes overall plant growth (Xu et al., 2020). In addition, adequate amounts of calcium and magnesium in the soil increase the resistance of apricot trees to diseases and promote fruit set. Soil diversity in different regions is an important factor in apricot farming; therefore, conducting local soil analysis helps farmers to determine the most appropriate farming practices (Zhao et al., 2018).

In apricot cultivation, effective management of soil carbon (C) and nitrogen (N) cycles is essential, as these directly influence plant nutrition and soil fertility. The cycling of carbon and nitrogen is intricately linked to microbial activity, organic matter content, and nutrient dynamics within the rhizosphere (Lal, 2015). Mineralization of organic matter enhances soil fertility by converting nitrogen into plant-available forms, while carbon sequestration contributes to improved soil structure and increased water-holding capacity. However, certain soil characteristics, particularly alkaline pH and elevated calcium carbonate levels, may restrict the efficiency of carbon and nitrogen turnover processes (Marschner, 2012). Notably, in calcareous soils, the bioavailability of micronutrients such as iron, zinc, and manganese is often reduced, potentially leading to nutritional imbalances and physiological disorders in plants.

Soil organic matter is at the center of the carbon cycle and is recognized as a component that improves soil health and increases productivity (Schlesinger and Andrews, 2000). Research focusing on apricot cultivation and soil organic carbon (SOC) dynamics is vital for enhancing both soil health and crop productivity. Organic carbon improves soil structure, enhances water retention capacity, and increases the bioavailability of essential nutrients. In particular, soils rich in organic matter significantly promote root development and nutrient uptake in apricot trees (Negușier et al., 2024). Empirical studies have demonstrated that the application of organic fertilizers can substantially increase SOC levels, thereby boosting apricot yields. For instance, Cai et al. (2020) reported that the integration of compost and manure led to a 15–20% increase in SOC and a corresponding yield improvement in apricot orchards. Moreover, sustainable land management practices—such as cover cropping and reduced tillage—not only support yield stability but also contribute to climate change mitigation by enhancing the soil's carbon sequestration capacity (Smith et al., 2018). Long-term field trials, such as those conducted by Zhang et al. (2022) and Du et al. (2022), have examined the comparative effects of organic versus conventional management on SOC trends in apricot-producing regions, offering specific recommendations to optimize carbon retention in orchard soils.

However, the management of soil organic matter and carbon dynamics in apricot farming regions is of great importance for productive and sustainable agriculture. Studies conducted in Turkey have revealed that soils with high organic matter content increase the growth and productivity of apricot trees (Yang et al., 2021). However, the organic matter content of the soil may vary according to the agricultural techniques used and the climatic characteristics of the region.

Studies on apricot agriculture and soil organic carbon (SOC) dynamics are critical for improving soil health, enhancing crop productivity, and ensuring long-term agricultural sustainability. Organic carbon contributes significantly to soil structure, increases water-holding capacity, and enhances the bioavailability of essential nutrients. In particular, soils with high organic matter content foster optimal root development and facilitate nutrient uptake in apricot trees (Negușier et al., 2024). Numerous studies have demonstrated that organic fertilizer applications can elevate SOC levels, thereby leading to substantial yield improvements in apricot orchards (Cai et al., 2020). Moreover, sustainable agricultural practices—such as compost application, reduced tillage, and cover cropping—not only enhance soil fertility but also play a pivotal role in climate change mitigation by increasing the soil's carbon sequestration potential (Smith et al., 2018). Long-term field experiments have evaluated the effects of various management strategies on SOC dynamics in apricot cultivation and have provided actionable recommendations to optimize carbon retention and overall soil functionality.

Effective soil analysis and nutrient management are vital components of apricot cultivation, as they directly influence both yield and fruit quality. In this context, conducting comparative soil studies across different agroecological regions is crucial for identifying region-specific nutrient deficiencies and optimizing fertilization strategies tailored to local conditions.

Soil surveys in the region show that soil organic matter and carbon dynamics are related to the productivity of apricot agriculture and these dynamics need to be improved for sustainable production (Yang et al., 2021). Battalgazi, Akçadağ, Hekimhan and Doğanşehir, which are four important districts of Malatya province, are among the regions where apricot production is intensive. The soil properties and organic carbon dynamics of these regions are of great importance for the sustainability of apricot production. Battalgazi, Akçadağ, Hekimhan and Doğanşehir districts show differences in terms of both climatic and soil structure. These differences are important factors affecting the applicability of apricot agriculture and soil management (Keesstra et al., 2016).

In this study, soil C and N dynamics and their interactions with soil pH, $CaCO_3$ content, and micro-macro nutrients were investigated in apricot cultivation in four important agricultural regions of Malatya (Battalgazi, Akçadağ, Doğanşehir, and Hekimhan). The main objective was to identify differences between these regions and to develop appropriate soil management strategies for agricultural production. Three main statistical methods were used in the study. Correlation analysis was applied to determine the relationships between soil carbon, nitrogen, and other nutrients. Principal Component Analysis (PCA) was used to identify the most dominant variables by determining the C and N dynamics of different regions. Hierarchical cluster analysis (HCA) was used to group the soils by identifying the similarities and differences between the regions. This study provides a powerful tool for a detailed study of the interactions between soil chemistry and nutrients, providing important information for sustainable soil management in apricot cultivation.

MATERIALS AND METHODS

Soil sampling procedure

In this study, soil samples taken from **Battalgazi, Akçadağ, Hekimhan and Doğanşehir** regions were subjected to laboratory analysis. Battalgazi, Akçadağ, Hekimhan and Doğanşehir districts in Malatya province are under the influence of continental climate and each of them has suitable climate and soil conditions for apricot cultivation (Table 1). The soils in these districts are generally calcareous, high in pH, rich in organic matter content, and show deep, well-drained structures suitable for apricot cultivation (Soydan, 2019; Bassi et al., 2024). The climatic and soil characteristics in these regions have the potential to ensure efficient and sustainable apricot production (Zhebentyayeva et al., 2012).

Table 1. Location of the study area and some climatic characteristics

District	Altitude (m)	Precipitation (mm/year)	Temperature (°C)	Climate	Coordinate
Battalgazi	~950	350-400	13-14	Temperate, arid, microclimate effective	38.3600° N, 38.3300° E
Akçadağ	~1,050	400-450	11-12	Dry, windy, harsher winters	38.6000° N, 38.5000° E
Hekimhan	~1,250	450-500	10-11	Harsh continental, high pH and lime	38.4000° N, 38.6000° E
Doğanşehir	~1,250	600-700	11-12	Moist, fertile, microorganism rich	38.5500° N, 38.7500° E

The climatic and geographical characteristics of the studied districts reveal distinct environmental conditions that directly influence apricot cultivation potential. Battalgazi and Akçadağ, with their relatively lower altitudes and moderate temperatures, offer favorable conditions for apricot growth, though limited precipitation and arid tendencies may necessitate irrigation and organic matter enrichment. Hekimhan's high elevation and harsh continental climate, coupled with alkaline and lime-rich soils, can challenge nutrient availability, requiring targeted fertilization strategies. In contrast, Doğanşehir stands out with its higher precipitation and fertile, microorganism-rich soils, presenting an ideal environment for sustainable and high-quality apricot production. These regional differences underscore the need for location-specific soil and crop management practices in apricot orchards (Table 1).

Soil samples were collected from the 0–30 cm layer, which is considered the most biologically active zone and crucial for nutrient uptake in apricot orchards. Following collection, the samples were air-dried and sieved through a 2 mm mesh to remove debris and homogenize the texture. To preserve microbial integrity, the samples were sealed in polyethylene bags to minimize moisture loss and stored at +4 °C until analysis

Soil analyses were conducted to evaluate key parameters including macronutrients (N, P, K), micronutrients (Fe, Zn, Cu, Mn), as well as pH, Electrical Conductivity (EC), Lime Content ($CaCO_3$), Organic Matter (OM), Sodium (Na), Soil Carbon and Nitrogen Dynamics, Soluble N, Biomass N, Soluble C, Biomass C, Cation Exchange Capacity were performed. By comparing the average values in each region, regional differences were determined and the effects of these differences on agricultural management were analyzed.

Soil reaction (pH) 1:2.5 (w/v) and electrical conductivity (EC) 1:5 (w/v) were measured in a soil water mixture (Jackson 1958). Texture analysis was done by hydrometer and after determining % clay, % sand and % silt, the class was determined in the texture triangle (Bouyoucos 1951). Calcium carbonate ($CaCO_3$, %) content was determined by Scheibler calcimeter in a closed system (Allison and Moodie, 1965). Organic carbon content was determined by wet combustion method based on potassium dichromate oxidation **(**Walkley and Black 1934). Total nitrogen content was determined by the Kjeldalh method (Bremner and Mulvaney 1982). Available Phosphorus (P) was extracted using the Olsen method (0.5 M $NaHCO_3$, pH 8.5) for neutral to alkaline soils. The phosphorus concentration in the extract was determined colorimetrically using the molybdenum blue method (Olsen., 1954). Potassium (K) was measured by extracting soil with 1 M ammonium acetate (NH_4OAc) at pH 7.0. The potassium content in the extract was determined using flame photometry. Sodium (Na) similar to potassium, sodium was

extracted using 1 M ammonium acetate, and quantified via flame photometry (Richards.,1954). Micronutrients such as Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn) were obtained using the DTPA (diethylenetriaminepentaacetic acid) extraction method (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA, pH 7.3). Concentrations were determined via atomic absorption spectrophotometry (AAS) (Lindsay and Norvel., 1978). Soluble Carbon (SC) and Soluble Nitrogen (SN): Extracted using 0.5 M K_2SO_4 and analyzed via spectrophotometry (Vance et al., 1987). Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN): Determined using the fumigation-extraction method (Vance et al., 1987; Joergensen et al., 1990)

Data Analyses

The SPSS 26.0 software (SPSS Inc.) and Origin pro 2024 were used to perform statistical analysis of the data. The fit of the data to a normal distribution for all properties measured was checked with the Kolmogorov-Smirnov test. Data were submitted to one-way repeated measures ANOVA as between-subject factors. Two-way ANOVA was performed to assess treatment effects and interactions, followed by Tukey's HSD test ($p < 0.05$). Principal Component Analysis (PCA) and Hierarchical Clustering Heat Map analysis were conducted to visualize soil biochemical relationships. All statistical analyses were performed using R (v4.2) and Python (v3.11) with ggplot2 and seaborn libraries for visualization.

RESULTS AND DISCUSSION

Soil Chemistry and pH Changes

Soil pH is one of the main parameters affecting the uptake of nutrients by plants. The pH values of the soils analyzed within the scope of the study varied between 7.53 and 7.92 and were generally in the neutral to slightly alkaline range. Previous studies have shown that the optimum pH range for apricot cultivation is 6.5-7.5 (Ercisli and Güleryüz, 2001). pH values above 7.5 may cause developmental problems, especially (Figure 1) by limiting the uptake of micronutrients (Fe, Zn, Cu, Mn) by plants (Marschner, 2012).

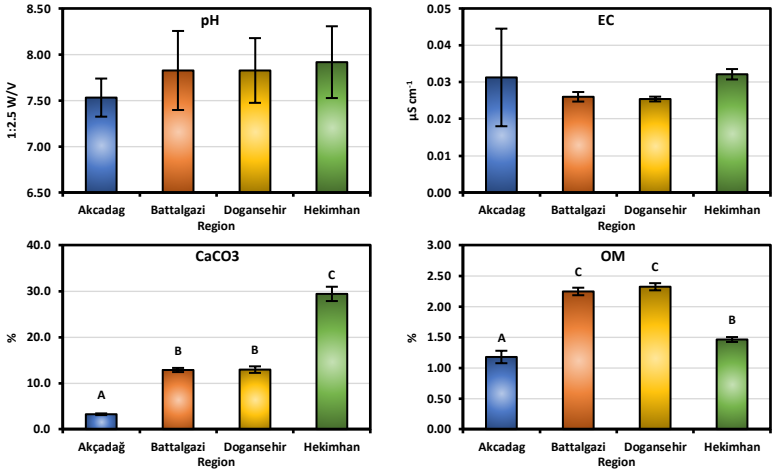


Figure 1. pH, EC, OM and $CaCO_3$ level of soils

The basic chemical properties of soils such as pH value, $CaCO_3$ content and OM content significantly affect the uptake of nutrients by plants. In this study, Hekimhan and Akçadağ regions were found to have high pH and lime content (Figure 1). Many studies have shown that calcareous soils reduce the uptake of micronutrients such as Fe, Zn, and Mn by plants (Marschner, 2012; Lindsay and Norvell, 1978). This inhibition typically occurs in soils with a pH above 7.5 and $CaCO_3$ content exceeding 15%, where micronutrients become increasingly unavailable due to precipitation and reduced solubility (Alloway, 2008; Kabata-Pendias, 2011).

Electrical conductivity analyses show that salinity levels are higher in Doğanşehir region (Figure 1). Soil salinity is one of the main factors limiting agricultural production, especially in arid and semi-arid regions (Rengasamy, 2010). High salinity disrupts soil structure due to the accumulation of Na and chlorine (Cl) ions and makes it difficult for plants to uptake water (Munns and Tester, 2008). Therefore, salinity management should be ensured by drainage practices and organic matter additions in areas with high EC values such as Doğanşehir.

The lime content in Malatya is highly variable, with the lowest measured in Akçadağ (3.23%) and the highest in Hekimhan (29.39%) (Figure 1). High lime content is often associated with micronutrient deficiencies, particularly Fe and Zn, and may result in visible symptoms such as chlorosis in apricot trees (Kacar and Katkat, 2010). However, explaining this phenomenon solely by lime content may be insufficient, as high total Zn levels in the soil do not necessarily ensure its bioavailability under alkaline conditions. In the Hekimhan district, for example, both lime and Zn contents were found to be high, yet zinc deficiency symptoms were observed, likely due to the reduced solubility and plant uptake of Zn in calcareous soils with elevated pH. To overcome these

challenges, targeted strategies such as the application of chelated micronutrient fertilizers or foliar feeding are recommended for sustainable apricot production in calcareous regions (Mengel et al., 2001).

Significant differences were observed between regions in terms of OM content. The highest value was found in Doğanşehir (2.32%) and the lowest value was found in Akçadağ (1.18%) (Figure 1). Previous studies have shown that soils with organic matter content above 2% have higher microbial activity and nutrient retention capacity (Lal, 2015). Increasing organic matter content is of great importance for the long-term sustainability of apricot cultivation in Malatya.

Soil organic matter is one of the main indicators of soil fertility and is directly related to nitrogen mineralization (Brady and Weil, 2008). In this study, a strong relationship between organic matter content and soil nitrogen (N) was observed. Similarly, studies have shown that an increase in soil organic matter content increases microbial activity and leads to the production of more mineral nitrogen for plants (Jenkinson and Ladd, 1981; Jilling et al., 2018). In Akçadağ and Hekimhan regions, organic matter content was relatively high, which means better biological activity and nutrient cycling. In contrast, regions such as Battalgazi and Dogansehir have lower organic matter content, which may require the use of organic fertilizers to maintain productivity in these regions in the long term (Lal, 2004).

Carbon and Nitrogen Dynamics

Carbon and nitrogen parameters are presented in Figure 2. According to the data obtained, soluble carbon (Soluble C) content shows a significant difference between the regions. The highest value was found in Akçadağ (105.95 mg/kg) and the lowest in Doğanşehir (19.26 mg/kg). This indicates that Akçadağ soils are richer in organic carbon and microbial activity may be higher in this region. The higher soluble carbon content suggests that the organic matter decomposition process is active and the microbial biomass of the soil may be higher.

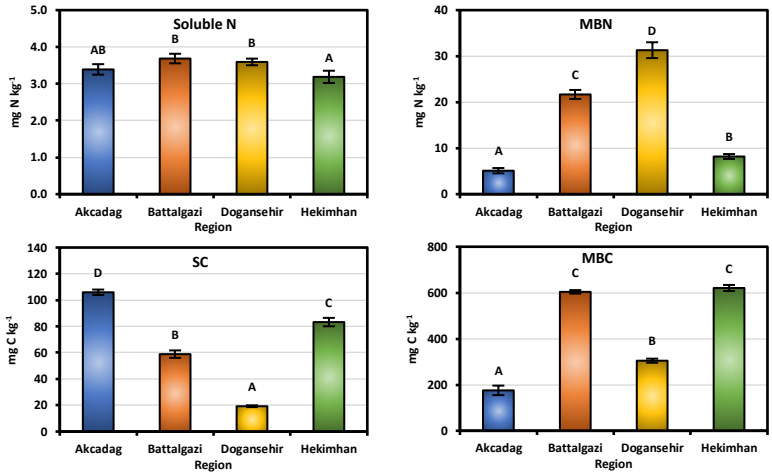


Figure 2. Soil C and N Dynamics

In terms of Total N content, the highest value was found in Doğanşehir with 0.12% and the lowest value was found in Akçadağ with 0.06%. Nitrogen is a critical macronutrient for plant growth and is directly related to shoot development, especially in apricot cultivation. The high total nitrogen content of Doğanşehir indicates that soil organic matter transformation and nitrogen mineralization processes may be more active in this region. When Biomass C and Biomass N are analyzed, Battalgazi and Akçadağ regions stand out as regions with higher microbial activity. Since biomass carbon and nitrogen contents reflect the activity of microorganisms in the soil and organic matter transformation processes, it can be said that microbial activities are more intense in these regions. When the relationship between organic matter content and nitrogen dynamics was analyzed, it was observed that there was a positive correlation between organic matter content and total nitrogen ($r = 0.67$). This finding indicates that soils rich in organic matter contain more nitrogen and this nitrogen becomes available for plants through mineralization.

Soil carbon and nitrogen cycling is a critical process for microbial activity, organic matter mineralization and plant uptake of nutrients. In this study, significant differences were found between soluble carbon and total nitrogen levels. The low levels of total nitrogen, especially in the Akçadağ region where soluble carbon is high, reveal the importance of biochemical interactions between carbon and nitrogen for regional soil management. Adequate soil carbon availability is critical for sustaining microbial activity and soil fertility (Schmidt et al., 2017).

Sufficient organic matter in the soil promotes nitrogen mineralization by enhancing microbial activities, thus allowing plants to take up more nitrogen. However, high lime content and pH level can reduce the bioavailability of some micronutrients, limiting the effective utilization of nitrogen by plants. This was particularly observed in

the Hekimhan region, where high $CaCO_3$ content was associated with relatively lower amounts of organic matter and nitrogen (Wright and Bailey, 2001).

The high total nitrogen levels in Dogansehir region indicate that the nitrogen cycle works more efficiently here. Nitrogen is a vital element for shoot development and fruit set in apricot cultivation, and yield and quality may decrease in nitrogen deficient soils (Fageria et al., 2010). In areas where nitrogen levels are inadequate, it is recommended to optimize nitrogen fertilization programs. However, high Na levels were detected in this region. Excess sodium can disrupt soil structure and hinder the uptake of water and nutrients by plants (Wakeel, 2013). Therefore, salinity management should be ensured through drainage practices and organic matter supplements in soils with high sodium content such as Doğanşehir.

The relationship between soil carbon and nitrogen is of great importance for sustainable soil management (Yuan et al., 2023). Increasing organic matter content and implementing balanced fertilization programs, especially in apricot farming regions, will help to maintain a healthy carbon and nitrogen cycle (Akın and Aygül, 2022).

Climate change and related environmental factors such as temperature fluctuations, irregular precipitation patterns, and increased evapotranspiration have significant implications for soil carbon (C) and nitrogen (N) dynamics. In semi-arid regions like Malatya, rising temperatures and decreasing soil moisture can accelerate organic matter decomposition, leading to rapid carbon loss and altered nitrogen mineralization processes (Davidson & Janssens, 2006; Smith et al., 2008). These changes may disrupt the balance between microbial activity and nutrient availability, thereby affecting plant nutrient uptake and soil fertility (Lal, 2004).

Moreover, increased frequency of droughts may limit nitrogen fixation and reduce microbial biomass carbon, while sporadic heavy rains may intensify nutrient leaching—particularly in soils with low water-holding capacity (Schimel et al., 2007). The interaction between high pH, lime content, and climatic stress further complicates micronutrient availability in certain regions like Hekimhan (Marschner, 2012).

Considering these vulnerabilities, adaptive soil management strategies that enhance organic matter retention, improve soil moisture conservation, and stabilize microbial ecosystems become increasingly important. Integrating climate-smart practices—such as cover cropping, reduced tillage, mulching, and organic amendments—can help buffer the negative impacts of climate change on soil C and N dynamics, ensuring long-term sustainability in apricot production (FAO, 2017).

Macro-Micro Nutrients and Apricot Yield

Macronutrients play an important role in apricot tree growth and fruit quality (Figure 3). Phosphorus content was highest in Hekimhan (11.45 mg/kg) and lowest in Akçadağ (2.36 mg/kg). Since P plays a critical role in root development and flowering processes, low phosphorus levels may limit fruit yield (Shen et al., 2011).

In terms of K content, the lowest value was found in Doğanşehir (167.17 mg/kg) and the highest in Hekimhan (207.66 mg/kg). Potassium is known to improve fruit quality and sugar accumulation (Wang et al., 2013). Especially the fact that Malatya apricot stands out in the world market with its high brix (sugar) content reveals the importance of potassium management (Duymuş, 2023).

In terms of Ca and Mg content, Akçadağ and Hekimhan stand out. Calcium is an element that increases fruit firmness and storage time, and Ca deficiency may cause softening and shorter shelf life of apricots (Fallahi et al., 2013). Sodium content was high in Akçadağ and Hekimhan (4.43-5.51 mg/kg). Excess sodium can degrade soil structure and reduce water holding capacity and nutrient uptake (Rengasamy, 2010). These results support the need for careful irrigation water management in Malatya.

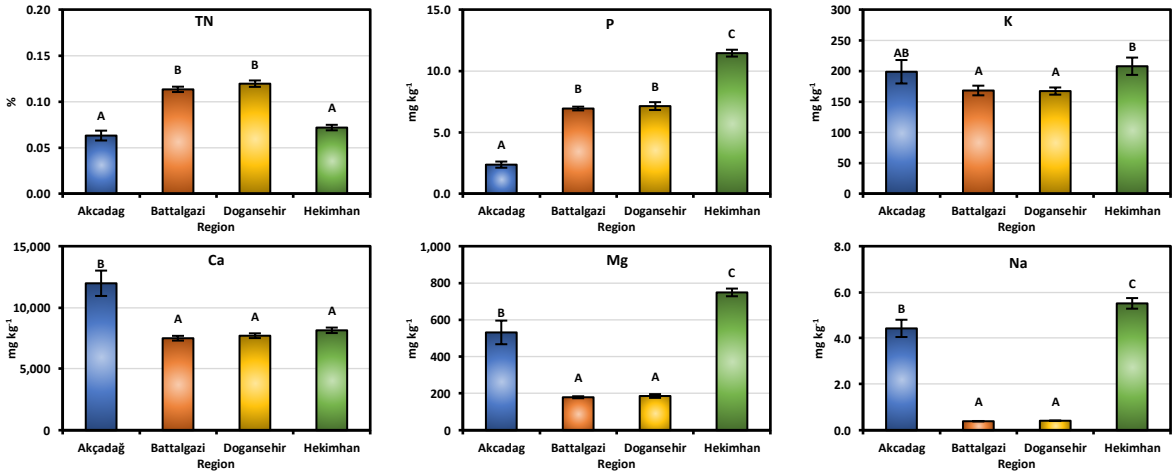


Figure 3. Macronutrient Levels in Soil (N, P, K, Ca, Mg, Na)

Iron, Zn, Cu and Mn are important micronutrients that affect quality and yield in apricot cultivation (Figure 4). When Fe value was analyzed; Akçadağ had the highest Fe content (5.55 mg/kg), while Hekimhan had the lowest (2.66 mg/kg). Iron deficiency causes leaf yellowing such as chlorosis and decreases photosynthetic capacity (Marschner, 2012). When Zn value was analyzed; the lowest zinc content was found in Akçadağ (0.26 mg/kg). Zinc deficiency may reduce apricot fruit setting by delaying flowering (Alloway, 2008).

When the Cu value is analyzed; Hekimhan (12.86 mg/kg) has the highest Cu content (Figure 4) and it is known that copper is important for lignin synthesis and plant resistance (Broadley et al., 2012). When the Mn value is analyzed; Mn deficiency is mostly observed in Hekimhan region (7.12 mg/kg). Manganese is a critical element for photosynthesis and carbohydrate metabolism (Millaleo et al., 2010).

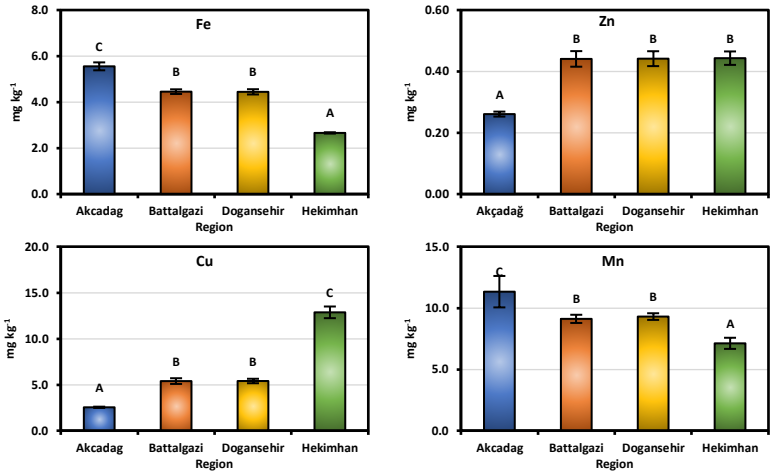


Figure 4. Soil Micronutrient Levels (Fe, Mn, Cu, Zn)

Soil nutrient analysis plays a critical role in planning soil management and fertilization programs. In this study, micronutrients, especially Fe and Zn, were found to be at low levels in Battalgazi and Doganshehir regions. Iron deficiency is a common problem, especially in calcareous soils with high pH, because Fe^{+2} ions are rapidly oxidized to Fe^{+3} form, making uptake by plants difficult (Lindsay, 1991).

Soil micronutrient deficiencies are one of the most important factors that reduce quality and yield in agricultural production. Especially Zn deficiency causes growth retardation in crops such as corn, wheat and rice (Alloway, 2008). Therefore, in Battalgazi and Doğanşehir regions, which have low Fe and Zn content, micronutrient support should be provided by foliar fertilization or soil conditioners.

Hierarchical Clustering Heatmap between Treatments and Soil

Hierarchical clustering and heat map methods were combined to compare soil properties in different regions of Malatya (Figure 5). The graph visualizes the similarities and differences of Battalgazi, Doğanşehir, Akçadağ and Hekimhan regions based on average soil parameters. The clustering analysis shows that Battalgazi and Doganshehir have similar soil characteristics, while Akcadag and Hekimhan are distinctly different from the other regions. The length of the clustering lines expresses the difference between the regions. Battalgazi and Doganshehir cluster at a short distance, while Akcadag and Hekimhan cluster at a longer distance. This suggests that Battalgazi and Doganshehir have more homogeneous soil structures, while Akcadag and Hekimhan have different chemical and physical properties (Zornoza et al., 2016).

The heat map shows the changes in soil parameters on a color scale. Yellow colors indicate high levels of a particular parameter, while blue-violet shades indicate lower levels. This distribution helps to determine regional soil management and fertilization strategies by revealing which regions are rich or poor in which nutrients. Battalgazi and Doğanşehir have high values for OM, N and Soluble C, indicating more fertile soils. However, K levels were lower in these regions compared to Akçadağ and Hekimhan. In terms of micronutrients,Cu and Zn levels were moderate. These findings indicate that Battalgazi and Doğanşehir are in good condition in terms of nutrient balance and have suitable soil properties for apricot cultivation (Yanardağ, 2023).

Akçadağ region has low values in terms of OM, soluble C, Fe and general nutrient content. This indicates that the soil is poor in organic matter and needs to be supplemented with fertilization strategies. pH and $CaCO_3$ levels are lower than in other regions, indicating that alkaline conditions are less dominant. However, Zn and Cu levels were also low, indicating that micronutrient supplementation is necessary in this region. It is recommended to optimize organic matter applications, green manuring and micronutrient fertilization to improve soil fertility (Lal, 2015).

The Hekimhan region has the highest $CaCO_3$ (lime) content. High pH levels can reduce plant uptake, especially of micronutrients. High lime content can limit the availability of elements (such as P and Fe) to plants, leading to

nutrient deficiencies (Marschner, 2012). Hekimhan is also the region with the highest Na levels. Excess Na can disrupt the soil structure, reduce the water holding capacity and cause salinity problems. This may adversely affect water uptake and root development of plants. Copper and Zn levels as micronutrients were found to be high, and chelated fertilization or foliar fertilization is recommended to increase plant uptake of nutrients in this region (Rengasamy, 2010).

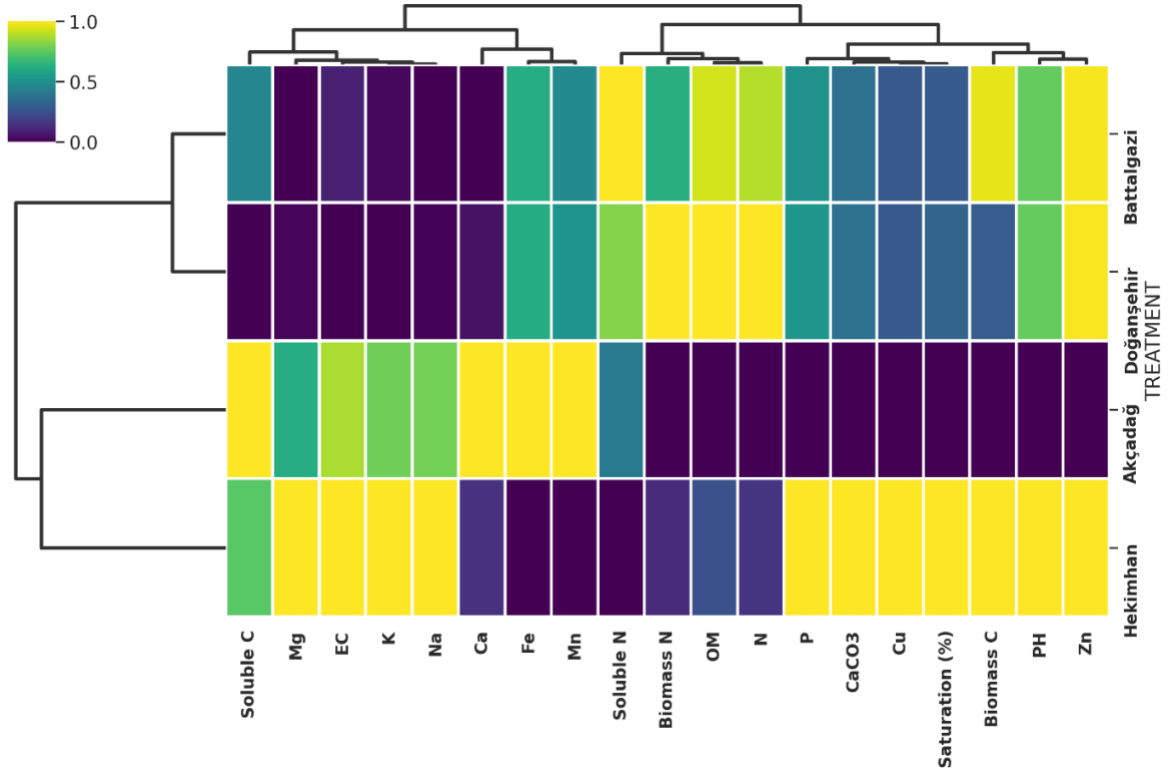


Figure 5. Hierarchical Clustering Heatmap between Treatments and Soil Properties

These findings indicate that there are significant differences in the nutrient content and chemical composition of soils in different regions of Malatya. Regional soil management and fertilization strategies should be optimized for apricot cultivation. Battalgazi and Dogansehir are rich in organic matter and nitrogen, and current fertilization strategies may be largely sufficient. Akçadağ should be supported due to micronutrient and organic matter deficiencies. Hekimhan needs special fertilization techniques to increase nutrient uptake due to high lime and salinity levels.

Soil management strategies are critical to increase apricot yields by maintaining plant nutrient balance. Especially water management and drainage practices are of great importance in Hekimhan where Na levels are high. In Akçadağ, increasing organic matter levels can be achieved through humus or compost-based fertilization. The most suitable regions for apricot cultivation are Battalgazi and Doğanşehir, which are rich in organic matter and have balanced nutrient content (Ercisli and Güleryüz, 2001).

In this study, soil similarities and differences between the regions were visualized using the hierarchical clustering method (Fig. 1). While Akçadağ and Hekimhan are in similar clusters in terms of soil characteristics, Battalgazi and Doğanşehir are located in different groups. Previous studies show that hierarchical clustering analysis is an important tool for agricultural management (Jiang et al., 2018). Hierarchical clustering methods, especially integrated with remote sensing and machine learning, provide important data for sustainable agriculture by mapping yields in large agricultural areas (Li et al., 2022).

The results show that agricultural management and fertilization programs need to be customized regionally. Different strategies should be applied for each region. For example, sulfur and acidic fertilizers can be used in high pH areas such as Hekimhan and Akçadağ, while organic matter increasing practices should be applied in Battalgazi and Doğanşehir. In areas with salinity and sodium excess (e.g. Doğanşehir), appropriate drainage systems should be established and salt tolerant plants should be grown. This study reveals the soil characteristics of different regions in Malatya and shows the necessity of optimized agricultural practices in apricot cultivation according to regional differences. In order to increase soil fertility and ensure sustainable apricot production, balanced management of micro and macro nutrients is of great importance.

Triangle Correlation Heatmap

This triangular correlation heat map was created to examine the relationships between different soil parameters. This analysis, visualized using the Viridis color palette, allows for easier interpretation of the correlations between parameters (Figure 6). Yellow tones indicate strong positive correlations, while blue and purple tones indicate negative correlations. A positive correlation indicates that two variables increase or decrease together, while a negative correlation indicates that one variable increases while the other decreases.

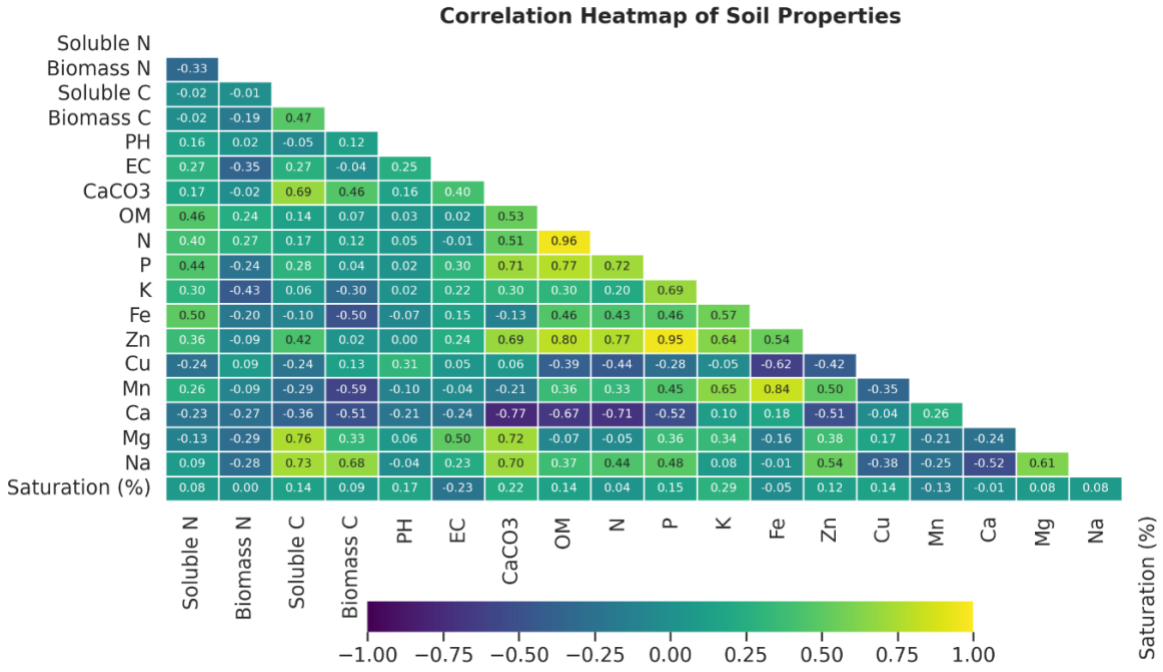


Figure 6. Correlation coefficient analysis of soil parameters in Alfisol, Entisol, and Mollisol soils.

One of the most remarkable findings in terms of soil chemistry is the strong positive correlation between OM and N (r = 0.51). This suggests that increasing the level of organic matter in soil can increase nitrogen levels. Soil organic matter is a critical part of the nitrogen cycle and helps microorganisms mineralize nitrogen into forms that plants can use (Lal, 2015). Especially in apricot cultivation in Malatya, the effectiveness of organic matter management is of great importance for soil fertility.

Another important finding was the negative correlation between pH and micronutrients. In particular, pH was negatively correlated with Zn (r = -0.72), indicating that zinc intake decreases as pH increases. This supports the literature findings that high pH levels can lead to micronutrient deficiencies (Marschner, 2012). Application of chelated micronutrient fertilizers or foliar fertilization methods are recommended to address such micronutrient deficiencies in calcareous soils.

The map also reveals a positive correlation (r = 0.98) between $CaCO_3$ and P. In soils with high lime content, the solubility of phosphorus decreases and its uptake by plants becomes difficult (Shen et al., 2011). In areas with high lime content such as Malatya, acidic forms of phosphorus fertilizers or application close to the root zone is recommended. This may prevent the binding of phosphorus and allow easier uptake by plants.

In terms of soil salinity, there is a strong positive correlation (r = 0.48) between EC and Na. This is in agreement with the literature that increasing Na level increases salinity (Rengasamy, 2010). Salinity can limit plant growth, especially by making water uptake difficult, and can cause serious yield losses in apricot cultivation. To reduce this problem, gypsum applications, proper drainage systems and the use of salinity-resistant rootstocks are recommended.

The positive correlation between K and EC (r = 0.43) indicates that electrical conductivity increases with increasing potassium in soil. This suggests that potassium plays an important role in ion balance and may be associated with salinity (Wang et al., 2013). Considering that potassium deficiency can reduce fruit quality in apricot cultivation, fertilization strategies should be optimized taking this factor into account.

In conclusion, this correlation analysis reveals the relationships between soil chemistry and plant nutrients in Malatya, contributing to the identification of appropriate management strategies for apricot cultivation. Issues such as the effect of pH on micronutrient uptake, the link between organic matter and nitrogen cycling, and salinity management should be considered in regional agricultural strategies. These findings can help to develop sustainable agricultural practices and optimize regional fertilization programs.

Many studies on soil chemistry show that the positive correlation between pH and $CaCO_3$ has critical effects on soil nutrient balance (Lindsay and Norvell, 1978). High pH can cause deficiencies of minerals such as Fe, Zn and Mn, especially by reducing the solubility of micronutrients (Alloway, 2008). This is confirmed by the negative correlation between Fe and pH in our analysis.

The strong relationship between organic matter and nitrogen indicates that soil organic matter content is a critical factor for agricultural productivity (Paul et al, 2013). Increasing organic matter increases soil nitrogen content by promoting nitrogen mineralization and improves nutrient uptake by plants (Jenkinson and Ladd, 1981; Büyükkılıç Yanardağ et al., 2020).

The positive correlation between Na and EC shows how increasing Na ions in saline soils degrades soil structure (Munns and Tester, 2008). High sodium levels can disrupt the structural integrity of the soil, reducing its water-holding capacity and inhibiting plant root growth. Soils with high $CaCO_3$ can be deficient in essential macronutrients such as potassium (Talaab et al., 2019). Therefore, potassium fertilization programs need to be carefully planned in areas with high lime content.

The Principal Component Analysis (PCA)

The results from Principal Component Analysis (PCA) biplot analysis indicate that differences in soil properties between regions can directly affect plant growth. High $CaCO_3$ and pH levels can limit the uptake of micronutrients, especially Fe and Zn, and therefore, micronutrient supplementation is recommended in areas with high lime content such as Hekimhan (Yadav et al.)

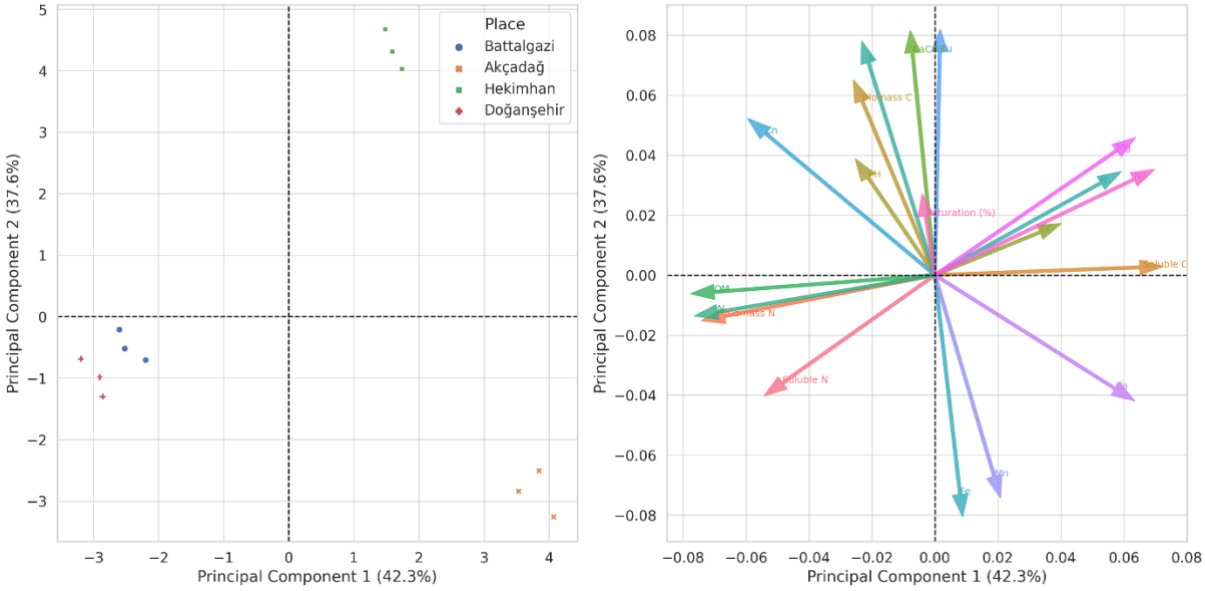


Figure 7. Principal Component Analysis (PCA) between Treatments and Soil Properties

The PCA biplot shown in the right panel shows the orientation of soil properties on the PCA axes and their influence on the variance. Each arrow represents a soil parameter and the length of the arrows indicates how much that parameter contributes to the PCA components.

By reducing multidimensional data into fewer components, the PCA method helps us understand the underlying patterns among soil properties and differences between regions (Jolliffe and Cadima, 2016). The graph shown in the left panel reveals the position of Battalgazi, Akçadağ, Hekimhan and Doğanşehir regions according to soil components. The X-axis (PC1) explains 42.3% of the total variance and the Y-axis (PC2) explains 37.6%, meaning that these two components represent about 80% of the total variability in the data. This is a very high explanatory rate in PCA analysis and shows that the analyzed parameters offer a good discrimination (Abdi and Williams, 2010).

This PCA analysis provides critical information in understanding soil chemical differences between regions and the effect of nutrients in variance (Figure 7). In terms of apricot cultivation, Battalgazi and Dogansehir have similar soil composition and therefore similar fertilization strategies can be applied. Hekimhan has a markedly different soil structure, especially high in lime, alkaline pH and may be low in some micronutrients. Chelated micronutrient fertilizers and foliar fertilization strategies are recommended in this region (Singh et al., 2013). In areas where EC and Soluble C are high, organic matter applications should be made to support the carbon cycle (Murphy, 2015). In areas with high Ca, Mg and Na, it is recommended to manage soil salinity and remove excess ions with appropriate drainage systems (Rhoades, 1974). In areas with Fe and Mn deficiencies, pH management and micronutrient fertilization should be optimized (Zuo and Zhang, 2011).

When the regional distribution is analyzed, it is seen that Battalgazi and Doğanşehir regions are located close to each other and have similar soil properties. Akçadağ and Hekimhan are more distinctly differentiated on different components, indicating that the soil chemical compositions are different in these regions. Especially Hekimhan region is located further away from other regions, indicating that it has different soil chemical structure. This differentiation suggests that factors such as $CaCO_3$, EC and pH may affect soil composition in this region (Shen et al., 2011).

The prominent findings of PCA Analysis (Figure 7) ; $CaCO_3$, pH and Cu show similar trends, indicating that these variables are directly related to high lime content. As lime content increases, pH increases and micronutrient uptake changes. This may lead to micronutrient deficiencies such as Zn, Fe and Mn, especially in alkaline soils with high pH (Marschner, 2012).

Electrical conductivity and Soluble C are located in the same direction, indicating that there may be a link between soil organic matter content and salinity (Figure 7). Under high salinity conditions, carbon cycling and microbial activity are known to be altered (Lal, 2015). Sodium, Mg and Ca move in the same direction, indicating that these elements are related to the ion balance and water holding capacity in the soil. Such high ion content can adversely affect soil structure and cause yield reduction in apricot cultivation (Rengasamy, 2010). Soluble N and OM are located in opposite directions, indicating that soil nitrogen cycling may vary depending on organic matter mineralization. In soils with high levels of organic matter, biological processes are known to be more active, which in turn affects nitrogen levels (Lal, 2015).

It is seen that the differences in soil properties have similar and different aspects when compared with previous studies. Sakin and Yanardağ (2023) found that Zn and Fe deficiency was common in soils with high $CaCO_3$ content, but Fe deficiency was more pronounced in this study. This may be due to regional climate differences and soil management practices. In terms of salinity level (EC and Na), Doğanşehir seems to be risky. In the study by Zornoza et al. (2016), high Na content was reported to restrict plant growth and reduce soil microbial activity. In this study, Na levels were found to be particularly high in Doğanşehir and it was recommended to improve drainage systems. These results reveal the importance of salinity management, especially in arid and semi-arid regions.

It was observed that Hekimhan region was richer in terms of organic matter content and carbon cycle compared to other regions. Sakin and Yanardağ (2023) evaluated the effect of organic matter content on soil fertility and stated that high organic matter levels increase soil biological activity and contribute positively to nitrogen cycling. Similarly, in this study, it was found that biological nitrogen content was higher in Hekimhan where high organic matter levels were found. On the other hand, low organic matter content in Akçadağ region may decrease soil fertility and organic fertilization methods should be encouraged.

In terms of macro and micronutrients, due to Zn and Cu deficiencies in Battalgazi and Doğanşehir regions, micronutrient support should be provided by foliar fertilization or soil conditioners. Zornoza et al. (2016) reported that Zn deficiency caused chlorosis symptoms in plants and fertilization programs should be planned accordingly. This result coincides with our study and appropriate fertilization methods should be applied to eliminate micronutrient deficiencies, especially in Battalgazi and Doğanşehir regions.

CONCLUSION

This study evaluated the dynamics of carbon (C) and nitrogen (N) in apricot-cultivated soils across four distinct regions in Malatya, using statistical techniques including correlation analysis, Principal Component Analysis (PCA), and hierarchical clustering. The results demonstrated that regional differences significantly influence soil chemical composition, and that these variations are closely related to nutrient dynamics.

A strong positive correlation was found between organic matter (OM) and total nitrogen (N), indicating that OM content plays a central role in the nitrogen mineralization process. Additionally, the observed relationships between soluble carbon, biomass carbon, and nitrogen parameters highlight the importance of microbial activity and carbon cycling in nitrogen availability.

Regional comparisons revealed that Battalgazi and Doğanşehir exhibit similar soil characteristics, while Akçadağ and particularly Hekimhan differ due to elevated pH and lime content. These findings underscore the need for region-specific soil management approaches in apricot cultivation.

Based on the outcomes of the study, several tailored recommendations are proposed to enhance soil quality and nutrient availability in apricot cultivation. In areas characterized by low organic matter content, it is recommended to increase soil fertility through the use of organic fertilizers, green manuring, and composting practices. For regions with high soil pH and elevated lime content, the application of chelated fertilizers and foliar nutrient sprays can significantly improve micronutrient uptake by the plants. In salt-affected areas, implementing effective drainage systems and adopting appropriate salinity management practices are essential to mitigate adverse effects on plant growth. Furthermore, the development and implementation of region-specific fertilization strategies are crucial for ensuring a balanced and adequate supply of both macro and micronutrients, thereby promoting sustainable orchard management.Moreover, given the sensitivity of C and N dynamics to environmental conditions, integrating climate considerations into soil management strategies will be essential for future resilience.

In conclusion, adapting soil management practices to regional soil characteristics in Malatya will contribute to enhanced apricot yield and quality. Long-term soil monitoring and optimization of regional fertilization plans are critical components for achieving sustainability in apricot production.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Optimization and sensory analysis of a beetroot, apple, and oat-based beverage using response surface methodology

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Abstract

This study aimed to optimize a functional beverage formulation comprising beetroot juice, apple juice, and oats, with a focus on enhancing taste, consistency, and overall acceptability. Optimization was carried out using Response Surface Methodology (RSM) to identify the ideal proportions of ingredients that would maximize sensory quality. The developed regression models demonstrated high predictive accuracy, with R^2 values exceeding 0.94, indicating a strong correlation between the experimental data and model predictions. The optimized formulation 10.07% beetroot juice, 59.99% apple juice, and 8.86% oats yielded the highest sensory scores: 8.40 for taste, 7.19 for consistency, and 8.12 for overall acceptability. Lack-of-fit tests confirmed the adequacy and reliability of the models. Results revealed that apple juice effectively moderated the earthy flavor of beetroot juice, while oats improved texture when used at moderate levels but detracted from sensory appeal at higher concentrations. Overall, the study highlights the effectiveness of RSM in optimizing multi-ingredient beverage formulations based on sensory criteria.

Keywords: Fruit-vegetable beverage, Beetroot juice, Apple juice, Oats, Response surface methodology (RSM), Sensory analysis, Optimization

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INTRODUCTION

In recent years, there has been a growing consumer demand for healthy and natural beverages, which has, in turn, stimulated scientific research into the development of formulations incorporating plant-based ingredients (Tireki et al., 2021). These beverages are expected to provide not only nutritional benefits but also high levels of sensory acceptability (Gupta et al., 2023). Among the various plant-derived components, beetroot juice (*Beta vulgaris*) has garnered significant attention due to its rich content of betalain pigments, dietary nitrates, and antioxidant compounds, all of which have been linked to cardiovascular health and improved exercise performance (Zamani et al., 2020). Nevertheless, at elevated concentrations, beetroot juice can contribute a distinctly earthy and slightly bitter flavor, potentially diminishing overall consumer acceptance (Kardas et al., 2024).

To mitigate the sensory limitations associated with beetroot juice, the addition of sweet and acidic components such as apple juice (*Malus domestica*) is a commonly employed strategy to enhance the overall flavor profile of beetroot-based beverages (Petrović et al., 2021). The natural sugars and organic acids present in apple juice contribute to a more balanced taste and improved sensory appeal (Jakubczyk et al., 2024). Furthermore, oats (*Avena sativa*), known for their high soluble dietary fiber content, particularly β-glucan, offer functional benefits by improving texture and increasing viscosity (Kasim et al., 2022). While β-glucan aids in stabilizing the beverage matrix, its use at elevated concentrations may result in an undesirable sticky mouthfeel, thereby reducing consumer acceptability (Chakraborty et al., 2019).

In beverage formulation research, statistical optimization techniques such as Response Surface Methodology (RSM) are widely utilized to evaluate interactions among ingredients and to identify the most effective combinations for achieving desired product attributes (Wanyonyi et al., 2025). RSM facilitates the modeling of

complex, multivariate relationships and has been extensively applied in the development and optimization of food products (Kidane, 2021).

Building upon this approach, the present study aims to determine the optimal formulation of a functional beverage comprising beetroot juice, apple juice, and oats, focusing specifically on the sensory attributes of taste, consistency, and overall acceptability. The objective is to develop a formulation that maximizes consumer preference while maintaining nutritional integrity. Additionally, this study seeks to enrich the current body of knowledge by evaluating the sensory effects of combining these specific plant-based ingredients. During the optimization process, the individual and interactive effects of each component were assessed using statistical modeling, and the results were contextualized with findings from previous research. The outcomes are expected to provide valuable insights for the development of health-oriented beverages with enhanced sensory appeal.

MATERIALS AND METHODS

Materials

The raw materials beetroot, apple, and oat were procured from a local market in Şanlıurfa, Turkey, to ensure freshness and consistent quality for the beverage formulation..

Experimental design

The beverages formulated with beetroot, apple, and oat were blended in varying proportions and optimized using Response Surface Methodology (RSM), following the procedure outlined by Nayi and Kumar (2021). The proportions of beetroot juice, apple juice, and oats were treated as equidistant factors in accordance with the experimental design principles described by Montgomery (2017). Specifically, beetroot juice (10–30 mL), apple juice (40–60 mL), and oats (6–9 g) were designated as independent variables. The selection of these factor ranges was informed by preliminary trials, in which sensory attributes namely taste, consistency, and overall acceptability were evaluated and defined as dependent response variables.

Preparation of the Beetroot-Based Beverage

Freshly sourced fruits and vegetables were thoroughly washed under running tap water to eliminate surface contaminants. The beetroot was peeled and cut into small pieces prior to juice extraction using a centrifugal juicer (Philips HR1832/00). The resulting juice was subsequently filtered through muslin cloth to remove particulate matter and obtain a clear extract. Apple juice was prepared following the same procedure, utilizing the same juicer and filtration method to ensure consistency and clarity.

Subsequently, the two freshly extracted juices were combined with finely ground oats in varying proportions, as outlined in Table 1. The mixtures were homogenized using a high-speed blender (Tefal Powelix) to ensure uniform dispersion of the ingredients. Sensory evaluation was performed immediately after blending to assess the initial sensory properties of the formulations. The freshly prepared beverages were then transferred into pre-sterilized glass tubes, sealed with crown caps, and stored under frozen conditions for subsequent physicochemical and sensory analyses.

Sensory Evaluation

To evaluate the sensory attributes of the beverages, a composite scoring method and a 9-point hedonic scale were employed, following the protocols described by Dambalkar et al. (2015) and Ranganna (2004). The sensory panel comprised 15 trained individuals (8 females and 7 males), aged between 20 and 25 years, all of whom were associate degree students enrolled in the Food Technology Program. Panelists were selected based on their availability, interest in sensory analysis, and prior experience in similar evaluations. The demographic homogeneity of the panel was intentional, aimed at minimizing perceptual variability among participants. Prior to the evaluation, panelists were briefed on the study objectives and procedures, and verbal informed consent was obtained. All panelists received standardized training to evaluate the samples based on taste, consistency, and overall acceptability.

Statistical Analysis

The experimental design, data modeling, and optimization processes were conducted using Response Surface Methodology (RSM) with Design-Expert software (version 13.0; Stat-Ease Inc., Minneapolis, MN, USA). A reduced quadratic model was employed to evaluate the effects of beetroot juice, apple juice, and oat content on the sensory responses of taste, consistency, and overall acceptability. Analysis of variance (ANOVA) was used to assess the statistical significance of the models and the individual effects of each factor. Model adequacy was evaluated using lack-of-fit tests, as well as the coefficients of determination (R^2), adjusted R^2, and predicted R^2. All experiments were performed in triplicate ($n = 3$), and differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Sensory Properties of Beetroot-Based Beverages

The experimental design based on Response Surface Methodology (RSM) was employed to optimize the formulation of the beetroot-based beverages, and the results were systematically recorded. In accordance with the

design matrix, the mean values of triplicate measurements for each response variable, along with the corresponding sensory responses of the blended beverages, are presented in Table 1.

Table 1. Variables and Responses For Optimization of Beetroot Based Beverages

Variables				Responses		
Run	**Beetroot juice(mL)**	**Apple juice(mL)**	**Oat (g)**	**Taste**	**Consistency**	**Overall acceptability**
1	20	50	6	6.54	6.85	6.72
2	20	60	3	7.67	5.25	5.98
3	10	40	6	7.47	6.92	6.16
4	30	40	6	6.42	6.32	6.41
5	30	50	3	6.33	5.51	5.43
6	20	50	6	6.54	6.85	6.72
7	20	50	6	6,54	6.85	6.72
8	20	40	3	5.45	5.14	5.57
9	30	50	9	6.65	5.78	6.21
10	10	50	9	8,35	7.11	7.05
11	20	50	6	6.54	6.85	6.72
12	20	50	6	6.54	6.85	6.72
13	20	60	9	7.11	7.02	7.16
14	20	40	9	7.32	7.14	6.14
15	10	60	6	8.24	7.08	8.12
16	10	50	3	7.49	5.05	5.27
17	30	60	6	7.45	6.14	6.21

The sensory attributes of the blended beverages taste, consistency, and overall acceptability were observed to vary within the ranges of 5.45 to 8.35, 5.05 to 7.14, and 5.27 to 8.12, respectively (Table 1). Response surface plots were generated by varying one ingredient across its specified range while maintaining the other variables at constant levels. A steep slope or pronounced curvature in the response surface indicates a high sensitivity of the response to that particular ingredient, whereas a relatively flat slope suggests minimal sensitivity to changes in that component.

To visualize the combined effects of the three independent variables on each response, three-dimensional (3D) surface plots were generated based on the fitted models. The experimental data were analyzed using linear, quadratic, and interaction models, and statistical evaluations were conducted for each sensory attribute (Tables 2–4).

Table 2. ANOVA results for the Taste model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model (Reduced Quadratic)	8.69	6	1.45	57.14	<0.0001
A - Beetroot Juice	2.76	1	2.76	108.89	<0.0001
B - Apple Juice	1.81	1	1.81	71.55	<0.0001
C - Oat	4.65	1	4.65	142.42	<0.0001
AB	1.17	1	1.17	28.6	0.0003
AC	0.25	1	0.25	6.13	0.0328
A^2	0.27	1	0.27	8.34	0.0148
Residual	0.36	11	0.0327		
Lack of Fit	0.36	7	0.0513		
Pure Error	0.0	4	0.0		
R^2	0,9547				
Adjusted R^2	0,9043				
Predicted R^2	0,7214				

The analysis of variance (ANOVA) revealed that the reduced quadratic model was statistically significant for the taste response ($p < 0.0001$). The model accounted for approximately 95.47% of the total variation in the data ($R^2 = 0.9547$), and the high adjusted R^2 value (0.9043) further confirmed the model's robustness and goodness of fit. Additionally, the lack-of-fit test was non-significant ($p > 0.05$), indicating that the model adequately described the experimental data. Among the variables, significant linear and interaction effects particularly those involving oat and beetroot juice demonstrated their strong influence on taste. The predicted R^2 value of 0.7214 also supported the model's reliability in predicting future observations.

Table 3. ANOVA results for the Consistency model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model (Reduced Quadratic)	8.8	5	1.76	53.92	<0.0001
A - Beetroot Juice	0.726	1	0.726	22.23	0.0006
C - Oat	0.775	1	0.775	30.56	0.0003
AC	0.801	1	0.801	24.53	0.0004
A^2	1.47	1	1.47	58.04	<0.0001
C^2	2.26	1	2.26	69.17	<0.0001
Residual	0.2536	10	0.0254		
Lack of Fit	0.2536	6	0.0423		
Pure Error	0.0	4	0.0		
R^2	0,9473				
Adjusted R^2	0,9157				
Predicted R^2	0,7415				

The ANOVA results indicated that the reduced quadratic model for consistency was statistically significant ($p < 0.0001$), with an R^2 value of 0.9473, suggesting that approximately 94.73% of the variation in consistency scores was explained by the model. The adjusted R^2 value of 0.9157 further supported the model's robustness by accounting for the number of predictors. The predicted R^2 value of 0.7415 confirmed the model's capacity to generate reliable predictions. Moreover, the non-significant lack-of-fit ($p > 0.05$) indicated that there was no considerable discrepancy between the model and the observed data. Among the independent variables, oat content (C) and its quadratic term (C^2) exhibited the strongest influence on beverage consistency. Additionally, the interaction between beetroot juice and oat (AC) was also found to be statistically significant.

Table 4. ANOVA results for the Overall Acceptability model.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model (Reduced Quadratic)	7.33	6	1.22	29.95	<0.0001
A - Beetroot Juice	0.6844	1	0.6844	16.79	0.0022
B - Apple Juice	1.27	1	1.27	31.19	0.0002
C - Oat	2.32	1	2.32	56.94	<0.0001
BC	1.48	1	1.48	58.21	<0.0001
B^2	0.3144	1	0.3144	12.4	0.0055
C^2	1.63	1	1.63	40.05	<0.0001
Residual	0.4078	10	0.0408		
Lack of Fit	0.4078	6	0.068		
Pure Error	0.0	4	0.0		
R^2	0,9608				
Adjusted R^2	0,9430				
Predicted R^2	0,7810				

The ANOVA results indicate that the reduced quadratic model for overall acceptability is statistically significant ($p < 0.0001$), explaining 96.08% of the variability in the data ($R^2 = 0.9608$). The adjusted R^2 (0.9430) confirms that the model fits well even after adjusting for the number of predictors, while the predicted R^2 (0.7810) demonstrates satisfactory predictive performance. The non-significant lack-of-fit test ($p > 0.05$) indicates that the model sufficiently represents the experimental data. Among the variables, oat (C) and its quadratic effect (C^2), as well as the interaction between apple juice and oat (BC), showed highly significant influence on overall acceptability. These results suggest that a balance between sweetness (apple juice) and texture (oat) is essential for optimizing sensory appeal.

Effect of Beverage Formulation on Taste

The taste scores of the beverage formulations ranged from 5.45 to 8.35. The three-dimensional (3D) surface plots presented in Figure 1 illustrate the effects of beetroot juice, apple juice, and oat content on sensory perception. These plots were generated by systematically varying one independent variable while holding the others constant, thereby allowing visualization of the individual influence of each component on taste.

Apple juice played a pivotal role in balancing the taste profile of the beverage by counteracting the intense aromatic notes of beetroot juice, thereby enhancing overall consumer acceptability. However, when used in excessive amounts, apple juice imparted an overly sweet flavor, which negatively affected sensory appeal. Although oats did not directly influence the taste, they modulated taste perception through their impact on the beverage's texture. At moderate concentrations, oats contributed to a smooth and creamy mouthfeel, whereas higher levels resulted in an undesirable sticky texture, ultimately diminishing sensory acceptance.

The response surface plots indicate that a well-balanced formulation, achieved through the optimal combination of beetroot juice, apple juice, and oats, can significantly improve taste quality and maximize consumer acceptability. These findings underscore the critical role of ingredient optimization in the development of functional beverages that meet consumer sensory expectations.

These findings are largely consistent with previous studies reported in the literature. El-Dakak and Youssef (2017) observed that while beetroot juice imparts a distinctive sensory profile to beverages, its use at high concentrations intensifies earthy flavor notes, thereby diminishing consumer preference. Similarly, Tobolková et al. (2020) demonstrated that apple juice plays a crucial role in balancing the flavor profile, effectively softening the dominant taste attributes of beetroot juice when incorporated at optimal levels.

Additionally, studies by Chakraborty et al. (2019) indicated that oats can enhance the sensory acceptability of beverages; however, excessive use may result in an undesirable sticky mouthfeel, negatively affecting consumer perception. Consistent with these findings, the present study confirms that a well-balanced combination of beetroot juice and apple juice contributes positively to the beverage's taste profile, while oats enhance consistency but may lead to adverse sensory responses when used beyond an optimal threshold.

The regression equation representing the relationship between the ingredient ratios and taste scores is given below:

$$Y_{Taste}=6.57-0.5875A+0.4763B-0.3219C-0.6071BC+0.5904A^2+0.2729B^2$$

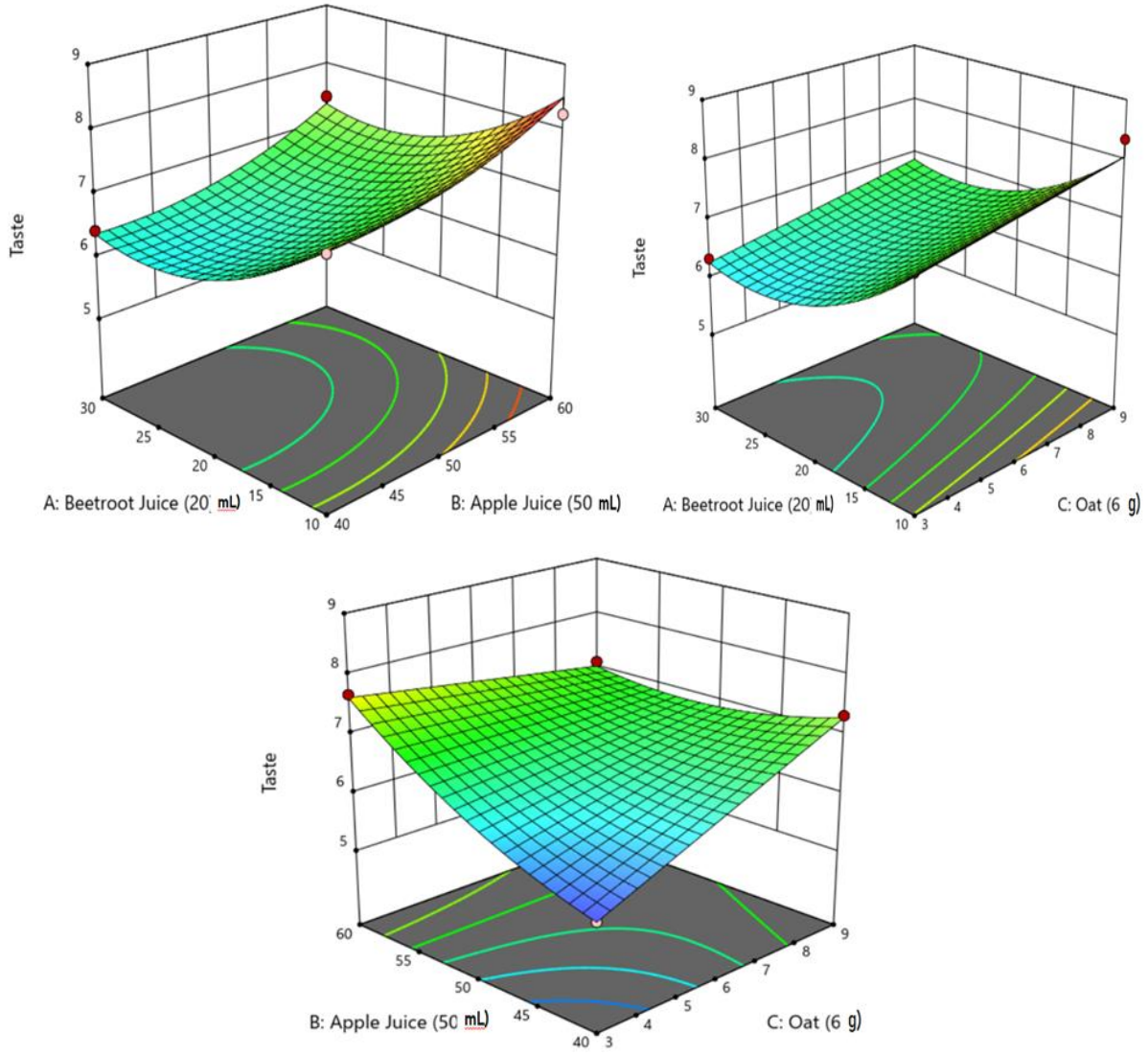


Figure 1. 3D Surface Plots Illustrating the Effect of Beverage Formulation on Taste

Effect of Beverage Formulation on Consistency

The consistency scores of the formulated beverages ranged from 5.05 to 7.14. Response surface analysis (Figure 2) revealed that beetroot juice alone had a minimal impact on viscosity. However, in combination with other components particularly oats significant changes in texture were observed. While the interaction between

beetroot juice and apple juice exhibited limited influence on consistency, oat content emerged as the primary factor contributing to increased viscosity, especially when combined with either beetroot or apple juice.

Oats, which are rich in soluble dietary fiber particularly β-glucan significantly contributed to increased beverage thickness. While moderate levels of oats imparted a desirable creamy texture, higher concentrations reduced fluidity and produced a sticky mouthfeel, adversely affecting sensory acceptability. Therefore, precise control of oat concentration is essential to achieve optimal consistency without compromising consumer perception.

These findings are in agreement with previous studies. El-Dakak and Youssef (2017) reported that while apple juice had minimal impact on texture, it played a key role in balancing flavor. Chakraborty et al. (2019) emphasized the contribution of oats to increased viscosity and texture stabilization, noting, however, that excessive amounts compromised drinkability. Similarly, Kumar and Kumar (2015) demonstrated that although the rheological behavior of beetroot juice is influenced by its soluble solids content, its contribution to viscosity is relatively limited compared to that of oats. In line with these reports, the present study confirms that oat concentration is the primary determinant of beverage consistency.

Therefore, to achieve optimal beverage consistency, the oat content must be carefully regulated to preserve the sensory balance between beetroot and apple juices while avoiding excessive viscosity that could diminish consumer acceptability. The regression equation describing the relationship between ingredient proportions and consistency values is presented below:

$$Y_{Consistency}=6.70-0.3012A+0.7625C-0.4475AC-0.2539A^2-0.7314C^2$$

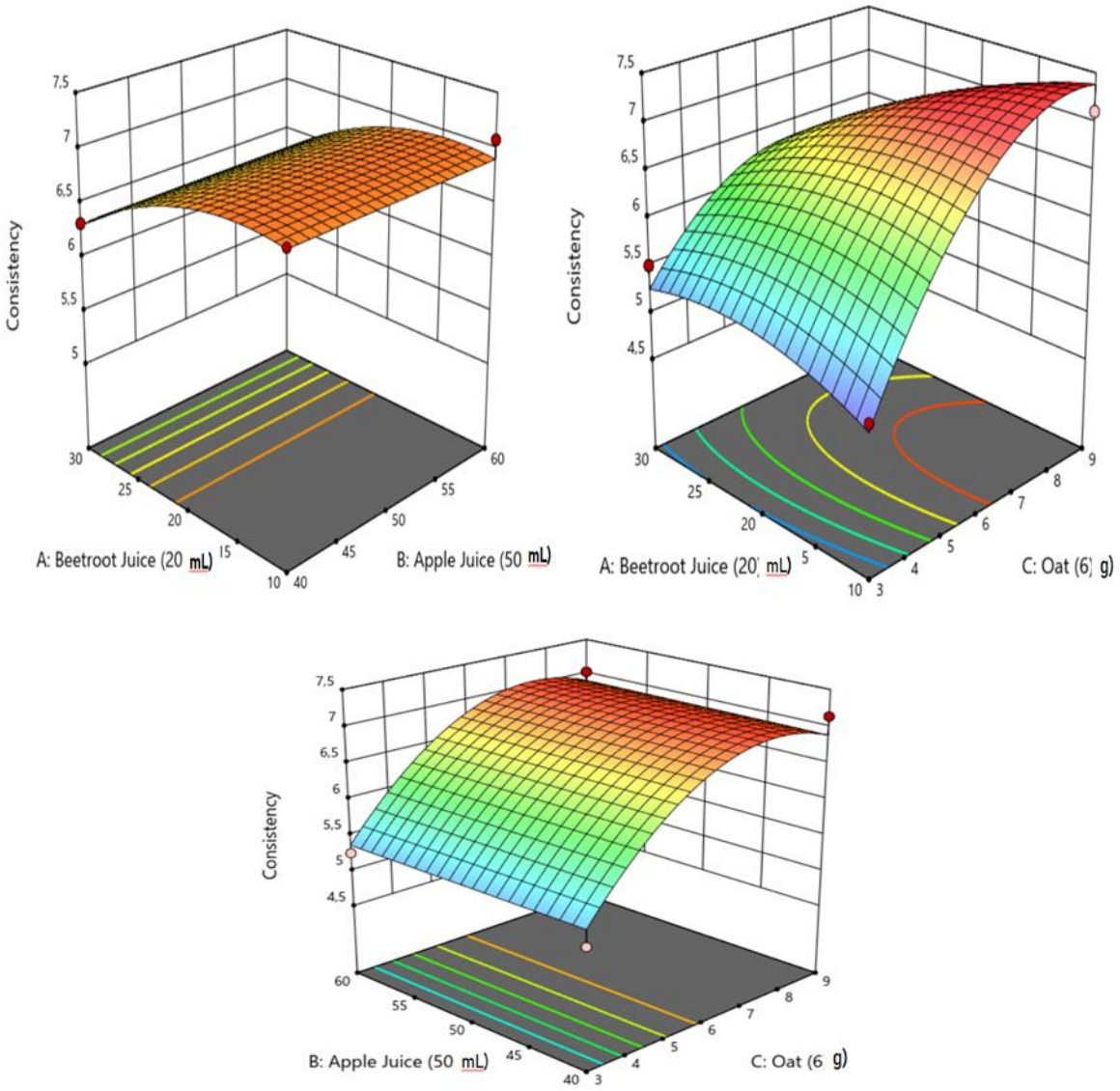


Figure 2. 3D Surface Plots Illustrating the Effect of Beverage Formulation on Consistency
Effect of Beverage Formulation on Overall Acceptability

The overall acceptability scores of the formulations ranged from 5.27 to 8.12. Response surface plots (Figure 3) indicated that increasing the concentration of apple juice had a positive effect on overall preference, likely due to its natural sweetness and acidity, which helped to mask the earthy flavor notes associated with beetroot juice.

Moderate levels of oats enhanced overall acceptability by improving the beverage's texture; however, higher concentrations led to a sticky mouthfeel, which negatively affected consumer satisfaction. Similarly, the combined effect of apple juice and oats contributed positively to overall acceptability only up to a certain threshold, beyond which excessive viscosity diminished sensory appeal.

These findings underscore the importance of achieving an optimal balance among ingredients to enhance sensory performance. Apple juice plays a key role in moderating the dominant flavor characteristics of beetroot juice, while oats contribute to mouthfeel and body. Determining the ideal proportions of these components is essential for developing a beverage with high consumer acceptability.

The results of this study are consistent with previous research. El-Dakak and Youssef (2017) reported that incorporating 30% apple juice enhanced acceptability in beetroot–carrot–apple blends. Bhavya et al. (2019) found that a 10% beetroot concentration yielded the most favorable results in tropical juice mixtures. Similarly, Michiu et al. (2024) observed the highest consumer acceptance in whey–beetroot beverages containing 20% beetroot juice. In alignment with these findings, the present study confirms that achieving a balanced formulation particularly between beetroot and apple juice and maintaining controlled oat content are critical for maximizing consumer approval in plant-based beverages. The regression equation representing the relationship between ingredient proportions and overall acceptability is provided below:

$$Y_{\text{Overall Acceptability}} = 6.72 - 0.2925A + 0.3988B + 0.5388C - 0.5400AB - 0.2500AC - 0.6210C^2$$

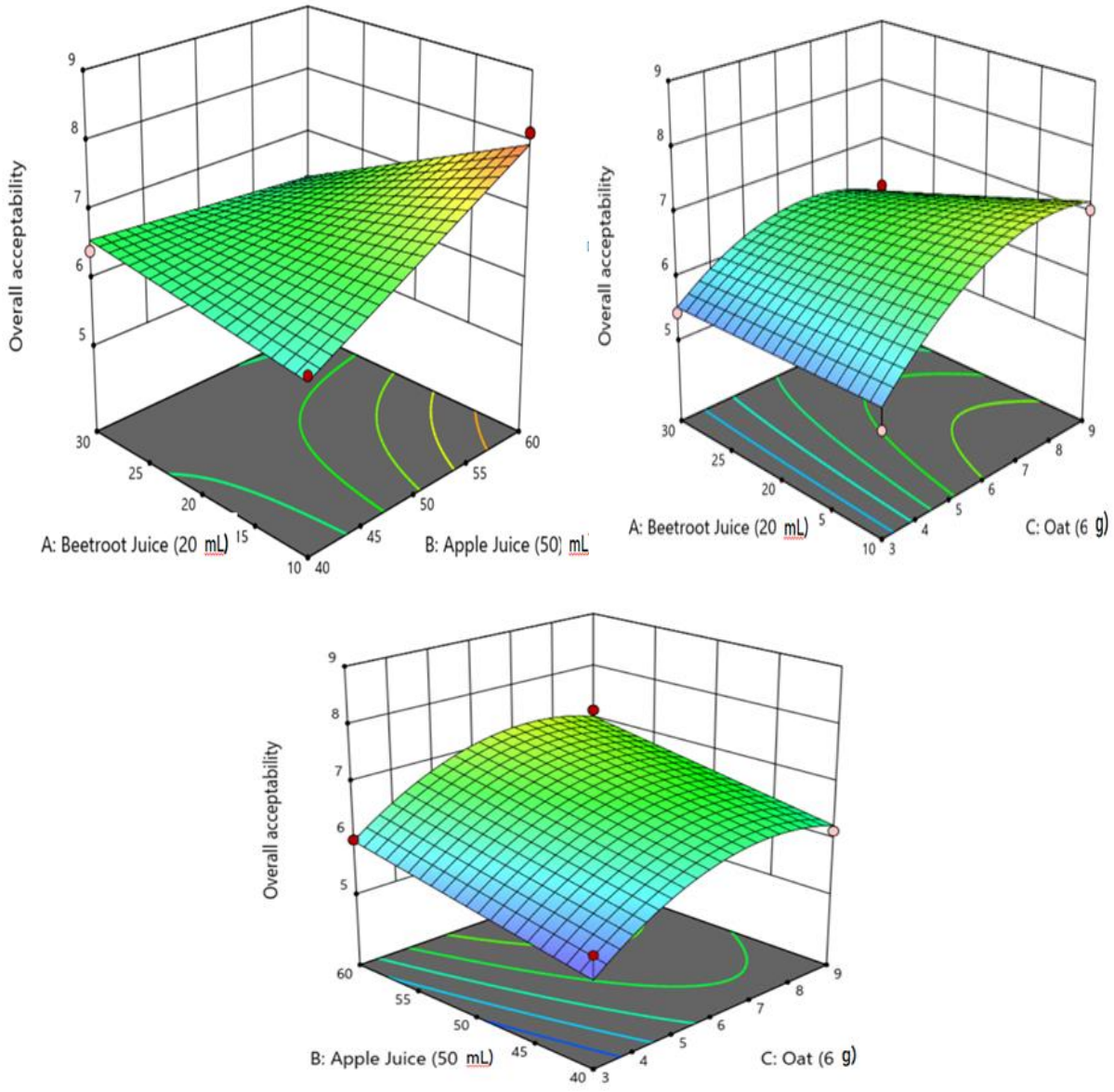


Figure 3. 3D Surface Plots Illustrating the Effect of Beverage Formulation on Overall Acceptability

Optimization of the Beetroot-Based Beverage

In this study, the effects of beetroot juice, apple juice, and oat content on taste, consistency, and overall acceptability were optimized using Response Surface Methodology (RSM). The F-values for all models were statistically significant at the 5% confidence level, indicating that the model terms were relevant and that the selected variables had a significant influence on the sensory and physicochemical properties of the beverage formulations.

To evaluate the goodness-of-fit of the models, the coefficients of determination (R^2) were examined. The R^2 values for taste, consistency, and overall acceptability were 0.9717, 0.9608, and 0.9473, respectively. These results indicate that the models account for more than 94% of the variability in the experimental data, thereby confirming their high predictive accuracy. Furthermore, the minimal discrepancy between the predicted and observed values reinforces the validity and reliability of the regression models.

The adequacy of the model was further confirmed by the lack-of-fit test, which yielded a non-significant F-value, indicating that the model provides a reliable fit within the studied range of factor levels. Based on these results, it was concluded that an optimal balance of beetroot juice, apple juice, and oat content can effectively maximize taste, consistency, and overall acceptability in the beverage formulation.

As a result of response surface optimization, the optimal ingredient ratios that maximized sensory acceptability were identified and are presented in Table 5. Furthermore, the industrial production potential of the optimized formulation was evaluated. These findings underscore the effectiveness of mixture design-based modeling as a robust approach for optimizing both sensory and physicochemical properties in beverage development.

Table 5. Coded and Actual Ranges of Independent Variables Used in the RSM Design

Constraints	**Goals**	**Optimization**	
		Lower limit	**Upper limit**
Factor			
A- beetroot Juice	in range	10	30
B- Apple Juice	in range	40	60
C-Oat	in range	3	9
Response			
Taste	maximize	5.45	8.35
Consistency	maximize	5.05	7.14
Overall Acceptability	maximize	5.27	8.12

For the simultaneous optimization of multiple responses, Design-Expert® software (version 10.0.6; Stat-Ease Inc., Minneapolis, MN, USA; http://www.statease.com) was employed using numerical optimization techniques. The variables were subjected to specific constraints based on predefined targets for each ingredient and response parameter, and the optimal formulation was subsequently determined.

During the optimization process, the objective was to maximize the sensory responses of taste, consistency, and overall acceptability by varying the proportions of beetroot juice, apple juice, and oats within the defined experimental limits. As shown in Table 6, the optimal beverage formulation was determined to consist of 10.07% beetroot juice, 59.99% apple juice, and 8.86% oats. Under these optimized conditions, the predicted sensory scores were 8.40 for taste, 7.19 for consistency, and 8.12 for overall acceptability.

Table 6. Optimum levels and predicted optimum values of responses.

Variable	Optimum value	Response	Predicted value
A- beetroot Juice	10.07	Taste	8.40
B- Apple Juice	59.99	Consistency	7.19
C-Oat	8.86	Overall Acceptability	8.12

The optimized formulation was validated through confirmation tests, thereby demonstrating the reliability and predictive accuracy of the model. These results indicate that the optimal combination of ingredients maximizes consumer acceptance based on both sensory and physicochemical parameters. The formulation recommended by the Design-Expert software serves as a promising foundation for the development of beverages with high sensory appeal and enhanced stability, offering valuable potential for application in the beverage industry.

CONCLUSION

In this study, a beverage formulated with beetroot juice, apple juice, and oats was optimized for taste, consistency, and overall acceptability using Response Surface Methodology (RSM). All regression models were found to be statistically significant, with R^2 values exceeding 94%, indicating strong predictive accuracy. The optimal formulation comprising 10.07% beetroot juice, 59.99% apple juice, and 8.86% oats achieved the highest sensory scores across all evaluated parameters.

The findings confirm that apple juice plays a crucial role in balancing the flavor profile, beetroot juice contributes a distinctive sensory character when used at appropriate levels, and oats primarily affect texture. While moderate amounts of oats enhance mouthfeel, excessive concentrations lead to increased viscosity, thereby reducing overall sensory appeal. The validity of the optimization model was supported by a non-significant lack-of-fit analysis, confirming its reliability. Overall, this study demonstrates that Response Surface Methodology (RSM) is an effective tool for developing sensory-optimized beverages and offers practical insights for formulating products aligned with consumer preferences. One limitation of the present study is that the optimized formulation was not re-prepared and subjected to independent sensory validation. Future research should address this gap by reproducing the optimized formulation and comparing predicted versus observed sensory scores to further confirm the model's accuracy.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Determination of the usability of jam factory fruit-vegetable wastes in silage making

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Abstract

Recently, many countries have focused on utilizing industrial food residues to reduce environmental pollution. The utilization of waste products as animal feed is important in terms of being a cheap feed source and preventing environmental pollution. This study was carried out with the aim of preventing the waste from turning into garbage and increasing the feed value of the straw by making silage with wheat straw of the pulp and wastes left from the fruits and vegetables (strawberry, mulberry and carrot) used by the jam factory located in the organized industrial zone of Bingöl province. The residues of strawberry (S), mulberry (M) and carrot (C) were mixed with wheat straw (10%, 20% and 30%) and urea (0.5%) in different proportions and silaged using plastic drums. After an average fermentation period of 100 days, the silages were opened. The pH, dry matter (DM), fleig score values, ADF (Acid Detergent Fiber), NDF (Neutral Detergent Fiber), crude protein (CP), crude ash (CA), Ca, Mg, P and K contents were determined. As a result of the study, it was determined that the ideal pH values were obtained from S10 and S20 and the ideal DM values were obtained from S20, C10, C20 and C30 treatments and all treatments except C10 and C20 treatments were in the I-excellent class, which is the best value in terms of fleig score. It was determined that the lowest ADF and highest crude protein ratios were obtained from M10, the lowest NDF and highest crude ash ratios were obtained from M10, M20 and M30, the highest Ca and P ratios were obtained from S10 and C10, and the highest Mg and K ratios were obtained from all mulberry group silages. In line with this information, it was concluded that all three plant production wastes to which straw was added can be used in silage production.

Keywords: Strawberries, Mulberry, Carrots, Waste, Straw, Silage

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INTRODUCTION

Recently, many countries around the world have focused on recycling industrial by-products to reduce environmental pollution (Mirzaei-Aghsaghali et al. 2011; Köksal et al., 2021). The use of agricultural by-products and food industry by-products with high water content as feedstuffs in animal nutrition after being dried or ensiled by various methods is also called alternative silage materials (FAO, 2013; Sargın and Denek, 2017).

Considering the industry, climate and vegetation of our country, there are many residues and by-products that can be utilized either as silage main material or as pulp material. Residues and waste of vegetables and fruits collected in fields and gardens, bark, stems, fruits and leaves of trees are products that can be used in silage production (İbrahimoğlu and Saruhan, 2019).

Fruit pulps obtained as a by-product in industrial production branches (fruit juice, jam, canned food) deteriorate in a short time under normal environmental conditions, making it impossible to use them in animal nutrition. For this reason, it is important to make silage of fruit pulps in terms of both their use in animal feeding and their utilization as a source of quality roughage (Yalçınkaya et al., 2012). Since fruits contain some important antioxidants, carotenoids, anthocyanins, anthocyanins, fatty acids, flavanoids and phenolic acids, and are rich in

vitamins and minerals (Velioglu et al., 1998), their pulps also make important contributions to silage quality (Ülger et al., 2015).

Food factory residues such as apple, orange, lemon, tomato and grape pulp are used for silage production (Yalçınkaya et al., 2012). In many parts of the world, the use of industrial by-products such as tomato pomace, pomegranate pomace, grape pomace, citrus pomace, etc. in ruminant feeding has greatly increased as a result of increasing high-cost feeds (Besharati et al., 2017; Başar and Atalay, 2020). Şengul et al. (2021) reported that the addition of dried mulberry pulp to the ration can also be used without any problems.

The utilization of waste products as animal feed is important in terms of being a cheap feed source and preventing environmental pollution caused by waste (Özdemir and Okumuş, 2021).

Adsorbents such as cereals or straw can be added during ensiling to protect fruit and vegetable pulp and residues from deterioration and nutrient loss during storage periods and to increase dry matter ratios (Denek and Can, 2005; Sargın and Denek, 2017).

Straws cannot provide the needs of high-yielding animals due to their high cellulose, low nutrient content and digestibility (Gürsoy, 2023). However, straws are mostly used as a roughage source in animal nutrition; to close the roughage deficit, to ensilage plants with high water content and to give animals a feeling of satiety with its high lignin-cellulose content (Gemalmaz and Bilal, 2016; Gürsoy, 2023).

In Table 1, silage researches using fruit-vegetable pulps in the last 5 years are given together with their sources.

Table 1. Silage Researches Conducted With Fruit and Vegetable Wastes in the Last 5 Years in Turkey (Özdemir and Okumuş, 2021).

Main material	Additives	Reference
Fresh tomato pulp	Molasses dry sugar beet pulp	(Sargin and Denek, 2017)
Orange, tangerine, lemon, maize straw, beet pulp	-	(Beyzi et al., 2018)
Maize	Pistachio husk	(Paydaş, 2019)
Lathyrus	Apple, lemon, pear	(İbrahimoğlu and Saruhan, 2019)
Citrus Pulp -		(Başar and Atalay, 2020)
Alfalfa	Waste jam	(Yayla and Soycan Önenç, 2021)
Meadow grass	White mulberry pulp	(Köksal et al., 2021)

Examining Table 1 and the studies in the literature, it was seen that apple, peach and citrus pomace were generally used in silages made with organic wastes, strawberry and carrot were not encountered, and white mulberry pomace was found in very few studies, but the silage formed with straw was not tried.

Considering that the fruit-vegetable pulps that occur as by-products in industrial production branches (fruit juice, jam, canned food) deteriorate in a short time under normal environmental conditions and also considering that mulberry molasses is a tradition especially for the region, not only for factory residues but also as a result of this traditional production, mulberry pulp is produced every year; in this study, it was aimed to prevent the waste of the leftovers of the fruits and vegetables (strawberries, carrots and, mulberries) used by a jam factory located in the organized industrial zone of Bingöl province during the year from being thrown away and to be used in silage production with a product with low feed value such as straw.

MATERIALS AND METHODS

The factory has about 1200 kg of strawberry, 100 kg of carrot and 97 kg of mulberry pulp left over from the strawberries, carrots and mulberries that are cleaned for jam making each season, which the factory disposes of as garbage. In the experiment, strawberry, carrot and mulberry residues used for making the silages were obtained from the factory and wheat straw and urea were obtained commercially. Wheat straws contain 1-2% digestible crude protein, over 50% crude cellulose, 1-2% crude fat and 28-50% N-free extracts in its dry matter, but it is very deficient in mineral and vitamin content (Ak and Akbay, 2018). In addition, some nutrient contents of wheat straw are presented in Table 2.

Table 2. Some Nutrient Contents of Wheat Straw (%).

Straw type	Dry matter	CP	Crude cellulose	Crude ash (CA)	(Gürsoy, 2023)
Wheat	92.4	4.1	37.1	16.4	
Straw type	**Digestible dry matter**	**CP**	**ADF**	**NDF**	(Çaçan and Kökten, 2023)
Wheat	53.9	5.18	45	70.5	

Urea is used to enrich silage in terms of nutrients and to increase the nitrogen content of silage (Yalçınkaya et al., 2012). In addition, straws are also treated with urea to increase their feed value and to use them effectively in

rations (Gürsoy, 2023). Silages were prepared in 3 replicates, using 0.5% urea and grouped in the ratios and mixtures indicated in Table 3 (Table 3).

In the preliminary experiment, when more than 30% straw was added, the ratio of fruit and vegetable wastes in the mixture was very low and the moisture to ensure fermentation was found to be insufficient. For these reasons and in order not to exceed the desired 35% dry matter rate in silages, the straw ratios in the mixtures were determined as 10%, 20% and 30%.

Table 3. Silage Groups and Straw Ratios Formed in The Experiment

	Groups
1.	**S10**: Strawberry waste + 10% straw
2.	**S20**: Strawberry waste + 20% straw
3	**S30**: Strawberry waste + 30% straw
4.	**C10**: Carrot waste + 10% straw
5	**C20**: Carrot waste + 20% straw
6.	**C30**: Carrot waste + 30% straw
7.	**M10**: Mulberry waste + 10% straw
8.	**M20**: Mulberry waste + 20% straw
9	**M30**: Mulberry waste + 30% straw

The periods when the wastes consisting of strawberries, carrots and mulberries obtained by the factory during the season were followed. These residues were mixed with straw and urea at the determined ratios in plastic containers and filled in an airtight container (Figure 1).



Figure 1. Images of Carrot, Strawberry and Mulberry Wastes Used in Silage Preparation.

The silages were left for fermentation for an average of 100 days.

Analysis and Evaluation of Silage Samples

At the end of the waiting period, the silages were opened and pH and dry matter (DM) were determined. After drying, the silage samples were ground to pass through a 1 mm sieve and stored in a nylon bag to prevent moisture absorption.

Acid detergent insoluble fiber (ADF), neutral detergent insoluble fiber (NDF), crude ash content and crude protein content of the samples were determined by NIRS (Near Infrared Spectroscopy).

Determination of Silage pH Content: In order to determine the pH value of silages, 25 g of silage sample was taken from each bin and homogenously mixed, weighed to 0.01 g on a sensitive scale and placed in the mixer. After the sample was homogenized by adding 100 ml of pure water and mixed in a magnetic mixer for 5 minutes, 30 ml of the mixture was filtered and the pH of this filtrate was measured with a digital pH meter (Polan et al., 1998).

Dry Matter (%): Approximately 50 g silage samples were placed in tared containers and dried at 70 °C for 4-5 days until thoroughly dried. At the end of the drying process, the container containing silage material was weighed. Dry matter content of silages formula (AOAC, 1990):

$$DM\% = 100 - Moisture\% \quad (I)$$

$$\%\,Moisture = ((A1 - D) - (A2 - D)) / A1 * 100 \quad (II)$$

(A1: Silage + container tare (g), D: Container tare (g), A2: Dry matter + container tare (g))

Fleig Score: It is a scoring system used to determine silage quality by using the KM and pH contents of silage material. Fleig Score was calculated as follows (Kılıç, 1986).

$$\text{Fleig Score} = [220 + (2 \times \%\,\text{DM} - 15)] - 40 \times \text{pH} \quad \text{(III)}$$

The Flieg score obtained from the above equation was used to classify the quality of the silages according to the score criteria given by Nauman and Bassler (1993) and Yalçınkaya et al. (2012) (0-20; V-Bad, 21-40; IV- Low, 41-60; III- Medium, 61-80; II- Good, 81-100; I- Excellent).

Determination of ADF, NDF, crude protein (CP) and crude ash (CA) ratios: Determined with the help of NIRS (Near Infrared Spectroscopy) device.

Statistical Analysis: Analysis of variance (ANOVA) was performed on all data obtained by measurements and analyzes in the study and the averages obtained were compared with Tukey test (5%) (JMP 13.2.0).

RESULTS AND DISCUSSION

pH, Dry Matter and Fleig Score

pH, KM and Fleig scores of silages made with jam factory wastes (strawberry, carrot and mulberry residues) and wheat straw are given in Figure 2. Among the analyzed traits, pH, KM and Fleig score were found to be statistically very significant (P<0.01).

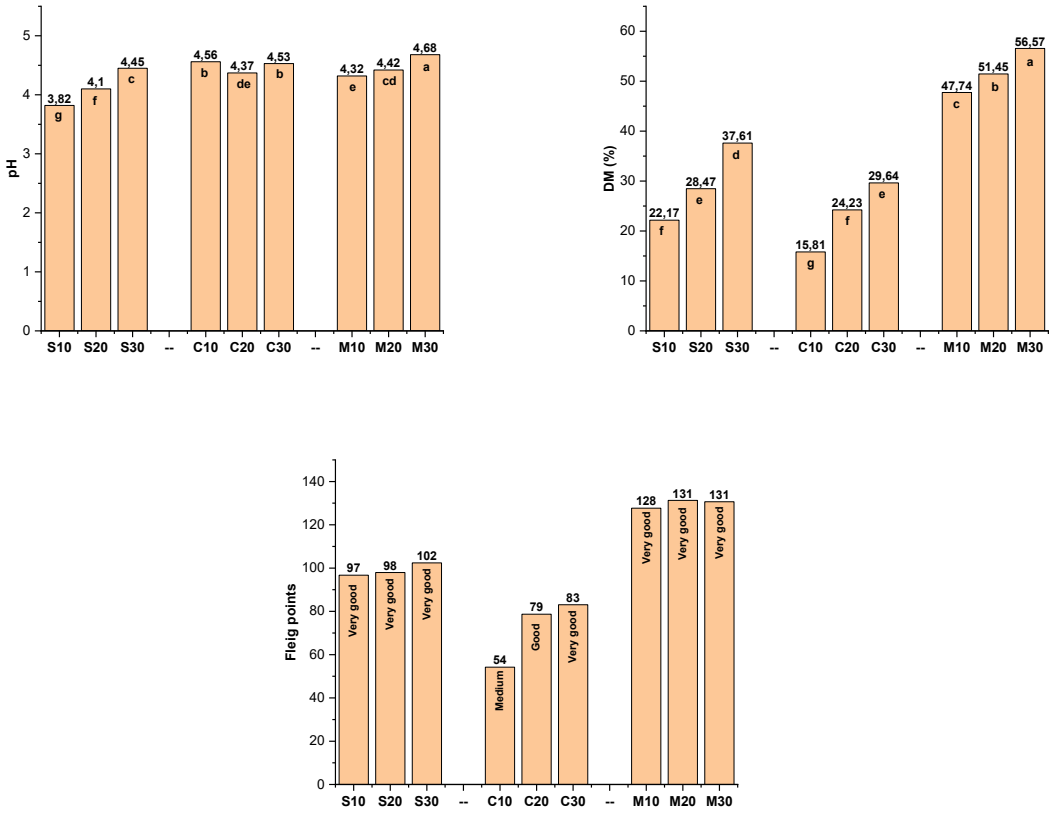


Figure 2. pH, KM and Fleig Scores of Silages Made From Strawberry, Carrot and Mulberry Residues.

The lowest pH value (3.82) was determined in the S10 group, while the highest pH value (4.68) was determined in the M30 group. The desired pH levels in a quality silage are in the range of 3.80-4.30 (Mafakher et al., 2010; Ülger et al., 2020). In this context, among the silage groups, only the 10% and 20% straw added group made with strawberry residues was at the desired pH level. The pH values of fruit-vegetable residues affected the silage pH value. Oğuz and Pırlak (2019) reported that some strawberry juice pH levels were in the range of 3.09-3.63, Kiracı and Padem (2015) reported the average pH value of carrot juice as 6.61, and Budak (2015) reported the pH value of white mulberry juice as 6.04. Ülger et al. (2015) determined pH values in the range of 3.59-4.35 in apple, lemon, orange, tangerine and peach silages made by silaging fresh beet pulp with fruit juice industry residues. Başar and Atalay (2020) found pH values in the range of 2.72-3.48 in crusted and uncrusted citrus silages. Kızılşimşek et al. (2011) reported that the pH level of alfalfa silage was significantly reduced with the addition of fruit pulp in their study.

The lowest DM value (15.81 g) was determined in C10 silage group while the highest DM value (56.57 g) was determined in D30 group. In all silage groups, the increase in the straw ratio caused an increase in the DM ratio. Only four silage groups (S20 and C10, C20, C30) were within the average DM (25-35%) content range reported

for silages (Kaynar and Atalay, 2023). Klamem et al. (2005) and Panyasak and Tumwasorn (2015) reported that dry matter content should be between 25-40% for a silage to be considered as good quality. A dry matter content of more than 40% means a high cellulose and hemicellulose content in the feed, which makes it difficult to digest and reduces its taste. In addition, high dry matter content makes it difficult to compress the silage, requiring more precise ensiling to eliminate the risk of spoilage. On the other hand, low dry matter content reduces the carbohydrate content of silage and causes spoilage (Panyasak & Tumwasorn, 2015; Burgu & Mut, 2023). The DM content of the silages formed by ensiling different ratios of molasses dry sugar beet pulp and tomato pulp varied between 21.48-35.10% (Sargın & Denek, 2017). Yalçınkaya et al. (2012) found DM content in the range of 32.93-35.03% in silages made by mixing apple, peach and apricot pomace with straw.

The quality classification of the silages was made according to the Flieg score obtained and the score criteria given by Nauman & Bassler (1993) and Yalçınkaya et al. (2012) and presented in Figure 2. The silages belonging to C10 straw group were in III-medium class, C20 straw group was in II-good class and all other silage groups were in I-excellent class. Since DM and pH values were used in the calculation of fleig score, since straw increased the DM ratio, fleig scores were found to be high accordingly. Yalçınkaya et al. (2012) stated that the fleig scores of straw and urea added groups were higher in apple, peach and apricot silages. In silages made by mixing strawberry, carrot and mulberry residues with straw, fleig scores varied between 54.21 and 131.29. İbrahimoglu and Saruhan (2019) found fleig scores between 76.22-85.12 in silages made with apple, pear and lemon fruits added at various rates of damson plant. Seydoşoğlu and Gelir (2019) found the highest fleig score of 109.23 in silages made with different ratios of damson and barley mixture. It is seen that these results obtained by the researchers are partially similar to the results of this study.

ADF, NDF, CP and CA values (%)

ADF, NDF, CP and CA values of silages made with jam factory wastes (strawberry, carrot and mulberry residues) and wheat straw are given in Figure 3. Among the analyzed traits, ADF, NDF, CP and CA were found to be statistically very significant (P<0.01).

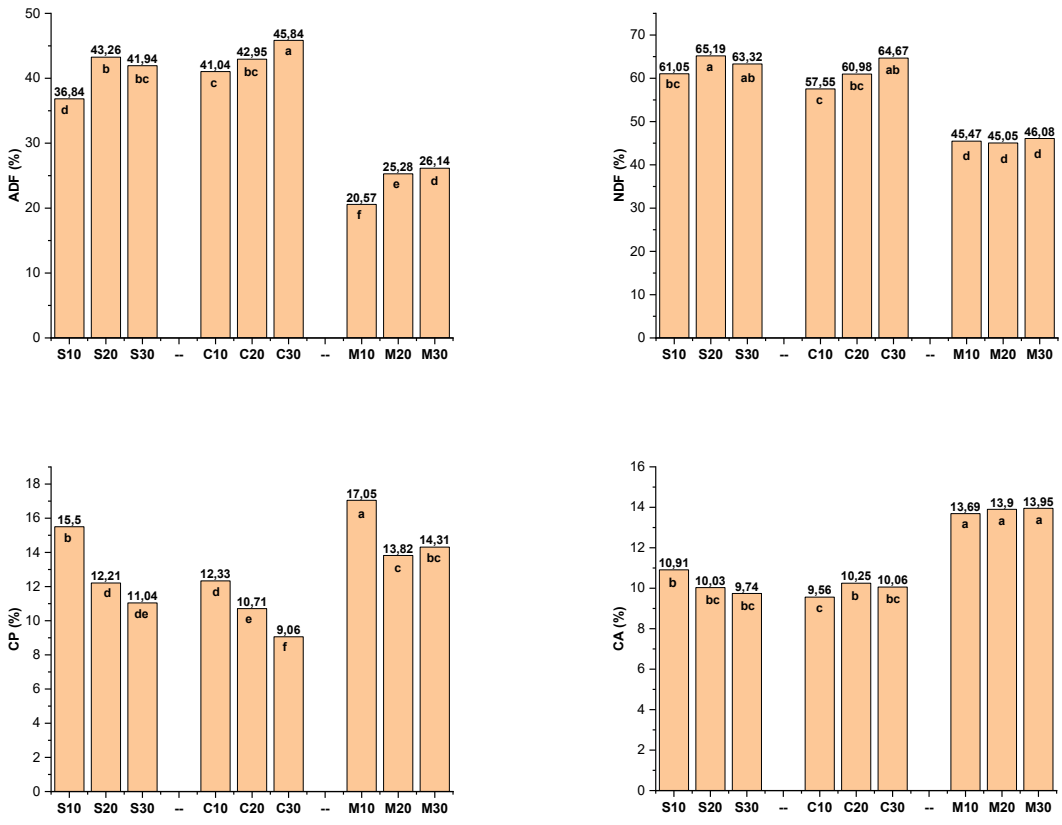


Figure 3. ADF, NDF, CP and CA Values of Silages Made From Strawberry, Carrot and Mulberry Residues.

The addition of straw to vegetable and fruit wastes caused an increase in ADF ratios in all silage groups. The lowest ADF rate (20.57%) was obtained in M10 group and the highest ADF rate (45.84%) was obtained in C30 group. ADF ratio is an important parameter that gives information about the degree of digestibility of silages and the higher the ADF ratio, the lower the degree of digestibility of that feed (Erişci et al., 2022). Acikbas and Ozyazici

(2019) reported that the average ADF rate in wheat straw was 52.31%. It is seen that especially mulberry residues help to decrease the ADF rate of straw. Doğan (2019) reported that the ADF content of silages decreased with the addition of apple pulp.

The lowest NDF ratios (45.05-46.08%) were obtained in silages containing 10%, 20% and 30% straw made with mulberry wastes, while the highest NDF ratio (65.19%) was obtained from the S20 straw group. The highest NDF ratios were found in the strawberry residue group, which is thought to be due to the fact that most of the strawberry residues consisted of sepals of strawberry fruit. Wardiny et al. (2021) found 40.8% NDF in Morinda citrifolia fruits, while in leaves they found 50.1%, higher than the fruit.

While the lowest crude protein content was determined in C30 silages, the highest crude protein content was determined in M10 silages. Mulberry group silages had a higher protein ratio than the other groups. This is thought to be due to the fact that strawberry residues were mostly composed of sepals, carrot residues were mostly composed of carrot stems, and mulberry residues contained the seeds of the fruit. Mulberry pulp (MP) is a by-product of mulberry juice production such as jam, molasses, etc. and consists of the seeds and peeled parts of the mulberry (Elmacı and Altuğ 2002). Kır and Temel (2017) found that the crude protein ratios in the seeds of the plants were higher than the plant stems. Carrot group silages had lower crude protein content. Kaya and Kamalak (2019) examined the nutrient composition of root and tuberous market residues and found that the crude protein content of carrot was considerably lower than other plants.

The lowest crude ash content (9.56%) was obtained in silages containing C10, while the highest crude ash content (13.69-13.95%) was obtained from silage groups containing 10%, 20% and 30% straw made with mulberry residues. Similar results for crude ash ratios were obtained in apple pomace silages added to alfalfa silages (Ünal et al., 2024) and residual jam particles silages added to alfalfa silages (Yayla and Soycan Önenç, 2021).

Ca, Mg, P and K contents of silages (%)

Ca, Mg, P and K values of silages made with jam factory wastes (strawberry, carrot and mulberry residues) and wheat straw are given in Figure 4. The differences between Ca, Mg, P and K values were found to be statistically very significant (P<0.01).

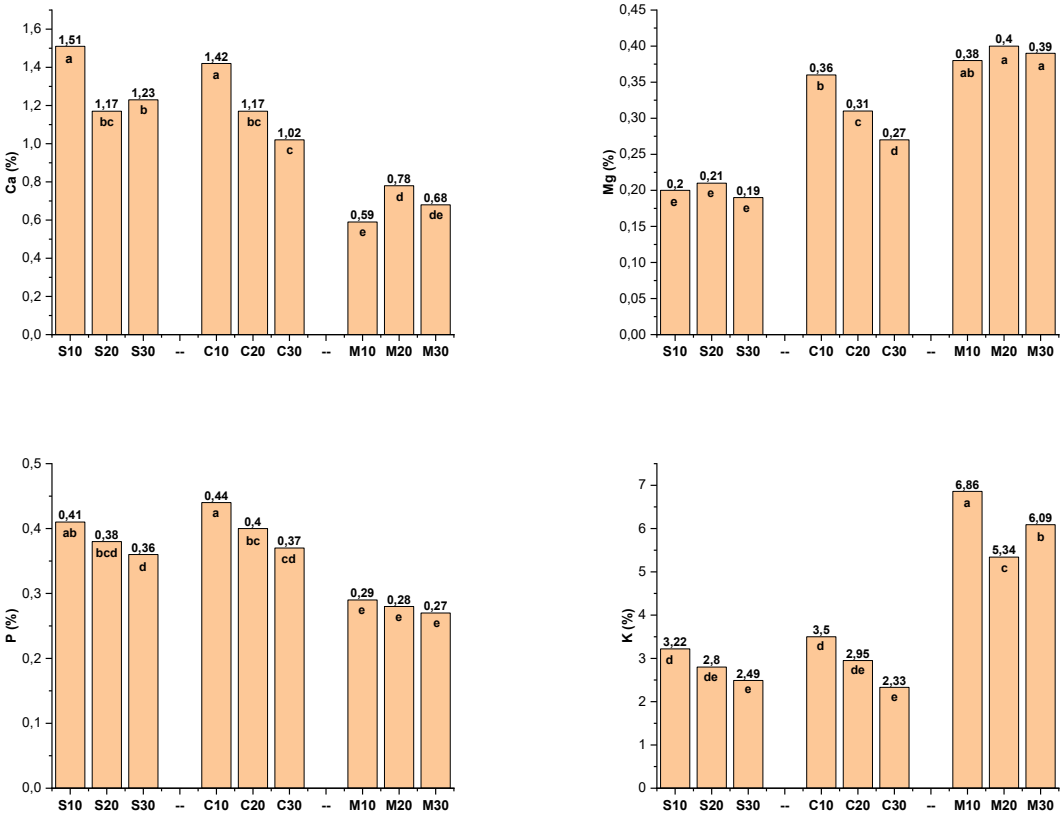


Figure 4. Ca, Mg, P and K Contents of Silages Made From Strawberry, Carrot and Mulberry Residues.

The Ca content of the silages varied between 0.59% and 1.42%. The silage group with the lowest Ca content was M10, while the groups with the highest Ca content were S10 and C10. In terms of ruminant health, Ca content of quality roughages should be between 0.18 - 0.44% (Kidambi et al., 1989; Burgu and Mut, 2023).

The Mg content of silages varied between 0.19%-0.40%. The lowest Mg content was in the S30 group and the highest Mg content was in the M20 group. It has been reported that magnesium content in feeds should be between 0.12% and 0.20% for healthy animal breeding (Anonymous, 1980; Başbağ et al., 2018).

The silage groups met the minimum recommended Ca and Mg contents in forages and also had Ca and Mg contents above the maximum recommended Ca and Mg contents. Jones et al. (1991) reported that some forage crops such as sorghum and maize had Ca and Mg contents of 0.20-0.30 and 0.10-0.15%, respectively, while strawberries and carrots had at least 1% for Ca and Mg, respectively. It is thought that Ca and Mg ratios are lower in ensiled plants than in fruits and vegetables and the ratios recommended for silage are generally determined according to the nutrient content of the silage plants. In this study, it is thought that fruit and vegetable wastes increased the Ca and Mg content of silages, resulting in higher ratios.

While the P contents of the silages varied between 0.27%-0.44%, the group with the highest P content was C10. K content of silage groups varied between 2.33%-6.86%. While the silage group with the lowest K content was C30, the group with the highest K content was M10 straw.

Forages for ruminants should have a P content of at least 0.21% (Kidambi et al., 1989) and a K content of at least (Tejada et al., 1985) 0.8%, (Mut et al., 2020). In all silage groups obtained by mixing strawberry, carrot and mulberry residues with straw at different ratios, P and K contents were found to have the ratios required by the researchers.

CONCLUSION

In this study in which the usability of jam factory fruit and vegetable wastes for silage production was investigated, it was observed that the ideal pH values were obtained from S10 and S20 and the ideal DM values were obtained from S20, C10, C20 and C30 treatments. It was determined that all treatments except C10 and C20 treatments were in the I- excellent class, which is the best value in terms of fleig score. The lowest ADF and highest crude protein ratios were obtained from M10, the lowest NDF and highest crude ash ratios were obtained from M10, M20 and M30 treatments. In terms of macro elements, the highest Ca and P ratios were obtained from S10 and C10, and the highest Mg and K ratios were obtained from M10, M20 and M30 treatments. In accordance with this information, it was concluded that all three crop production residues to which straw was added can be used in silage making.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

There is no conflict of interest between the authors in this study.

Author contribution

Conception and design of the study: HŞİ, ST, EÇ, Eİ; sample collection: HŞİ, Eİ; analysis and interpretation of data: HŞİ, EÇ ; statistical analysis: HŞİ, EÇ; visualization: HŞİ, ST,EÇ; writing manuscript: HŞİ, EÇ.

Ethics committee approval

We declare that there is no need for an ethics committee for this research.

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Comparison of the winter bud productivity, rooting and shoot development parameters of some *Vitis* spp. with single bud

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Abstract

Grapes belonging to the *Vitis* species are growing worldwide for many years for different purposes. Determining the bud productivity values of varieties belonging to this species for product programming during winter pruning is of great importance for high-quality production. Therefore, our study investigated bud productivity, rooting and shoot development parameters of single-bud cuttings taken from the first and ninth buds of 12 grape varieties with different species and polyploidy levels. In the study, Atak 77, Muscat Bleu, Rizpem, Kyoho (4N), Regent, Isabella, Heukboseok (4N), Özer Beyazı, Superior Seedless, Crimson Seedless, Kismish Rozovyi and Müşküle varieties were compared. Winter bud productivity was evaluated to clarify the winter pruning levels. In addition to cluster number, root development level and shoot length parameters were compared in the same single-bud cuttings. According to the values obtained from all buds regarding bud productivity, especially the interspecific hybrid Regent variety and the *V. labrusca* species, Isabella variety stood out as more productive than other varieties. In addition, very low bud productivity values were obtained in seedless varieties. Regarding the rooting status of single bud cuttings, Regent and Muscat Bleu, interspecific hybrids, were the most successful varieties. In evaluating shoot length, the interspecific hybrid Muscat Bleu variety gave the longest shoot according to the values obtained from almost all buds. According to these results, varieties obtained from cross-breeding different *Vitis* species, in particular, could generally have better bud productivity and rooting and shoot development values. This situation should be considered for quality and efficient production, especially during pruning applications.

Keywords: Buds, Grape, Interspecies varieties, Quality production, Crop loading, Bud fertility

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INTRODUCTION

Grapes are among the most cultivated fruits in the world, and their cultivation is important for the economies of many countries, including Türkiye (Shahzad et al., 2019; Daskalakis et al., 2018). Türkiye, among the gene centres of cultivated grapevines, is an important country in world viticulture with its rich grapevine genetic resources and ecological characteristics for viticulture culture (Atak et al., 2021). Viticulture is the source of income for many producers throughout the country due to the suitability of climate and growing conditions. Grape varieties with different botanical properties and rich in health components are widely consumed in many countries (Akram et al, 2019).

Grape varieties and genotypes of the *Vitis* species have been cultivated for many years for different purposes. The bud productivity values of the varieties belonging to this species should be determined for product programming during winter pruning. Bud productivity values of different grape varieties belonging to the *Vitis* species differ. This difference should be determined, and the most appropriate winter pruning should be decided. Otherwise, it may not be possible to reach the desired yield values. For this purpose, different researchers have determined the bud productivity values of grape varieties.

In viticulture, researchers use different pruning and loading applications to efficiently use unit areas and obtain higher-quality products (Başaran, 2006). The term yield in vineyard refers to the number of clusters the buds carry on annual shoots. There may be 0-4 cluster primordia in the primary bud of the winter buds of the vine. However, usually 1-2 cluster primordia can be seen (Çelik, 1999; Şen and Atak, 2020). Grape yield in vineyards vary depending on the winter bud positions on annual branches. Nodes at different levels are important in determining winter bud productivity (Dardeniz and Kısmalı, 2005). To ensure yield and quality, pruning levels should be determined according to the bud productivity of the varieties (Akın et al., 2011). Pruning balances between vegetative development and product yield (Şen and Atak, 2020). Knowing the bud productivity helps to adjust the number of buds to be left on the vine and the fruit load during winter pruning and also to increase fruit quality (Çelik and Kök, 1998; Dardeniz and Kısmalı, 2005).

Different methods are used to determine the bud fertility of grapevine. Among these, the most preferred method is forcing bud bursts and detecting clusters on each bud. Most of the researchers preferred forcing bud bursts and detecting clusters (Akın et al. 2011; Taşçı, 2015)

In this study, the winter bud productivity and rooting/shoot development parameters were examined to determine the optimum pruning and vine loads of 12 grape varieties belonging to different *Vitis* species. It is aimed to recommend the appropriate pruning level to grape producers who produce with these varieties to obtain high yield and quality products.

MATERIAL and METHOD

In this study, cuttings were taken from one-year-old shoots of a total of 12 grape varieties belonging to different species and polyploidy levels from the Vineyard Genetic Resources parcel located in the Yalova Atatürk Horticultural Central Research Institute in winter period. The trial area is situated on the borders of Yalova center, between 40◦39′40″ North latitude and 29◦17′22″ East longitude. The altitude is 3 m above the sea level, and the distance to the sea is 150 m. These cuttings were kept in the cold storage of the Department of Horticulture, Faculty of Agriculture, Bursa Uludağ University, at +4°C and 80% humidity until the time of the study (Figure 1).

Material

The study material consisted of 12 grape varieties belonging to different *Vitis* species. Two of these varieties are tetraploid (4N), while the other grape varieties are diploid (2N). In addition, 4 of the varieties are seedless, while the other eight are seeded (Table 1). The study evaluated the 1st and 9th buds of these 12 grape varieties.

Table 1. Grape varieties and species used in the study.

No	Variety	Species	Poliploidy Level	Seeded/Seedless
	Atak 77	*V.vinifera*	2N	Seeded
	Crimson Seedless	*V. vinifera*	2N	Seedless
	Heukboseok	Türlerarası melez	4N	Seeded
	Isabella	*V. labrusca*	2N	Seeded
	Kismish Rozovyi	*V. vinifera*	2N	Seedless
	Kyoho	Türlerarası melez	4N	Seeded
	Muscat Bleu	Türlerarası melez	2N	Seeded
	Müşküle	*V. vinifera*	2N	Seeded
	Özer Beyazı	*V. vinifera*	2N	Seedless
	Regent	Türlerarası melez	2N	Seeded
	Rizpem	*V. labrusca*	2N	Seeded
	Superior Seedless	*V. vinifera*	2N	Seedless

Method

Determination of Bud Productivity

To determine the winter bud productivity of the varieties, the method of counting the cluster primordia on the shoots that emerged by forcing the bud awakening was used according to the method used by Şen and Atak (2020). Cuttings were prepared by cutting them as single buds in the Climate Room of the Horticulture Department of the Faculty of Agriculture of Bursa Uludağ University by paying attention to the bud rows. The prepared cuttings were planted in plastic containers to determine the bud productivity and root and shoot developments. A small amount of activated charcoal was also added to the plastic containers. The containers were covered with plastic film to fix the single bud cuttings and ensure they all contacted the water equally.

Planting operations were carried out in plastic containers with 3 replications for each variety and 3 buds in each replication. When all the buds were considered for a variety, 90 single bud cuttings were used. A total of 90*12= 1080 single bud cuttings were used in the study. The buds on the shoot were cut according to the sequence number starting from the cutting points and planted in the container according to this bud order (Figure 2). The containers were filled with pure water so the bottom parts of the planted single-bud cuttings could contact the

water as equally as possible. The planted containers were kept in the climate room at +25°C for 4 weeks (Figure 3).

The dormant single-bud cuttings of the varieties were forced to shoot at the end of 4 weeks. After the buds burst, the bud productivity (number of buds/bud), rooting status of the buds and shoot lengths were evaluated according to Şen and Atak 2020 by counting the cluster primordia formed on the shoot.



Figure 1. Cuttings taken by pruning and storage in cold storage.

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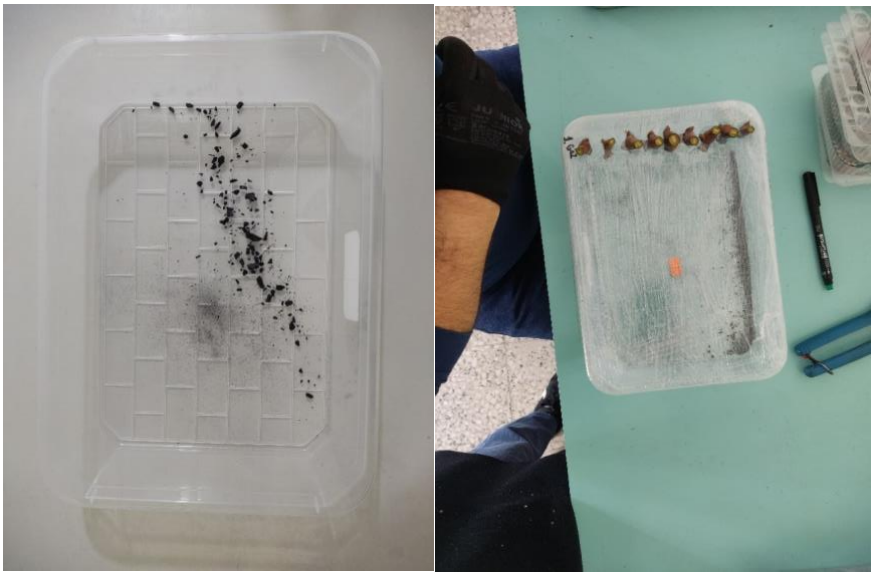


Figure 2. Preparation of plastic containers for planting to determine bud productivity



Figure 3. Photos of buds planted in plastic containers in the climate chamber

Evaluated Parameters

Rooting Development Level (0-3 Scale): The root development level in cuttings of various varieties was evaluated using a 0-3 scale. Roots of cuttings developing 0.5 cm and above were evaluated according to the scale values given in Figure 4.



Figure 4. Rooting scale used in the study for single bud cuttings (0-3)

Shoot Length (cm): The lengths of shoots sprouting from single-bud cuttings were measured. The distance between the bud and the shoot tip was measured with the help of a ruler (Figure 5).

Number of Cluster (Number of cluster/Bud): The cluster primordia on the developing shoots following the burst of the buds were counted, and the bud productivity of each variety and bud was determined (Figure 5).

Statistical Analyses

The experiment was established according to the completely randomized plots experimental design with 3 replications. The obtained data were subjected to variance analysis in the JPM-16 package program and the differences between the applications were statistically evaluated using the LSD test at a significance level of 0.05.



Figure 5. Photos of varieties with shoot length and cluster numbers counted

RESULTS AND DISCUSSION

In the study, while determining the bud productivity for each variety, data on root development level and shoot length were also determined. According to the results obtained, root development level (0-3 scale), shoot length and cluster number data were found to be statistically significant.

When the varieties were compared in terms of the location of the buds (according to the cluster number) in determining the bud productivity, the most productive variety in the 1st bud was the Regent variety, which is an interspecific hybrid. The Superior Seedless and Kishmish Rozovyi varieties from the *V. vinifera* species were determined as the varieties with the lowest bud productivity in the 1st bud. The Kyoho variety, an interspecific hybrid with a 4N polyploidy level, was found to be the most productive variety in the 2nd bud. The Superior Seedless variety from the *V. vinifera* species was determined as the variety with the lowest bud productivity in the 2nd bud. In the interspecific hybrid Regent variety, the 3rd bud is the most productive variety, while the Superior Seedless and Kishmish Rozovyi varieties belonging to the *V. vinifera* species are seen to have the lowest 3rd bud yield. In the Isabella variety belonging to the *V. labrusca* species, the 4th bud was found to be the most productive. The Superior Seedless and Kishmish Rozovyi varieties belonging to the *V. vinifera* species were found to have the lowest 4th bud yield. The most productive 5th bud was the Isabella variety belonging to the *V. labrusca* species, and the least productive variety was the Superior Seedless variety belonging to the *V. vinifera* species. While the Atak 77 variety belonging to the *V. vinifera* species had the highest bud productivity in terms of the 6th bud, the 6th bud was the lowest in the Kishmish Rozovyi variety from the same species. The Regent variety, an interspecific hybrid, was found to be the most productive variety in terms of the 7th, 8th and 9th buds. The lowest yielding variety was Superior Seedless, belonging to the *V. vinifera* species, for the 7th bud and the lowest yielding variety was Kishmish Rozovyi, belonging to the same species, for the 8th bud. The lowest yielding variety was Heukboseok, which is an interspecific hybrid and has 4N polyploidy, for the 9th bud (Table 2). The productivity values of the buds by variety are given in Figure 6. When the results obtained in determining the most productive buds based on varieties were examined, the following results were obtained: In the interspecific hybrid and 4N polyploidy level Kyoho variety, the most productive bud was determined as the 2nd bud. In contrast, the least productive bud was determined as the 5th bud (Figure 6). In the Atak 77 variety belonging to the *V. vinifera* species, the most productive bud was determined as the 7th bud. The same variety's least productive buds were determined as the 1st and 2nd buds. In the Özer Beyazı variety belonging to the *V. vinifera* species, the 8th bud was productive, while the 1st bud was the least productive.

Table 2. The bud productivity values of the varieties.

NUMBER OF BUNCHES*									
VARIETY	**1st bud**	**2nd bud**	**3th bud**	**4th bud**	**5th bud**	**6th bud**	**7th bud**	**8th bud**	**9th bud**
Kyoho (4N)	0,78^{e}	**2,17^{a}**	0,57^{e}	1,00^{d}	0,38^{g}	0,63^{d}	0,83^{d}	0,43^{f}	0,50^{e}
Atak 77	0,44^{h}	0,44^{g}	0,88^{c}	1,25^{c}	1,25^{b}	**1,40^{a}**	1,57^{c}	1,44^{c}	1,22^{c}
Özer Beyazı	0,14^{j}	0,40^{g}	0,22^{f}	0,56^{g}	0,44^{f}	0,50^{e}	0,56^{f}	0,67^{e}	0,44^{e}
Heukbseok (4N)	0,29^{i}	0,11^{j}	0,60^{e}	0,22^{i}	0,57^{e}	0,14^{g}	0,20^{i}	0,20^{j}	0,00^{h}
Regent	**2,11^{a}**	1,43^{c}	**1,38^{a}**	1,56^{b}	1,14^{c}	0,88^{c}	**2,38^{a}**	**2,50^{a}**	**2,44^{a}**
Superior Seedless	0,14^{j}	0,00^{k}	0,00^{g}	0,00^{j}	0,00^{i}	0,11^{g}	0,00^{k}	0,33^{h}	0,11^{g}
Crimson Seedless	0,89^{d}	0,67^{f}	1,00^{b}	1,00^{d}	0,56^{e}	0,11^{g}	0,38^{g}	0,38^{g}	-
Isabella	1,63^{b}	1,00^{e}	1,00^{b}	**2,00^{a}**	**2,00^{a}**	1,20^{b}	1,75^{b}	2,00^{b}	2,00^{b}
Rizpem	0,63^{f}	0,33^{h}	0,22^{f}	0,44^{h}	0,29^{h}	0,30^{f}	0,22^{i}	0,25^{i}	0,50^{e}
Kishmish Rozovyi	0,14^{j}	0,25^{i}	0,00^{g}	0,00^{j}	0,25^{h}	0,00^{h}	0,33^{h}	0,11^{k}	0,33^{f}
Müşküle	1,00^{c}	1,14^{d}	**1,33^{a}**	0,75^{e}	0,75^{d}	0,29^{f}	0,11^{j}	0,71^{d}	-
Muscat Bleu	0,56^{g}	1,50^{b}	0,78^{d}	0,67^{f}	1,25^{b}	0,67^{d}	0,78^{e}	0,67^{e}	1,00^{d}

* Lowercase letters indicate that the difference between varieties is statistically significant. LSD is significant at the 0.05 level.

In the interspecific hybrid and 4N polyploidy level Heukboseok variety, the most productive bud was the 3rd bud, while the least productive bud was the 9th bud. In the interspecific hybrid Regent variety, whose 8th bud was the most productive, the 6th bud was found to be the least productive. The 8th bud of the Superior Seedless variety belonging to the *V. vinifera* species was determined to be the most productive. All the other buds except the 1st and 9th buds of the same variety were the least productive. It was observed that the 6th bud of the Crimson Seedless variety belonging to the *V. vinifera* species, whose 3rd and 4th buds were productive, was least productive. It was determined that the 4th, 5th, 8th and 9th buds of the Isabella variety belonging to the *V. labrusca* species were productive, while the 2nd and 3rd buds were least productive. It was determined that the 1st bud of the Rizpem variety belonging to the same species was productive, while the 3rd and 7th buds were least productive. It was observed that the 7th and 9th buds of the Kishmish Rozovyi variety belonging to the *V. vinifera* species were productive, while the 3rd, 4th and 6th buds were least productive. In the Müşküle variety of the same species, the 3rd bud was found to be productive, while the 7th bud was determined to be least productive. In the interspecific

hybrid Muscat Bleu variety, the 2nd bud was found to be productive, while the 1st was determined to be least productive.

Along with bud productivity, root development level in single-bud cuttings was also evaluated based on bud and variety. The following results were obtained in the evaluations based on bud: 1st bud of interspecific hybrid varieties Muscat Bleu and Regent showed the best result in root development level. It was determined that the root development level in all buds was weak in the Atak 77 variety belonging to *V. vinifera* species. Root development level in 2nd bud of interspecific hybrid varieties Muscat Bleu and Özer Beyazı varieties belonging to *V. vinifera* species showed the best result in root development level in 3rd bud of interspecific hybrid varieties Muscat Bleu. Interspecific hybrid Regent showed the best result in root development level between the 4th and 9th buds. Isabella variety belonging to *V. labrusca* species stood out with 5th bud in root development level (Table 3).

Table 3. Average root development level values of the buds based on variety

ROOT DEVELOPMENT LEVEL*									
VARIETY	**1st bud**	**2nd bud**	**3th bud**	**4th bud**	**5th bud**	**6th bud**	**7th bud**	**8th bud**	**9th bud**
Kyoho (4N)	2,44^{e}	1,89^{d}	2,11^{f}	1,67^{g}	2,11^{d}	2,22^{d}	1,56^{g}	0,89^{i}	1,22^{g}
Atak 77	1,22^{i}	1,11^{h}	0,78^{j}	0,88^{i}	0,56^{i}	0,50^{k}	0,50^{k}	0,44^{k}	0,33^{i}
Özer Beyazı	2,57^{c}	**2,90^{a}**	2,67^{b}	2,89^{b}	2,78^{b}	2,44^{c}	2,78^{c}	2,67^{c}	2,56^{c}
Heukboseok (4N)	1,86^{g}	1,44^{g}	2,10^{g}	2,00^{e}	2,00^{e}	1,43^{j}	1,43^{i}	1,20^{h}	0,75^{h}
Regent	**3,00^{a}**	2,33^{b}	2,67^{b}	**3,00^{a}**	**2,89^{a}**	**2,89^{a}**	**2,88^{a}**	**3,00^{a}**	**3,00^{a}**
Superior Seedless	2,00^{f}	2,11^{c}	1,89^{h}	2,22^{d}	2,11^{d}	1,89^{g}	2,22^{e}	2,22^{d}	1,67^{e}
Crimson Seedless	1,56^{h}	1,78^{e}	2,22^{e}	1,89^{f}	1,89^{f}	2,00^{f}	2,00^{f}	1,56^{f}	-
Isabella	2,50^{d}	2,33^{b}	2,44^{d}	2,88^{b}	**2,89^{a}**	2,20^{e}	2,80^{b}	2,90^{b}	2,00^{d}
Rizpem	2,00^{f}	2,11^{c}	1,56^{i}	1,56^{h}	2,50^{c}	1,80^{h}	1,22^{j}	0,88^{j}	1,50^{f}
Kishmish Rozovyi	1,56^{h}	2,11^{c}	2,22^{e}	1,89^{f}	1,44^{h}	2,00^{f}	2,78^{c}	1,89^{e}	2,67^{b}
Müşküle	2,67^{b}	1,67^{f}	2,56^{c}	0,89^{i}	2,00^{e}	1,78^{i}	1,44^{h}	1,38^{g}	-
Muscat Bleu	**3,00^{a}**	**2,90^{a}**	**3,00^{a}**	2,78^{c}	1,75^{g}	2,78^{b}	2,33^{d}	1,56^{f}	1,71^{e}

* Lowercase letters indicate that the difference between varieties is statistically significant. LSD is significant at the 0.05 level.

In the evaluations of root development level based on varieties, the following results were obtained (Figure 7); While the 1st bud of the Kyoho variety, which is an interspecific hybrid and has a 4N polyploidy level, gave the best result in root development level, the 8th bud showed the weakest root development. In the Atak 77 variety belonging to the *V. vinifera* species, the 1st bud showed the best result in root development level. It was observed that the root development level of the 2nd bud of the same variety was the weakest bud. In the Özer Beyazı variety belonging to the *V. vinifera* species, the 2nd bud gave the best result in root development level. In contrast, the weakest root development was detected in the 6th bud. In the Heukboseok variety, an interspecific hybrid with a 4N polyploidy level, the 3rd bud gave the best result in root development level. The 9th bud of the same variety showed the weakest root development. In the Regent variety, an interspecific hybrid, the 1st, 4th, 8th, and 9th buds gave the best result in root development level, while the weakest root development was detected in the 2nd bud. The 4th, 7th and 8th buds of the Superior Seedless variety belonging to the species *V. vinifera* gave the best results in terms of root development, while the 9th bud showed the weakest root development. In the Crimson Seedless variety belonging to the same species, the 3rd bud showed the strongest root development, while the 1st and 8th buds showed the weakest root development. The 8th bud of the Isabella variety belonging to the *species V. labrusca* gave the best results in terms of root development, while the 9th bud showed the weakest root development. In the Rizpem variety belonging to the same species, the 5th bud showed the strong root development, while the 8th bud showed the weakest root development. The 9th bud of the Kishmish Rozovyi variety belonging to the species *V. vinifera* gave the best results in terms of root development, while the 5th bud showed the weakest root development. While the 1st bud of the Müşküle variety belonging to the same species stands out with the strongest root development, the weakest root development was detected in the 4th bud. In the interspecific hybrid Muscat Bleu variety, the 1st and 3rd buds gave the best results in terms of root development, while the 8th bud showed the weakest root development.

In determining bud productivity, the lengths of shoots growing on single-bud cuttings were also evaluated based on bud and variety. When we examined the bud basis, the following results were obtained: Regarding the length of shoots growing between 1 and 8 buds, the interspecific hybrid Muscat Bleu variety gave the best results.

The data obtained from shoot lengths varied depending on the position of the bud. While the lowest shoot length values were obtained in some buds in the Atak 77 and Crimson Seedless varieties belonging to the *V. vinifera* species, the lowest shoot length values were also obtained in some buds in the Rizpem variety belonging to the *V. labrusca* species. It was determined that the Heukboseok variety, an interspecific hybrid with a polyploidy level of 4N, gave the shortest shoots in the 7th bud. When the shoot lengths of the 9th bud were examined, the Özer Beyazı variety belonging to the *V. vinifera* species gave the best result. It was determined that the Kyoho and Heukboseok varieties, which are interspecific hybrids and have a polyploidy level of 4N, the Superior Seedless

variety belonging to the *V. vinifera* species and the Rizpem variety belonging to the *V. labrusca* species gave the shortest shoots in the same bud (Table 4).

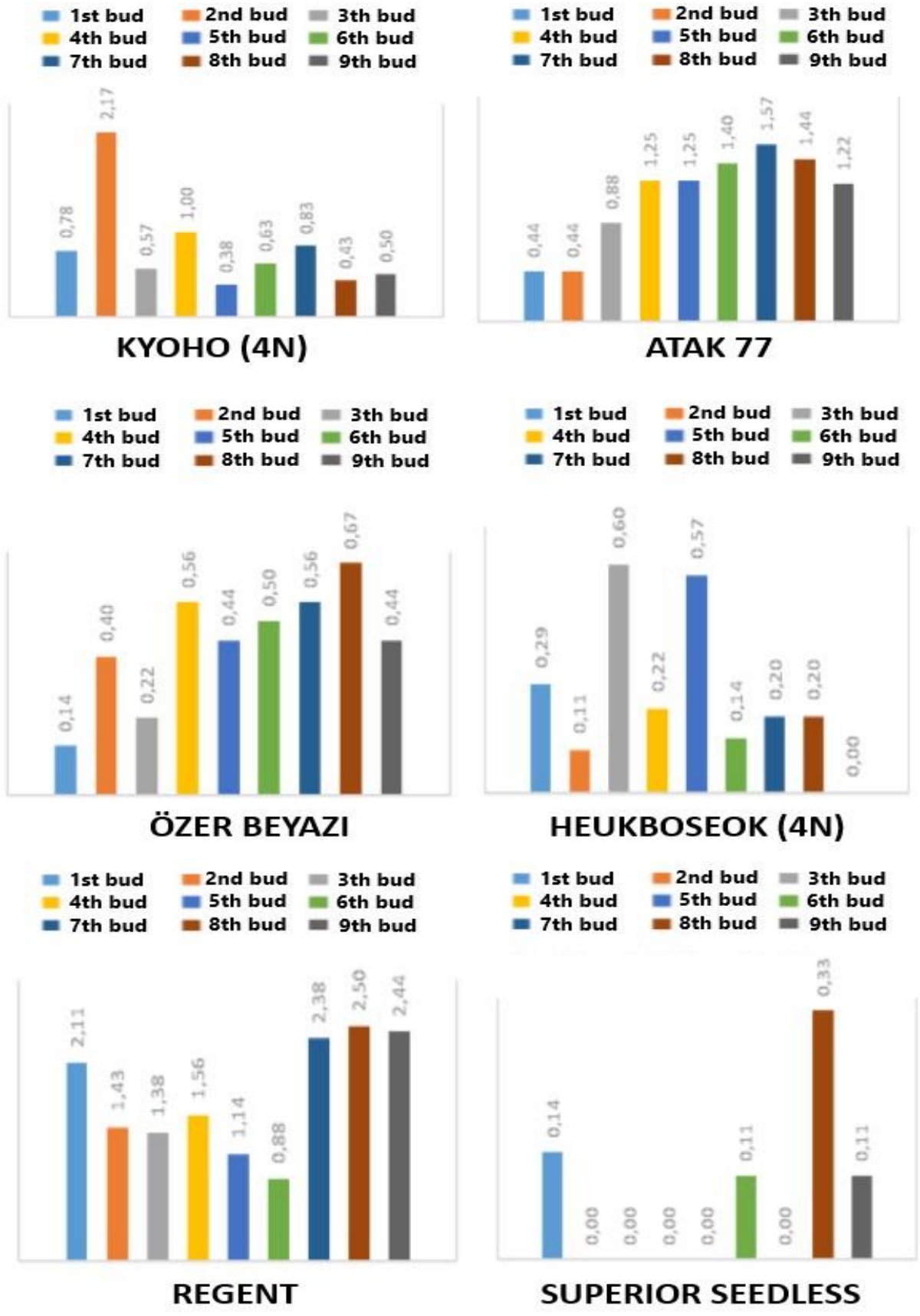
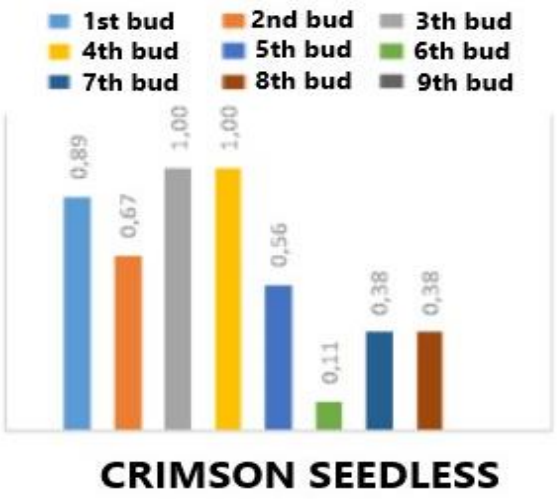
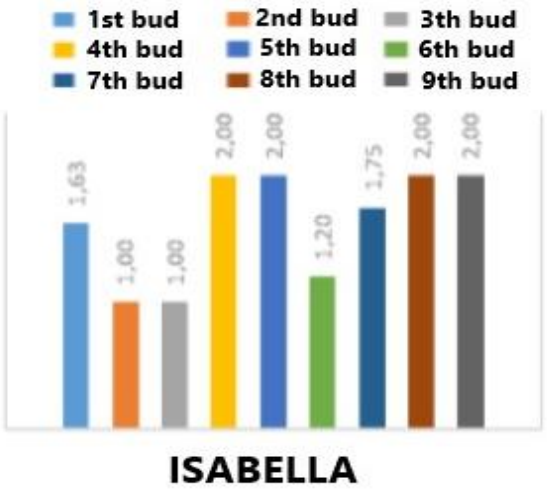
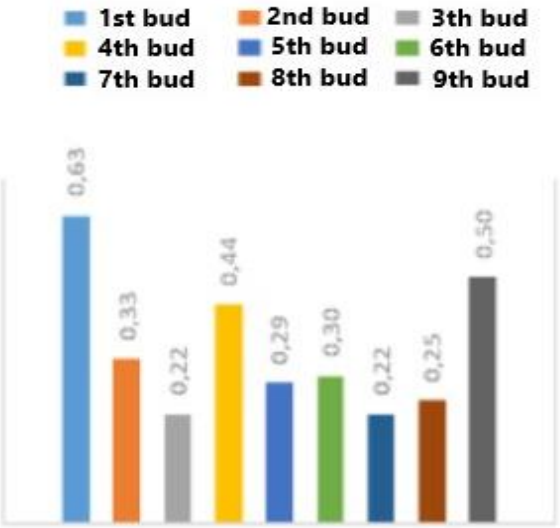
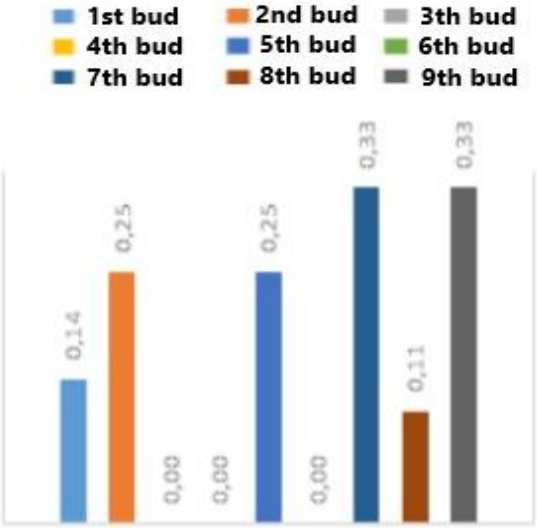


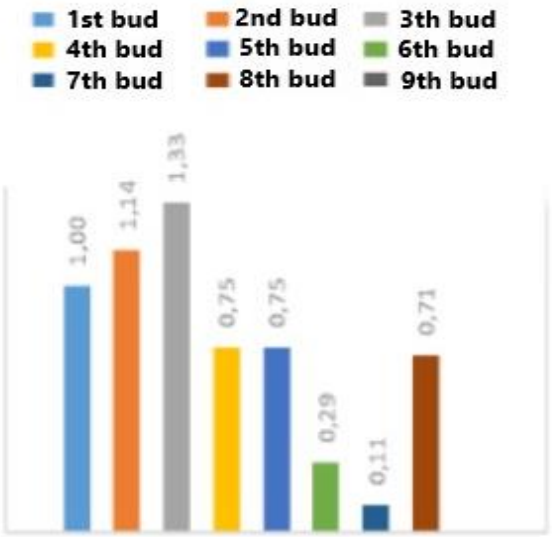
Figure 6. Average bud productivity values between 1st and 9th buds of varieties.











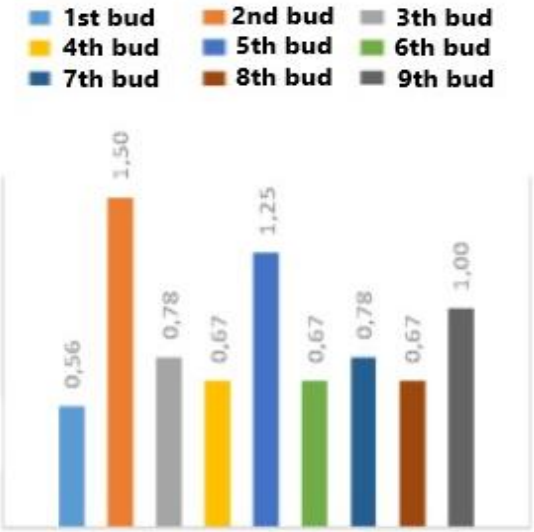


Figure 6. Average bud productivity values between 1st and 9th buds of varieties (continue).

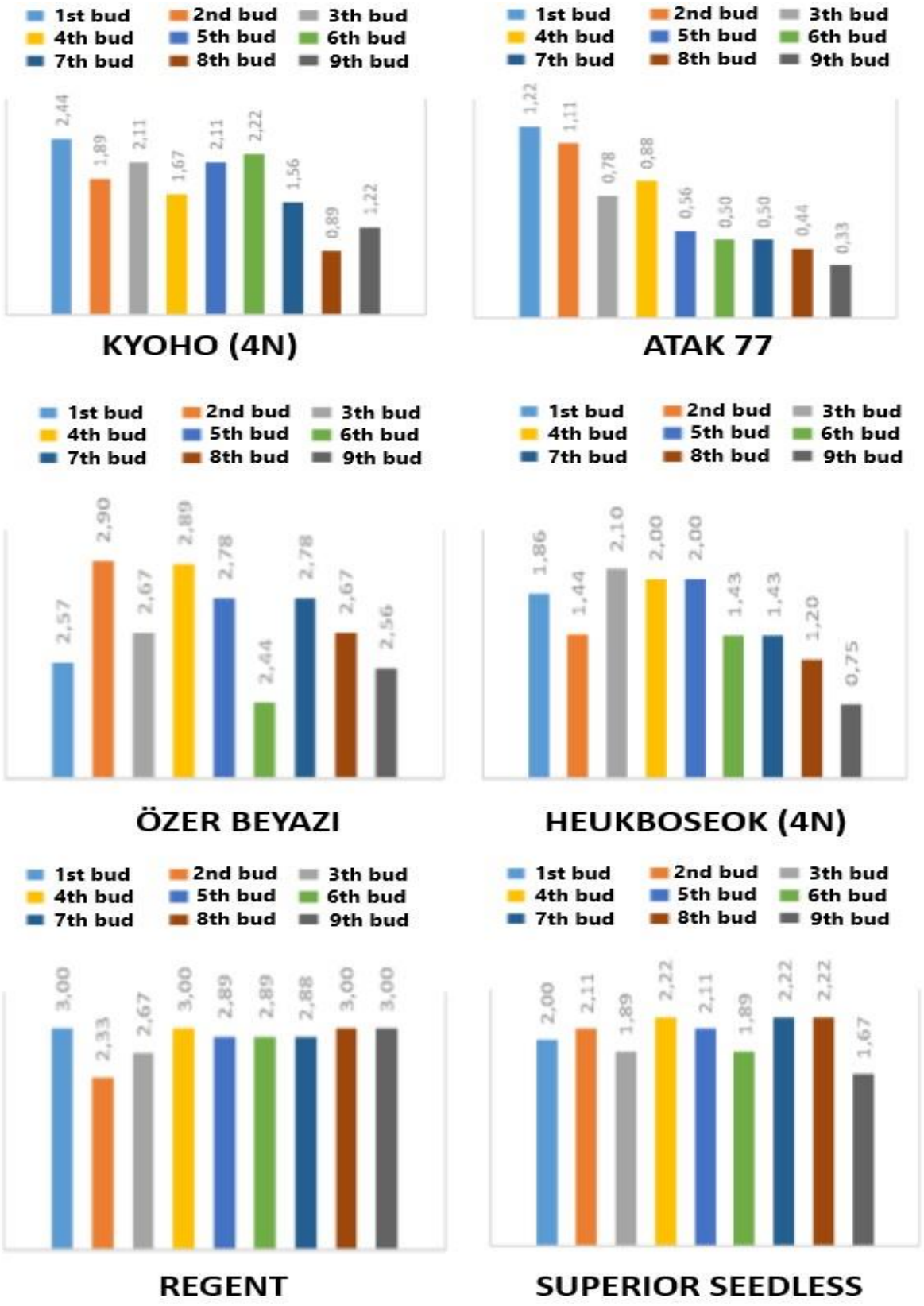


Figure 7. Root development level values of the buds according to the variety. a) Kyoho (4N), b) Atak 77, c) Özer Beyazı, d) Heukboseok (4N), e) Regent, f) Superior Seedless, g) Crimson Seedless, h) Isabella, ı) Rizpem, j) Kishmish Rozovyi, k) Müşküle and l) Muscat Bleu

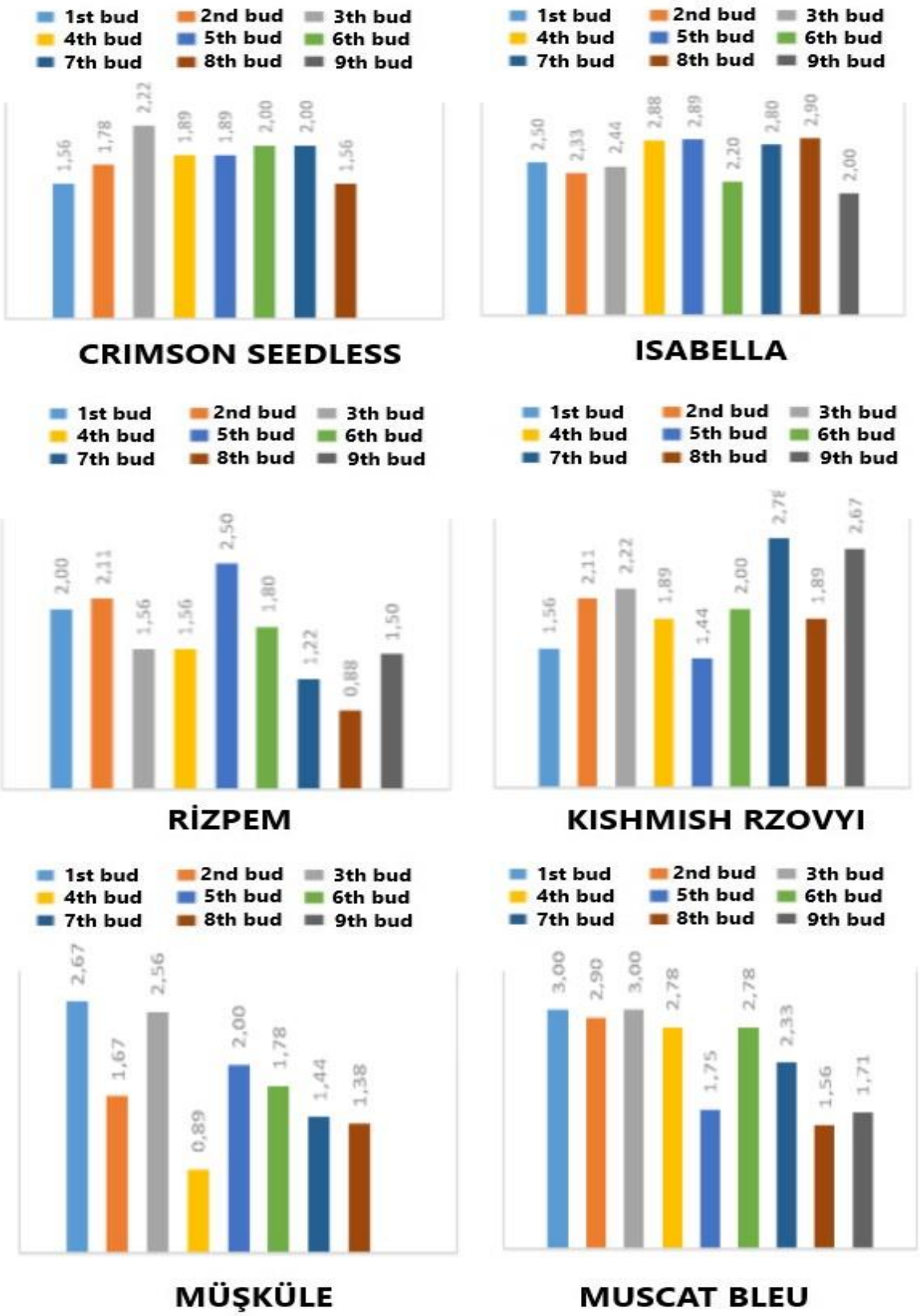


Figure 7. Root development level values of the buds according to the variety. a) Kyoho (4N), b) Atak 77, c) Özer Beyazı, d) Heukboseok (4N), e) Regent, f) Superior Seedless, g) Crimson Seedless, h) Isabella, ı) Rizpem, j) Kishmish Rozovyi, k) Müşküle and l) Muscat Bleu (continue).

When we evaluate the shoot lengths on a variety basis, the results are as follows (Figure 8): While the best result was obtained in the shoot length parameter of the Kyoho variety, which is an interspecific hybrid and has a 4N polyploidy level, the 4th bud gave the best result, the shortest shoots were detected in the 9th bud. When the shoot length values of the Atak 77 variety belonging to the *V. vinifera* species were examined, it was observed that the shoot extending from the 4th bud gave the best result, while the 5th bud had shorter shoots. In the Özer Beyazı variety belonging to the same species, it was observed that the shoot length gave the best result in the 3rd bud, while the 7th bud gave the shortest shoots. While the best result was obtained in the shoot length of the Heukboseok variety, which is an interspecific hybrid with a 4N polyploidy level, the 7th and 9th buds had the shortest shoots.

Table 4. Average shoot length values of the buds based on variety

LENGTH OF SHOOT*									
VARIETY	**1st bud**	**2nd bud**	**3th bud**	**4th bud**	**5th bud**	**6th bud**	**7th bud**	**8th bud**	**9th bud**
Kyoho (4N)	1,33^{h}	1,67^{e}	1,71^{f}	2,20^{d}	1,38^{f}	1,33^{i}	1,43^{h}	1,14^{j}	1,00^{e}
Atak 77	1,00^{k}	1,44^{f}	1,44^{i}	1,63^{g}	0,78^{l}	1,40^{g}	1,57^{f}	1,22^{g}	1,22^{d}
Özer Beyazı	1,86^{d}	2,30^{b}	2,67^{b}	2,33^{c}	1,78^{c}	1,67^{e}	1,56^{g}	2,11^{b}	2,00^{a}
Heukboseok (4N)	1,57^{f}	1,67^{e}	2,20^{d}	2,00^{e}	1,63^{e}	1,43^{f}	1,00^{k}	1,20^{i}	1,00^{e}
Regent	1,78^{e}	1,22^{h}	1,56^{h}	1,56^{h}	1,13^{j}	1,25^{j}	1,63^{e}	1,22^{h}	1,22^{d}
Superior Seedless	1,38^{g}	1,44^{g}	1,56^{h}	1,56^{h}	1,33^{g}	2,11^{c}	1,67^{d}	1,56^{e}	1,00^{e}
Crimson Seedless	1,11^{j}	0,89^{i}	1,44^{i}	1,13^{j}	1,00^{k}	1,22^{k}	1,25^{i}	0,75^{l}	-
Isabella	2,00^{b}	1,50^{f}	2,22^{c}	2,33^{c}	1,71^{d}	1,75^{d}	1,71^{c}	2,00^{c}	**2,00^{a}**
Rizpem	1,25^{i}	1,22^{h}	1,11^{j}	1,33^{i}	1,14^{i}	1,10^{l}	1,10^{j}	1,00^{k}	1,00^{e}
Kishmish Rozovyi	1,78^{e}	1,89^{d}	2,11^{e}	2,38^{b}	2,11^{b}	2,33^{b}	2,22^{b}	1,89^{d}	1,78^{c}
Müşküle	1,89^{c}	2,00^{c}	1,67^{g}	1,67^{f}	1,22^{h}	1,33^{h}	1,56^{g}	1,25^{f}	-
Muscat Bleu	**3,00^{a}**	**2,90^{a}**	**2,78^{a}**	**2,89^{a}**	**3,00^{a}**	**2,67^{a}**	**2,67^{a}**	**2,22^{a}**	1,86^{b}

* Lowercase letters indicate that the difference between varieties is statistically significant. LSD is significant at the 0.05 level.

While the best result was obtained in the shoot length of the Regent variety, which is an interspecific hybrid, in the 1st bud, the shortest shoots were observed in the 5th bud. In the Superior Seedless variety belonging to the species *V. vinifera*, the best shoot length was given in the 6th bud, while the shortest shoots were detected in the 9th bud. While it was observed that the best shoot length was given in the 7th bud of the Crimson Seedless variety belonging to the same species, it was determined that the shortest shoots were given in the 8th bud. In the Isabella variety belonging to the species *V. labrusca*, the 4th bud gave the best shoot length. The shortest shoot length was seen in the 2nd bud in the same variety. While the best results were obtained in shoot length in the Rizpem variety belonging to the same species, the shortest shoots were found in the 8th and 9th buds. While the 4th bud of the Kishmish Rozovyi variety belonging to the species *V. vinifera* showed the best result regarding shoot length parameter, it was determined that the 1st and 9th buds gave the shortest shoots. In the Müşküle variety belonging to the same species, the best shoot length was seen in the 2nd bud, and it was observed that the shortest shoots were given in the 5th bud. In the interspecific hybrid Muscat Bleu variety, the best results were obtained from the 1st and 5th buds in shoot length, while the shortest shoot was found to be from the 9th bud.

Leaving enough bunches on the annual branches during winter pruning is important to create a crop load. One of our main goals in this study was to determine the productivity of the buds on the annual branches and to reveal the most appropriate pruning method. When we look at the studies conducted by different researchers, it is reported that bud productivity values can give different results depending on the variety and the location of the bud on the annual branch. In general, it is stated that there is an increase in bud productivity towards the middle level of the one-year-old branches, but productivity decreases towards the ends of the branch (except for seedless varieties) (Akın et al., 2011; Piras et al., 2014; Uyak and Doğan, 2023). These results are mainly in line with the results obtained in our study. However, it is not very correct to generalize, and it is still helpful to determine the most productive buds with preliminary trials on variety of bases. This way, it can clearly state which varieties should be pruned short, medium and long. Şen and Atak (2020) used some of the table grape varieties we used in our study in their study to determine the bud productivity of some table grape varieties. In the study, while the most productive buds showed similar results to ours in some varieties, they obtained different results in the Atak 77 (2nd or 3rd bud) variety. Different researchers report that many factors affect bud productivity values and that factors such as climate, location, soil and nutritional conditions may be effective.

Also, Çelik et al. (2015) reported that the bud fertility of genotypes belonging to *V. labrusca* species was higher than that of genotypes belonging to *V. vinifera* species. Their study found bud productivity values ranging from 0.34 to 3.82 in *V. labrusca* genotypes. Similarly, in our study, interspecific hybrid varieties carrying *V. labrusca* blood and the *V. labrusca* species İsabella variety showed higher bud productivity than *V. vinifera* varieties.

Gutiérrez-Gamboa et al. (2018) evaluated bud productivity with a Chilean variety (Carménère). Bud productivity reached an average of 1.3 clusters per bud, min. 0.9 and max. 1.7. Accordingly, Carménère showed

low productivity in base buds. In our study, this situation varied by variety and generally, higher bud productivity values were reached

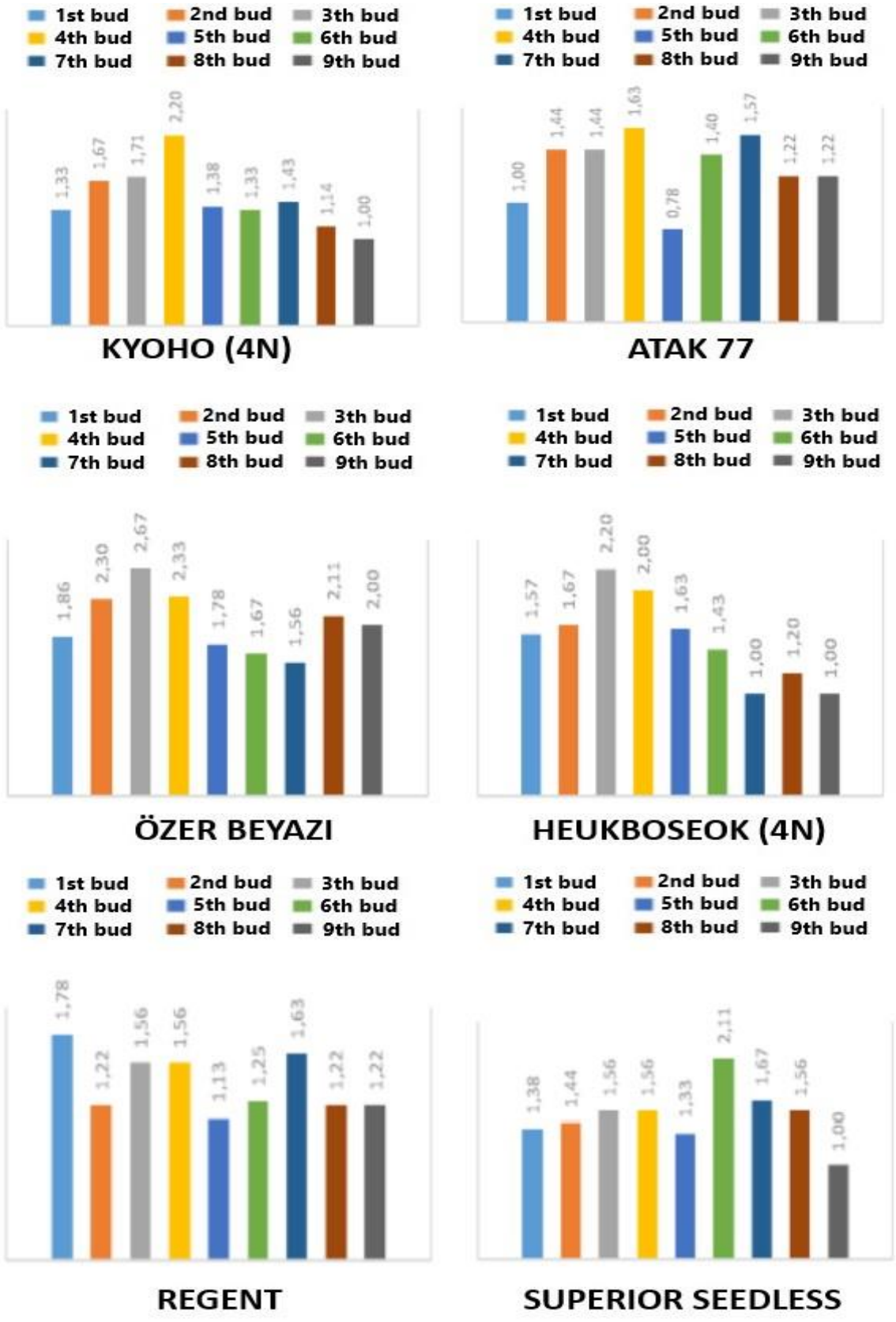


Figure 8. Average shoot length values of the buds based on variety. a) Kyoho (4N), b) Atak 77, c) Özer Beyazı, *d) Heukboseok* (4N), e) Regent, f) Superior Seedless, g) Crimson Seedless, h) Isabella, ı) Rizpem, j) Kishmish Rozovyi, k) Müşküle and l) Muscat Bleu

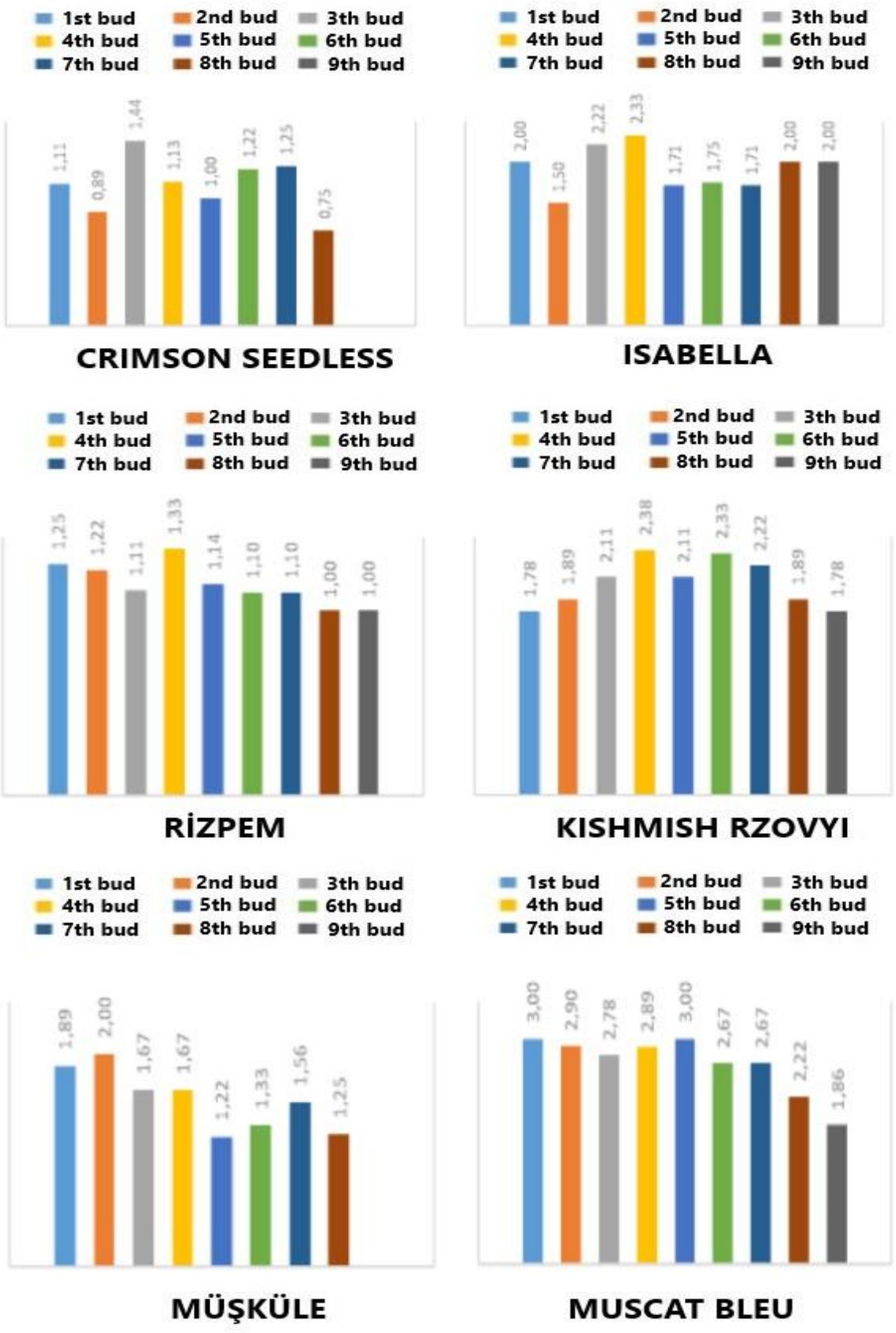


Figure 8. Average shoot length values of the buds based on variety. a) Kyoho (4N), b) Atak 77, c) Özer Beyazı, *d) Heukboseok* (4N), e) Regent, f) Superior Seedless, g) Crimson Seedless, h) Isabella, ı) Rizpem, j) Kishmish Rozovyi, k) Müşküle and l) Muscat Bleu (continue).

Buttrose (1974) reported that when the buds on the annual branch experience water stress, the formation of the flower cluster primordia, which occurs due to carbohydrate deficiency in the buds at the nodes at the tip of the branch, decreases. Our study observed that the water requirement of single-bud cuttings increases when the buds burst and leaf formation begins. This situation may cause differences among varieties and changes in the number of cluster primordia, which may cause changes in bud productivity. According to this prediction, studies on bud productivity should be conducted in the long term, and more definitive results should be obtained with different pruning practices in the field. Kliewer and Dokoozlian (2005) reported that the number of clusters is primarily considered in determining bud productivity and that the difference in the number of clusters is effective not only on yield but also on the quality and size of the fruit. Leao et al. (2017) reported an increase in bud productivity according to the position of the buds on the annual branch in the varieties they studied, and this increase reached the highest productivity in the 8th bud. Similar results were observed in A1105 and BRS Clara seedless hybrid grapes. Similarly, in the seedless Kishmish Rozovyi, Superior Seedless, and Özer Beyazı varieties we used in our study, it was observed that the tip buds were more productive.

It is also reported that pruning practices performed during the growing season may have an effect on bud productivity. For example, it is reported that summer pruning practices (Türk and Köse, 2024) and autumn pruning (De Souza et al., 2024) may have a positive effect on bud productivity.

Zinni et al. (2023) investigated the effects of some applications on bud productivity in two different growing environments in the Michele Palieri grape variety during two vegetation periods. They reported that the 9th and 10th buds maintained in the climate chamber were productive, while the 1st and 2nd buds had the lowest productivity. It was determined that the 5th and 6th buds of the buds maintained in the vineyard were the most productive, while the lowest yield was in the 1st, 2nd, 11th and 12th buds. In our study, the fact that the bud productivity values between the 1st and 9th buds of the 12 varieties we maintained in the climate chamber conditions may sometimes differ from similar studies in the literature can also be explained by this study above. Because the conditions obtained in the field may sometimes not coincide with the results obtained in the climate chamber. For this reason, it is thought that it would be appropriate to make observations in the field for more reliable results in such studies. Şahin and Dardeniz (2023) reported in their study that bud productivity values may sometimes increase up to the 10th node. Therefore, it is recommended that these studies should be carried out with as many buds as possible, as in our study. Dardeniz and Kısmalı (2005) reported that bud productivity studies are carried out not only to reduce product load but also to determine the appropriate pruning to reduce summer pruning practices and positively affect quality. In varieties where sufficient product can be obtained with short pruning, medium or long pruning creates excessive product load and quality decreases. It has been reported that longer pruning can be done in some varieties (especially seedless) to obtain sufficient yield. Our study shows that short or medium pruning is sufficient in seeded varieties, while seedless varieties should be pruned longer. However, sometimes short pruning can be preferred, especially in seeded varieties, to avoid excessive product load and to ensure higher-quality grain formation.

CONCLUSION

Grape varieties and genotypes of the *Vitis* species have been produced for many years for different purposes. The bud productivity of varieties belonging to this species must also be determined for the purpose of product programming during winter pruning. Bud productivity is a very important feature determined by genetic and environmental factors, and adjusting the crop load on the vine with pruning is of great importance for quality production. Bud productivity is important in determining newly developed grape varieties with high yield and potential, but these values vary according to the buds' variety and location. Our study determined bud productivity values and the number of buds left on annual shoots during winter pruning for grape varieties with different species and polyploidy levels.

Crimson Seedless and Superior Seedless varieties belonging to the *V. vinifera* species are less productive than other seedless varieties. Sometimes, the desired results cannot be obtained from long pruning applications because their bud productivity values vary. Therefore, a mixed pruning application may yield better results for these two varieties. Different results were obtained on a variety basis when looking at seeded varieties. Especially if it is planned to obtain high yield with the trellis system, long pruning can give better results in some varieties (such as Atak 77 and Muscat Bleu) as an alternative to short or medium pruning. Short and medium pruning in other varieties seems sufficient when bud productivity values are considered. However, in varieties or years with high yields, sometimes this situation can affect product quality and thinning is needed. Therefore, short pruning applications can also be recommended to increase fruit quality and sometimes to save on thinning labour. Many factors can affect bud's productivity, including pruning, fertilisation, and other cultural practices performed in the previous season. So, it is recommended that all maintenance practices be carried out meticulously throughout the growing season.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

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Declaration of Interests

The authors state there is no competing interest.

Author contribution

The contribution of the authors to the present study is equal. Also this study includes some of the studies from Kevser Çorak's master thesis.

Data availability

Data will be made available on request.

Consent to participate

The authors consent to participate.

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Effect of low nitrogen stress on plant growth traits of double haploid melon (*Cucumis melo* var. *cantalupensis*) lines with different low nitrogen Tolerances

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Abstract

In recent years, excessive nitrogen (N) fertilizer application has adversely affected the ecosystem. Additionally, inefficient fertilizer use can diminish soil fertility and result in the loss of organic matter. Limiting access to N fertilizer leads to increased prices, which consequently results in reduced crop productivity. Developing/breeding nitrogen-efficient plants is another strategy to enhance N uptake and efficiency in crops. The aim of this study was to assess the genotypic differences of 27 double haploid (DH) melon genotypes under low nitrogen (0.3 mM) conditions based on biomass parameters in hydroponic growth conditions. Significant differences were determined among the genotypes in all the parameters investigated. At low nitrogen levels, the highest stem fresh weight was recorded in genotypes C19 (48.53 g/plant) and D10 (42.57 g/plant), while the highest leaf fresh weight was observed in genotypes C8 (51.57 g/plant) and C19 (51.37 g/plant). In plants subjected to low N, the highest stem dry weight was found in genotypes E13 and C19, whereas the lowest was recorded in genotypesB9 and C5 with 2.10 g/plant. Under low nitrogen conditions, genotypes I7 and CA7 exhibited the highest root fresh and dry weights, respectively. The average shoot/root ratio of melon genotypes under low nitrogen conditions was 1.40, with the highest ratio in genotype B5 (2.69) and the lowest in genotype I7 (0.76). Melon genotypes had an average root length of 4000.92 cm under low nitrogen conditions, with genotype I7 having the longest root with 8194.43 cm and genotype B5 having the shortest root with 1795.34 cm. Under low nitrogen stress, genotypes displayed significant variation in plant growth. In terms of shoot fresh weight, there was a two-fold difference between susceptible and tolerant genotypes, while for root fresh weight, the difference was four-fold. This indicates that tolerance to nitrogen is attributed to changes in the root system rather than the shoot system.

Keywords: Melon, Low nitrogen, Nitrogen efficiency, Hydroponic culture

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INTRODUCTION

Melon is an important species of the *Cucurbitaceae* family. The global melon production is 29.541.294 tons, with China being the largest producer at 14.454.741.3 tons, followed by India with 1.498.000 tons and Türkiye with 1.403.214 tons (FAO, 2024). The yield per hectare in China is 36.4 ton, while in Türkiye it is 25.3 ton. Melon yield varies from country to country and from different growing regions within the same country. Yield is highly dependent on climate, soil, moisture, plant nutrition, disease and pest control, variety, and many other factors (Gorski, 2019). In recent years, chemical fertilizers are the most important input sources that directly affect plant growth and crop yield in both high-input and low-input agricultural systems, and among these sources, nitrogen is

the most needed and widely used nutrient element in crop production (Ulas et al., 2012, 2019; Goyal & Huffaker, 2015; Yam et al., 2020).

Nitrogen is one of the most important macronutrients that promotes crop growth and yield by playing multiple roles in biochemical and physiological functions in plant development such as synthesis of amino acids, proteins and enzymes (Ma et al., 1997; Souri & Dehnavard, 2018; Xiong et al., 2018; Janpen et al., 2019). It has been reported that insufficient N in vegetable species leads to reduced leaf number, leaf size, leaf chlorophyll content, reduced fruit yield and biomass, and delayed plant development and fruit production associated with a decrease in photosynthetic rate (Castellanos et al., 2011; Peña-Fleitas et al., 2015; Souri & Dehnavard, 2018; Xiong et al., 2018; Cavalcante et al., 2019). High N fertilization increased vegetative growth in plants, but yield losses associated with N over-fertilization have been detected in crop plants such as lettuce, pineapple, watermelon, cherry, and strawberry (Neilsen et al., 2007; Andriolo et al., 2011; Peña-Fleitas et al., 2015). In addition, excess N can be toxic to plants by altering their biochemistry and accumulating in plants, resulting in an imbalance of different nutrients and poor fruit quality (Fernández-Escobar et al., 2006; Goyal & Huffaker, 2015). It has been shown by many researchers that there are large differences between genotypes of the same species, especially in nitrogen efficiency (N-uptake and N-utilization) (Ruiz et al., 2006; Hoque et al., 2010; Ulas et al., 2012; Inkham et al., 2021 This genotypic variation serves as a tolerance mechanism against various biotic and abiotic stresses (salt, pH, nutrient deficiency, heavy metals, etc.) that adversely affect plant growth, and has allowed the yield and quality of the plant to increase even under adverse environmental conditions (Coskun, 2023; Coskun, 2025). A genotype is considered nitrogen-efficient if it produces a yield higher than the average of other genotypes under very low nitrogen conditions (Graham, 1984). Conversely, a genotype that responds positively to additional nitrogen under high nitrogen conditions and achieves a higher yield than other genotypes is also characterized as a nitrogen-efficient genotype (Balcha, 2014; Ulas et al., 2019). Improving nitrogen use efficiency (NUE) of crops is important for sustainable agricultural input and crop production (Xu et al., 2011; Xiong et al., 2018). Low N utilization by plants indicates that uptake is inefficient or higher than the plant needs (Anas et al., 2020). Crops such as rice, wheat, and maize require large amounts of N for healthy growth and higher yields (Linquist et al., 2012). Therefore, varieties with higher NUE should be a priority for breeders developing new varieties (Balyan et al., 2016; Mălinaş et al., 2022). For this purpose, determining the nitrogen efficiency of existing genetic resources constitutes the first stage of the breeding studies to be initiated. The aim of this study was to determine the genotypic differences of melon genotypes in low nitrogen (0.3 mM) application according to biomass parameters under hydroponic conditions.

MATERIALS AND METHODS

Plant Material

The study was conducted in the R&D greenhouse of Kırşehir Ahi Evran University in 2023. In the hydroponic culture test, 27 double haploid (DH) melon genotypes were used as plant material and Çıtırex, Altınbaş, Margot and Isovac melon varieties were used as controls (Table 1). DH melon genotypes were selected from the genotypes produced in project number TOGTAG-1430. DH melon lines were developed by induction with irradiated pollen from genetic resources collected from protected melon cultivation areas in Türkiye (Sarı et al., 1999). The authors are grateful to the project coordinator Prof. Dr. Nebahat SARI for her support in providing seeds. The genotypes were grown in Kırşehir and seeds were produced by selfing.

Table 1. List of plant melon genotypes used in the study.

Code	Description	Code	Description
C13	Double Haploid	D25	Double Haploid
C10	Double Haploid	I5	Double Haploid
G34	Double Haploid	B5	Double Haploid
E13	Double Haploid	C19	Double Haploid
H14	Double Haploid	D5	Double Haploid
D10	Double Haploid	C8	Double Haploid
E25	Double Haploid	E21	Double Haploid
C7	Double Haploid	E34	Double Haploid
G7	Double Haploid	B9	Double Haploid
E19	Double Haploid	H5	Double Haploid
E8	Double Haploid	C5	Double Haploid
K2	Double Haploid	MO	Margot (Diploid)
G20	Double Haploid	ISO	Isovac (Diploid)
I7	Double Haploid	GA7	Altınbaş (Diploid)
H1	Double Haploid	CA7	Çıtırex (Diploid)
G35	Double Haploid		

Sowing Seeds and Growing Seedlings

The melon seeds used in the hydroponic culture test were sown in multipots filled with peat:perlite (2:1) mixture with an electrical conductivity of 0.4 dS/m and a pH of 5.8. Fifty seeds of each genotype were planted. After germination, the seedlings were grown in a greenhouse at 24/18˚C (day/night) and 60% relative humidity.

Hydroponic Culture Low Nitrogen Application

The roots of the seedlings at the 2-3 true leaf stage were washed with tap water and cleaned from the growing medium and then planted in 130 liter plastic pots with 14 plants in each pot. The top surface of the pots was covered with composite and the plants were placed in the holes on the composite with equal spacing. The pots were placed on the growing tables at 40 cm distances. The growing solution was regularly aerated with an air pump. The composition of the water culture nutrient solution in the experiment was 1125 µM $Ca(N0_3)_2$, 375 µM $(NH_4)_2SO_4$ (Low-nitrogen: 0.3 mM N), 750 µM K_2SO_4, 650 µM $MgSO_4$, 500 µM KH_2PO_4, 10 µM H_3BO_3, 0. 5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $MoNa_2O_4$, and 80 µM Fe EDDHA (modified Hoaglan solution). The electrical conductivity (EC) of the solution was kept at 1.80 dS/m and pH between 6.5-7. The experiment was carried out according to the random plots experimental design with 3 replications and 4 plants (two pots) in each replicate. The study was conducted under controlled greenhouse conditions (22-24°C day /16-18°C night and 60% relative humidity) for 30 days.

Parameters Investigated in the Hydroponic Culture Experiment

Root and shoot fresh and dry weight

After harvesting, fresh weights (g/plant) of roots, shoots and leaves were determined using a digital weighing scale with an accuracy of 0.01. To determine dry weights, plant organs were dried in an oven at 65°C until they reached constant weight and then weighed.

Shoot/root ratio

Root/shoot ratio was calculated by dividing the root dry weight by the shoot dry weight (stem and leaves) with the following formula. Shoot/root ratio= (stem dry weight+leaf dry weight)/root dry weight).

Root length and volume

At the end of the experiment, the harvested plant parts were separated into roots, leaf and stems, the fresh weight of the root was recorded and the whole root was cut into small pieces of 1 cm with scissors. Then, all of these small cut root pieces were placed in the tray of a special scanner connected to a computer and some water was added to spread the roots homogeneously in the tray. Afterwards, the scanner was closed and the root length (cm) and volume (cm^3) of the root morphological parameters were calculated with a special root imaging program (WinRhizo Regular LA2400, Regent Instruments).

Leaf area

After determining the number of leaves of the harvested plants, the total leaf area of each genotype was determined in cm^2 using the LI 3100 C Model leaf area measuring device.

Statistical Analysis

The data obtained from the study were analyzed by one-way analysis of variance (ANOVA) using SPSS 18.0 statistical software (IBM, Chicago, IL, USA) at 5% significance level and the difference between the means was determined by Duncan multiple comparison test. In addition to this the data were analyzed by principal component analysis and cluster analysis using XLSTAT Software (XLSTAT, USA) statistical program.

RESULTS

The results of stem and leaf (fresh and dry) weights of 31 different melon genotypes tested using a low nitrogen dose (0.3 mM) of hydroponics nutrient solution are given in Table 2. Stem and leaf fresh and dry weights were significantly affected by genotypes. At low nitrogen dose, the average stem fresh weight was 36.40 g/plant, while the highest stem fresh weight was measured in genotypes C19 (48.53 g/plant) and D10 (42.57 g/plant). Genotypes C5 (21.57 g/plant) and B9 (22.57 g/plant) had the lowest stem fresh weight. Under low N conditions, leaf fresh weight ranged from 51.57 g/plant to 26.00 g/plant. The highest leaf fresh weight was measured in genotypes C8 and C19 (51.57 and 51.37 g/plant, respectively) and the lowest in genotype G7 (26.00 g/plant). Similar to the fresh weights, significant differences in dry weight were determined among the genotypes with low N application. The highest stem dry weight was recorded in plants of E13 and C19, while the lowest was 2.10 g/plant in genotypes B9 and C5. Under low N conditions, the average leaf dry weight was 3.88 g/plant, while the highest leaf dry weight was 6.57 g/plant in genotype C19 and the lowest in genotypes B9 (3.60 g/plant), GA7 (3.63 g/plant) and E34 (3.77 g/plant) (Table 2).

Genotype variation was found significant for root development under low nitrogen stress. The average root fresh weight was 55.22 g/plant, while the average root dry weight was 2.48 g/plant. Genotypes I7 and CA7 had the highest root fresh and dry weights, respectively, under low nitrogen conditions. Genotypes B5 and B9 had the lowest root fresh weight, while genotypes B5, C10 and B9 had the lowest root dry weight. Under low nitrogen

conditions, the average shoot/root ratio of melon genotypes was 1.40, the highest shoot/root ratio was determined in genotype B5 (2.69) and the lowest ratio was determined in genotype I7 with 0.76 (Table 3).

Table 2. Stem and leaf (fresh and dry) weights of melon genotypes under low N conditions in the hydroponic culture.

Genotype	Stem Fresh Weight (g/plant) Mean±SE	Leaf Fresh Weight (g/plant) Mean±SE	Stem Dry Weight (g/plant) Mean±SE	Leaf Dry Weight (g/plant) Mean±SE
B5	28.27±3.50e-i	38.03±2.45a-h	2.90±0.40j-l	4.40±0.61e-h
B9	22.57±7.60hi	31.53±5.39d-h	2.10±0.87k	3.60±0.69h
C10	37.17±2.40b-d	29.40±7.17f-h	4.83±0.57a-e	4.03±0.25gh
C13	38.93±1.21b-d	40.50±2.94a-g	3.73±0.57c-j	4.97±0.40b-h
C19	48.53±3.19a	51.37±5.34a	5.57±0.45ab	6.57±0.83a
C5	21.57±1.16i	33.40±1.04b-h	2.10±0.20k	3.80±0.10gh
C7	36.87±1.16b-f	46.83±0.99ab	4.43±0.15b-h	5.30±0.10a-g
C8	38.07±0.85b-d	51.57±1.80a	4.40±0.20b-h	6.17±0.15a-c
CA7	41.13±2.40a-c	45.23±3.16a-d	4.77±0.21a-e	5.80±0.44a-e
D10	42.57±0.35ab	35.70±1.81b-h	4.20±0.20c-j	4.23±0.15f-h
D25	32.47±0.81c-g	31.13±1.53e-h	3.40±0.17g-l	4.03±0.32gh
D5	36.40±5.03b-f	34.30±11.33b-h	3.40±0.56g-l	4.50±1.14d-h
E13	40.90±2.69a-c	32.00±3.04c-h	6.00±0.40a	4.00±0.46gh
E19	31.77±7.16c-h	27.03±3.52gh	4.53±1.16b-g	4.33±0.25e-h
E21	38.63±3.36b-d	43.10±2.16a-f	4.07±0.31c-j	4.57±0.59d-h
E25	37.17±0.29b-d	39.43±3.95a-h	5.07±0.40a-c	4.77±0.49c-h
E34	34.63±2.61b-f	30.93±1.79e-h	3.57±0.47e-k	3.77±0.15h
E8	36.33±1.53b-f	43.33±1.53a-e	4.17±0.21c-j	4.50±0.00d-h
G20	27.50±0.99f-i	45.37±1.58a-c	3.53±0.06e-k	6.00±0.36a-d
G34	35.90±1.25b-f	35.13±0.67b-h	3.97±0.21c-j	3.87±0.21gh
G35	32.67±0.80c-f	39.70±2.19a-h	3.00±0.30i-l	4.63±0.31d-h
G7	29.87±7.23d-i	26.00±14.11h	3.83±0.42c-j	4.50±0.36d-h
GA7	23.20±1.00g-i	37.43±3.27b-h	2.33±0.29kl	3.63±0.46h
H1	32.60±0.70c-g	37.13±1.76b-h	3.10±0.20h-l	4.17±0.38f-h
H14	34.70±0.76b-f	36.77±2.12b-h	3.70±0.17d-j	4.07±0.06gh
H5	34.77±0.47b-f	40.03±1.57a-g	3.53±0.49e-j	4.53±0.15d-h
I5	31.30±0.44d-h	38.80±1.56a-h	3.17±0.12h-l	4.47±0.35e-h
I7	38.33±0.31b-d	47.03±1.25ab	4.23±0.15b-j	6.33±0.46ab
İSO	40.97±1.07a-c	34.87±2.14b-h	4.33±0.42b-i	4.97±1.04b-h
K2	4538±1.31e-i	34.13±1.63b-h	3.43±0.32f-l	4.10±0.00f-h
MO	38.87±1.22b-d	39.73±1.10a-g	5.00±0.44a-d	5.60±0.53a-f
Mean	34.60	37.97	4.65	3.88
Min	21.57	26.00	2.10	3.60
Max	48.53	51.57	6.00	6.57
p value	0.001	0.008	0.001	0.006

Means that do not share a letter are significantly different. ISO: Isovac, MO: Margot, GA7: Altınbaş, CA7: Cıtırex. SE: Standart Error. $P \leq 0.01$: Very strong evidence, the result is considered very significant. $P \leq 0.05$: Strong evidence, the result is considered significant. $P > 0.05$: Insufficient evidence, the result is not significant.

The average leaf area of melon genotypes grown at low nitrogen (0.3 mM) dose under hydroponics conditions was 1439.67 cm^2. The highest leaf area was found in genotypes C19 (2044.67 cm^2) and E25 (1964.67 cm^2), while the lowest leaf area was measured in genotypes B9 (946.00 cm^2) and D25 (1154.00 cm^2). Melon genotypes had an average root length of 4000.92 cm under low N conditions, the longest root length was found in genotype I7 with 8194.43 cm and the shortest root length was recorded in genotype B5 with 1795.34 cm. The average root volume under low N conditions was determined as 2.91 cm3, while the highest root volume was measured in genotype I7 (5.92 cm^3), and the lowest root volume was determined in genotype B5 (1.30 cm^3) as in root length (Table 4).

Principal component analysis (PCA) was used to classify melon genotypes based on biomass parameters in hydroponics. According to the analysis, two principal components (99.85% according to PC1 and 1.14% according to PC2) accounted for about 99% of the total variation. According to the PCA graphs, the genotypes on the left side of the red arrow in graph b produced less biomass under low nitrogen conditions than the genotypes on the right side of the arrow (Figure 1). Genotype C19 had the highest leaf area and shoot biomas in region I. of the graph (b), while genotype I7 had the highest root parameters in region IV. of the graph (b).

Table 3. Root (fresh and dry) weight and shoot/root ratio of melon genotypes under low N conditions in the hydroponic culture.

Genotype	Root Fresh Weight (g/plant) Mean±SE	Root Dry Weight (g/plant) Mean±SE	Shoot/Root RatioMean±SE
C13	24.63±2.23j	1.40±0.17k	2.69±0.11a
C10	36.50±12.48ij	1.57±0.55i-k	1.52±0.21b-f
G34	38.67±10.11h-j	1.53±0.55jk	1.77±0.27b-d
E13	70.83±11.62b-d	3.00±0.53b-f	1.15±0.25e-h
H14	57.07±6.49c-i	3.00±0.53b-f	1.76±0.19b-d
D10	37.87±1.86h-j	1.90±0.17f-k	1.45±0.07b-f
E25	63.07±4.91b-g	3.07±0.32b-e	1.33±0.10c-g
C7	67.63±5.49b-e	3.30±0.10a-c	1.33±0.07c-g
G7	82.13±15.65b	3.77±0.32ab	1.07±0.18f-h
E19	53.67±3.50d-i	1.93±0.12f-k	1.46±0.13b-f
K2	62.77±5.65b-g	2.87±0.12b-g	1.02±0.11f-h
G20	60.23±13.14b-h	2.60±0.70c-j	1.17±0.06e-h
I7	62.03±2.46b-g	2.33±0.21c-k	1.18±0.04e-h
H1	52.07±4.61d-i	2.63±0.15c-j	1.15±0.29e-h
G35	52.63±11.55d-i	2.60±0.61c-j	1.59±0.27b-e
D25	56.17±5.99c-i	2.17±0.23d-k	1.37±0.14c-g
I5	36.73±2.71ij	1.87±0.38g-k	1.80±0.25bc
B5	41.30±0.00f-j	2.60±0.00c-j	1.93±0.04b
C19	77.87±6.76bc	3.23±0.46a-d	0.94±0.10gh
D5	60.07±1.69b-h	2.23±0.15c-k	1.18±0.04e-h
C8	57.4±4.37c-i	2.27±0.25c-k	1.26±0.06d-h
E21	43.73±13.62f-j	1.90±0.27f-k	1.32±0.30c-g
E34	40.43±7.04g-j	2.07±0.23e-k	1.52±0.18b-f
B9	57.8±2.80c-i	2.37±0.25c-k	1.21±0.06e-h
H5	51.17±0.81d-i	1.73±0.21h-k	1.40±0.07c-g
C5	45.33±1.19e-j	2.20±0.36c-k	1.65±0.01b-e
E8	50.27±4.22d-i	2.70±0.44b-h	1.40±0.10c-g
GA7	112.43±6.49a	4.33±0.40a	0.76±0.04h
CA7	52.47±4.88d-i	2.67±0.12b-i	1.46±0.19b-f
MO	43.40±3.12f-j	1.77±0.12g-k	1.44±0.10b-g
İSO	63.60±4.17b-f	3.27±0.15a-d	1.24±0.11e-h
Mean	55.22	2.48	1.40
Min	24.63	1.40	0.76
Max	112.43	4.33	2.69
p value	0.001	0.001	0.001

Means that do not share a letter are significantly different. ISO: Isovac, MO: Margot, GA7: Antınbaş; CA: Cıtırex. SE: Standart Error. P ≤ 0.01: Very strong evidence, the result is considered very significant. P ≤ 0.05: Strong evidence, the result is considered significant. P > 0.05: Insufficient evidence, the result is not significant.

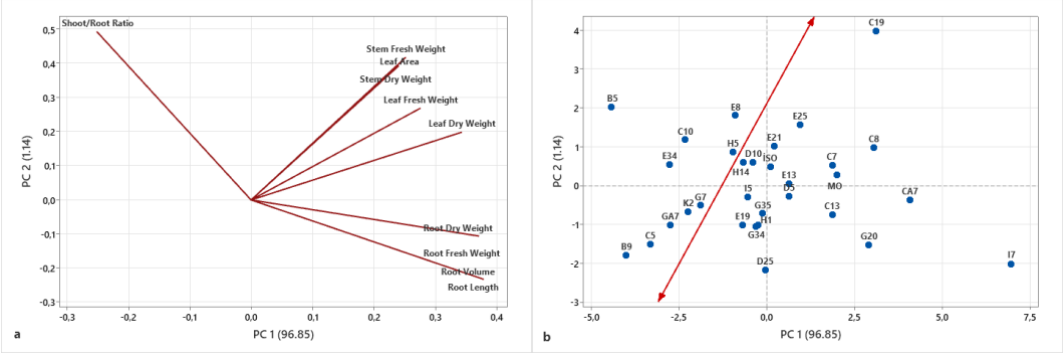


Figure 1. Two-dimensional PCA plot of melon genotypes with biomass traits under hydroponics low N conditions. ISO: Isovac, MO: Margot, GA7: Altınbaş, CA7: Cıtırex.

Cluster analysis was performed to determine the relationship between 31 melon genotypes using biomass parameters in hydroponics (Figure 2). In the dendrogram graph, 2 main clusters were formed, cluster I included

genotype I7, which had the highest values in terms of root parameters, while cluster II included 30 genotypes that were 35% similar to each other. Cluster II, which was 35% similar to each other, was divided into 2 sub-clusters. In the sub-cluster of cluster II with 63% similarity, genotypes with high values in terms of biomass parameters under low nitrogen conditions were located, while in the sub-cluster with 73% similarity, genotypes with low values in terms of biomass parameters were located.

Table 4. Leaf area, root volume, and diameter of melon genotypes under low N conditions in the hydroponic culture.

Genotype	Leaf Area (cm^2) Mean±SE	Root Length (cm) Mean±SE	Root Volume (cm^3) Mean±SE
C13	1237.00±75.80b-d	1795.34±162.50j	1.3±0.1174.00j
C10	946.00±41.10d	2660.21±909.00ij	1.92±0.65ij
G34	1305.33±139.70a-d	2818.13±737.00h-j	2.04±0.53h-j
E13	1396.67±25.20a-d	5162.51±847.00b-d	3.73±0.61b-d
H14	2044.67±185.00a	4159.16±473.00c-i	3±0.34c-i
D10	1203.33±77.80cd	2759.82±135.60h-j	1.99±0.098h-j
E25	1530.67±19.80a-d	4596.46±358.00b-g	3.32±0.26b-g
C7	1668.67±55.00a-d	4929.29±400.00b-e	3.56±0.29b-e
G7	1472.33±109.30a-d	5986.09±1141.00b	4.32±0.82b
E19	1373.67±114.50a-d	3911.36±255.00d-i	2.82±0.18d-i
K2	1154.00±18.10cd	4574.60±412.00b-g	3.3±0.29b-g
G20	1767.33±144.90a-c	4389.96±957.00b-h	3.17±0.69b-h
I7	1289.33±94.70b-d	4521.15±179.00b-g	3.26±0.19b-g
H1	1258.33±16.17b-d	3794.75±336.00d-i	2.74±0.24d-i
G35	1518.00±3.46a-d	3836.05±842.00d-i	2.77±0.61d-i
D25	1964.67±883.00ab	4093.57±437.00c-i	2.96±0.32c-i
I5	1297.67±96.50a-d	2677.22±197.00ij	1.93±0.14ij
B5	1500.00±0.00a-d	3010.05±0.00f-j	2.17±0.00f-j
C19	1557.33±49.20a-d	5675.12±493.00bc	4.1±0.36bc
D5	1223.67±26.50b-d	4377.81±122.90b-h	3.16±0.089b-h
C8	1418.67±101.40a-d	4183.46±319.00c-i	3.02±0.23c-i
E21	1386.00±24.20a-d	3187.40±993.00f-j	2.3±0.72f-j
E34	1329.00±109.10a-d	2946.88±513.00g-j	2.13±0.37g-j
B9	1390.67±97.80a-d	4212.61±204.00c-i	3.04±0.15c-i
H5	1797.67±853.00a-c	3729.16±58.09d-i	2.69±0.04d-i
C5	1530.00±42.90a-d	3304.01±86.30e-j	2.39±0.06e-j
E8	1479.33±75.60a-d	3663.56±308.00d-i	2.65±0.22d-i
GA7	1689.33±25.30a-d	8194.43±473.00a	5.92±0.34a
CA7	1297.67±31.20a-d	3823.90±356.00d-i	2.76±0.26d-i
MO	1201.33±26.70cd	3163.10±228.00f-j	2.28±0.16f-j
ISO	1401.33±82.20a-d	4635.33±304.0b-f	3.35±0.22b-f
Mean	1439.67	4024.92	2.91
Min	946.00	1795.34	1.30
Max	2044.67	8194.43	5.92
p value	0.001	0.001	0.001

Means that do not share a letter are significantly different. ISO: Isovac, MO: Margot, GA7: Altınbaş, CA7: Cıtırex. SE: Standart Error. $P \leq 0.01$: Very strong evidence, the result is considered very significant. $P \leq 0.05$: Strong evidence, the result is considered significant. $P > 0.05$: Insufficient evidence, the result is not significant.

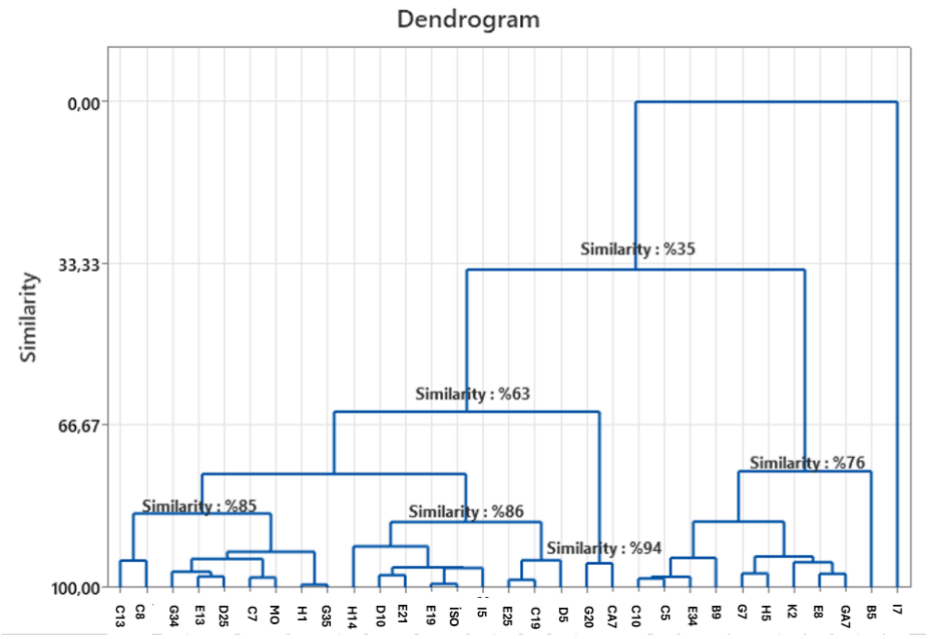


Figure 2. Dendrogram plot of melon genotypes with biomass traits under hydroponics low N conditions. ISO: Isovac, MO: Margot, GA7: Altınbaş, CA7: Cıtırex.

Regresyon analysis was performed to determine the relationship between stem, leaf, and root parameters of melon genotypes grown under low nitrogen conditions (Table 5). Since the significance level between root fresh weight, root length and root volume and stem fresh weight is $p<0.05$, the established regression (R^2) model is significant. According to the regression analysis results made for the prediction of the relationship, it is seen that root fresh weight, root length and root volume have a positive and weak effect on stem fresh weight. The R^2 value expressed as the explanatory power of all models was calculated as 0.187 ($R=0.433$; $R^2= 0.187$). This value shows that 18.7% of the stem fresh weight variable is explained by the independent variables in the model, namely root fresh weight, 18.7% by root length and 18.7% by root volume. 20.4% of the stem fresh weight variable is explained by the independent variable root dry weight. Since the significance level between root fresh weight, root dry weight, root length and root volume parameters and leaf fresh weight and leaf dry weight is $p<0.01$, the established regression model is significant. According to the results of the regression analysis performed to predict the relationship between these parameters, it is seen that the root fresh weight, root dry weight, root length and root volume parameters have a positive and strong effect on the leaf fresh weight and leaf dry weight parameters. Additionally, positive correlation (R) was found between all root parameters and stem fresh weight and leaf area parameters at 0.05 significance level, while positive correlation was found with leaf fresh and dry weight parameters at 0.01 level.

Table 5. Regresyon between stem, leaf and root parameters.

Parameters	Stem Fresh Weight			Leaf Fresh Weight			Stem Dry Weight			Leaf Dry Weight			Leaf Area		
	R	R^2	p	R	R^2	p	R	R^2	p	R	R^2	p	R	R^2	p
Root Fresh Weight	0.433	0.187	0.015	0.514	0.264	0.003	0.380	0.144	0.035	0.691	0.478	0.000	0.390	0.152	0.030
Root Dry Weight	0.451	0.204	0.011	0.649	0.421	0.000	0.391	0.153	0.030	0.809	0.654	0.000	0.396	0.157	0.027
Root Length	0.433	0.187	0.015	0.514	0.264	0.003	0.380	0.144	0.035	0.691	0.478	0.000	0.390	0.152	0.030
Root Volume	0.433	0.187	0.015	0.514	0.264	0.003	0.380	0.144	0.035	0.691	0.478	0.000	0.390	0.152	0.030

DISCUSSION

Under low nitrogen stress, significant changes in growth and development occur in most plant species. Previous studies have shown that plant adaptation to nutrient stress is mainly due to morphological changes and significantly suppresses plant growth (Zhao et al., 2005; J. Wang et al., 2019). In the present study, the responses of melon genotypes to low nitrogen stress in terms of biomass development were significantly different from each other. Since there is a positive correlation between root development and leaf and stem development, the genotypes with the highest root fresh and dry weight, root length, and volume had higher stem and leaf fresh and dry weight and leaf area than the other genotypes under low nitrogen conditions. Wahocho et al. (2017) reported that low nitrogen conditions reduced vegetative growth in two melon cultivars and the cultivars were affected differently by low nitrogen stress. Reducing nitrogen fertilization had a low effect on fruit marketable yield in tomato (-7.5%), but a significant effect on plant vegetative Growth (Bénard et al., 2009). In general, previous studies have reported that increasingly high N application has a negative effect on fruit quality and that reducing N application to a level that does not reduce yields can be achieved commercially in terms of quality and economically (less fertilizer input). In the case of prolonged N deficiency, plant leaves turn yellow, brown and die, eventually reducing the production and quality of agricultural products. According to the current literature, N deficiency-induced leaf chlorosis and reduced plant growth have been reported in many plants, including rice (Shin et al., 2018), potato (Wei et al., 2015), maize (Ning et al., 2018), sunflower (Agüera et al., 2010), tobacco (Wang et al., 2013), rapeseed (Pant et al., 2009), Arabidopsis (Scheible et al., 2004) and wheat (Cartelat et al., 2005). It has also been reported that limiting nitrogenous fertilizer to a level that does not reduce yield and plant growth has positive effects on fruit quality (Bénard et al., 2009; Wahocho et al., 2017). The results of this study confirmed previous studies and showed that there is a significant genetic variation and that low nitrogen conditions reduce plant growth in melon.

Approximately 199 million tons of NPK (Nitrogen-Phosphate-Potassium) were produced in the world in 2015, and nitrogen-based fertilizers accounted for the largest share at 57%. World nitrogen fertilizer production was 114 million tons in 2015, 52% of which was provided by China, India and the USA. In the same year, of the total NPK exports of 90.6 million tons, 38% of nitrogen (41 million tons) and 47% of phosphorus (17.5 million tons) were provided by China and Russia, and 51% of potassium (32.3 million tons) were provided by Canada and Russia. Nitrogen constitutes 43% of the total NPK imports of 88.5 million tons in the world. Of the 38.5 million tons of nitrogen imports, 36% was provided by the USA, India and Brazil. NPK fertilizer consumption, which increased by 20% compared to 2005, was approximately 184 million tons as of 2015; China ranked first with a consumption of 51 million tons. Türkiye increased its consumption amount, which was 2 million in 2005, by 7% in 2015 to 2.2 million tons (equivalent to 5.5 million tons in physical fertilizer consumption) (Tagem, 2018). Since nitrogen use and export are high in Türkiye, breeding of nitrogen-using plants is important for reducing the budget allocated to nitrogen in plant nutrition and for sustainable agriculture. For all these reasons, determining the responses of genotypes to low nitrogen conditions is important both economically and in terms of fruit quality.

CONCLUSION

It was found that low nitrogen doses in melon genotypes inhibited plant growth, yet the genotypes exhibited varied responses to low nitrogen conditions. Melon, which has a monoecious and andromoncios flower structure, has created great genetic diversity by cross-pollination with other melon species over the years. In this study, the genotypic differences of a population consisting of a limited number of DH melon genotypes against low nitrogen stress were revealed. Two and four-fold differences were detected in terms of shoot and root biomasses, respectively. The researchers believe that higher variation can be detected if larger populations are studied in terms of nitrogen use efficiency. It is thought that the determination of nitrogen-efficient genotypes in our study and similar studies will help develop high-quality melon varieties suitable for sustainable melon production in future research. Evaluation of the responses of genotypes showed superior characteristics in this screening experiment regarding vegetative growth, nitrogen use efficiency, yield and quality under varying nitrogen doses in the field or greenhouse environment will make this study meaningful.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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An investigation of the association of sleep quality and diet quality in adults

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Abstract

This study, which aimed to examine the relationship between sleep quality and diet quality in adults, was conducted on 50 adults residing in Bursa province and known not to use sleeping pills. The researchers via face-to-face interviews completed a questionnaire, querying the personal characteristics, sleep duration and sleep quality of the participants. The daily food consumption data of the participants were recorded using the retrospective recall method. Diet quality and sleep quality were measured using the Healthy Eating Index-2015 (HEI-2015) and Pittsburgh Sleep Quality Index (PSQI), respectively. Most of the participants were female (62%) and aged 25-44 years (68%). The groups with good and poor sleep quality significantly differed for caffeine intake ($p < 0.05$). No statistically significant correlation was observed between sleep quality and other nutrients including vitamin A, calcium and iron ($p > 0.05$). While no statistically significant correlation was determined between sleep quality and the HEI overall diet quality score ($p > 0.05$), the HEI components, the wholegrain score displayed a statistically significant negative correlation with the overall sleep quality score ($p < 0.05$). In this study, no statistically significant correlation was determined between overall diet quality and sleep quality, but caffeine intake and wholegrain consumption were ascertained to significantly affect sleep quality.

Keywords: Caffeine intake, Adult, Sleep quality, Diet quality, Wholegrain consumption

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INTRODUCTION

Sleep is an important biological function that is essential for optimum physical and mental health (Baranwal et al., 2023; Indrasari Utami et al., 2023). It is known that sleep disorders may increase the risk of certain types of cancer and death, as well as the symptoms of some gastrointestinal diseases (Medic et al., 2017).

In addition to homeostasis, which is effective in regulating sleep status, circadian rhythm is controlled by both genetic components and external factors such as nutrition and environment (Zhao et al., 2020; Cheon and Kim, 2022). Internal factors affecting sleep include genetic structure, hormones, and the presence of disease; external factors include the environment and nutrition (Pattnaik et al., 2022).

Although a link between sleep and diet has been reported, it has not been determined whether nutrition affects sleep or sleep affects nutrition (St-Onge et al., 2016). Nutrition affects not only the state of alertness during the day but also the quality of sleep, and it is known that proper nutrition significantly improves the quality of sleep (Sejbuk et al., 2022). The effect of diet on sleep has been associated with a variety of complex and interacting mechanisms (Zhao et al., 2020; St-Onge et al., 2023). Foods affect sleep quality primarily through serotonin and melatonin biosynthesis mechanisms in the body (Zuraikat et al., 2021). Tryptophan is the precursor of the serotonin neurotransmitter and melatonin hormone, which are involved in regulating sleep-wakefulness (St-Onge et al., 2023; Wilson et al., 2022). Many dietary metabolites can become bioactive and affect sleep directly or indirectly (Indrasari Utami et al., 2023). It is known that dietary intake of macronutrients (carbohydrates, fat and protein) can affect the time it takes to fall asleep, total sleep time and sleep quality via tryptophan metabolism (Cheon and Kim, 2022). High-carbohydrate diets may be associated with improved sleep quality and duration (Binks et al., 2020). The glycaemic index may play a role in shortening sleep onset latency and increasing the number of arousals

during sleep (Godos et al., 2021). In general, diets low in carbohydrates and high in fiber have been associated with better sleep quality (Wilson et al., 2022). In addition to macronutrients, caffeine can also affect sleep quality through parameters such as total sleep time and time to fall asleep (Gardiner et al., 2023). Caffeine has long been associated with sleep disruption (Alruwaili et al., 2023).

Further research is needed to confirm the hypothesis that nutrition-related variables are associated with sleep quality (Godos et al., 2021). Only very few studies have investigated overall diet quality indicators, instead of a single nutrient or food, for their correlation with sleep quality. In view of the lack of definitive literature data on the correlation of diet and sleep and the underlying mechanism of a possible correlation, the present study was designed to investigate the association of sleep quality with daily energy and macronutrient intake levels together with diet quality, as well as with food groups related to diet quality.

MATERIALS AND METHODS

This study was carried out on 50 adults, who had been referred to a private psychological counseling center, living in the Osmangazi district of Bursa province, between December 2020 and May 2021, were not diagnosed with any psychological disorder, were known not to be using any sleep-inducing or side effect-causing medication and were aged 18 years and older. Data were collected by face-to-face interviews and were analyzed with the SPSS software package. This study was conducted with the informed written consent of the participants, in accordance with the 1989 Declaration of Helsinki of the World Medical Association and was approved by the Ethics Board of the Medical Faculty of Bursa Uludag University.

The participants filled in a personal information sheet, including personal information such as age, sex and educational background.

The Pittsburg Sleep Quality Index (PSQI), which was developed and proven to be reliable and valid by Buysse et al. (1989) and adapted to Turkish by Agargun et al. (1996), was used to determine and assess sleep quality. PSQI is a sleep quality scale used to calculate seven individual component scores and an overall sleep quality score, based on participant responses given to 19 questions. This scale also includes questions addressing the inmate/sleeping partner of the respondent if present. It aims to assess sleep quality over the past month. Sleep quality is inversely proportional to the PSQI score. A PSQI score equal to or below five is consistent with good sleep quality, whilst a score above five is consistent with poor sleep quality.

The 24 hour-food consumption records of the participants were collected and assessed with an aim to determine their daily energy and nutrient intake levels. Recorded data were entered into the Nutrient Data Base (BEBIS) program, and energy and nutrient values were calculated. Diet quality was assessed using the Healthy Eating Index-2015 (Ruel, 2002), which was translated into Turkish. The HEI was developed by the United States Department of Agriculture's (USDA) Center for Nutrition Policy and Promotion (CNPP) and its most recent version was published in 2015 (Ruel, 2002). The HEI-2015 consists of 13 components, nine of which are related to diet adequacy and four of which are related to consumption moderation. Foods recommended to be included in the diet in adequate quantities include total fruits, whole fruits, greens and beans, total vegetables, wholegrains, dairy, seafood and plant proteins, total protein foods and fatty acids. On the other hand, foods recommended to be consumed at limited levels include refined grains, added sugar, sodium and saturated fats. The HEI score is directly proportional to the level of consumption of foods recommended to be included in the diet in adequate quantities and inversely proportional to the level of consumption of foods recommended to be consumed at limited levels. A total score equal to or below 50 points indicates a "poor diet quality", a total score between 51 and 80 points indicates "a diet quality that needs to be improved", and a total score between 81 and 100 points indicates a "good diet quality" (Krebs-Smith et al., 2018).

RESULTS AND DISCUSSION

Out of the 50 participants included in this study, 31 (62%) were female and 19 (38%) were male. While 42% of the participants were between the ages of 25-34, 26% were aged 35-44 years, 16% were aged 18-24 years, 12% were of 45-54 years of age and 4% were aged 55-64 years. Twenty-six (52%) participants had good sleep quality and 24 (48%) suffered from poor sleep quality. Most of the participants were university graduates (66%) and salaried employees (46%). The majority were living with their families (88%). The demographic characteristics of the participants, including sex, age, educational background, occupation, and lifestyle are listed in Table 1.

The daily energy and nutrient intake levels calculated using the 24 h-food consumption records of the participants, which were obtained using the retrospective recall method, are shown in Table 2. The mean dietary energy intake of the participants with good and poor sleep quality were calculated as 1,819.42 ± 779.61 kcal and 1,701.11 ± 732.25 kcal, respectively. The groups with good and poor sleep quality did not show any statistically significant difference for macronutrient and micronutrient levels ($p > 0.05$). The mean caffeine intake level of the participants with good sleep quality (64.14 ± 57.03) was significantly lower than that of the participants with poor sleep quality (112.35 ± 75.38) ($p < 0.05$).

Table 1. Distribution of the participants for their demographic characteristics

Characteristics	Number (n)	Percentage (%)
Sex		
Female	31	62.0
Male	19	38.0
Age (Years)		
18-24	8	16.0
25-34	21	42.0
35-44	13	26.0
45-54	6	12.0
55-64	2	4.0
Educational Background		
Primary school	1	2.0
Junior high school	1	2.0
High school	13	26.0
University	33	66.0
Advanced degree/Doctorate degree	2	4.0
Occupation		
Salaried employee	23	46.0
Student	13	26.0
Proprietor	4	8.0
Artist	1	2.0
Non-worker	9	18.0
Lifestyle		
Solitary	6	12.0
Living with family	44	88.0

The mean overall diet quality scores of the participants with good and poor sleep quality were 51.54 ± 15.07 and 49.11 ± 16.03, respectively. No statistically significant correlation was determined between the overall diet quality score and sleep quality (Table 3). The correlation between the components of the diet quality index and the overall sleep quality score is shown in Table 4. Among the HEI components, the wholegrain score displayed a statistically significant negative correlation with the overall sleep quality score ($p < 0.05$), whilst no such statistically significant correlation was determined for the other HEI components ($p > 0.05$).

Our findings demonstrated a significant difference in caffeine intake between groups with good and poor sleep quality. However, no statistically significant correlation was observed between sleep quality and other nutrients, including vitamin A, calcium, and iron. While no statistically significant correlation was determined between sleep quality and the overall HEI dietary quality score, a negative correlation was found between sleep quality and the HEI whole grains factor score.

An increased number of studies pointing out to a correlation between obesity and sleep has led to the proposition of several theories for an understanding of the causality of this correlation. One of these theories suggests that the dietary amounts and percentages of macronutrients affect sleep. In a previous study, using data of the Korea National Health and Nutrition Examination Survey, the correlation between the sleep duration and macronutrient intake of 14,111 participants aged 20-79 years was analyzed. Daily protein intake was found to be significantly correlated with short sleep duration in both men and women. Sleep duration was determined to be significantly short in men with high fat consumption levels and women with high carbohydrate consumption levels (Doo and Kim, 2016). In a research investigating the effects of a low-carbohydrate diet (30% carbohydrate, 35% protein, 35% fat) on sleep quality, sleep duration, severity of insomnia and risk of obstructive sleep apnea for a period of six months (three months of weight loss and three months of weight maintenance), it was ascertained that the low-carbohydrate diet was not correlated with the PSQI scores and had no effect on sleep quality, sleep duration, severity of insomnia and obstructive sleep apnea in obese adults (Kalam et al., 2021). In a previous study by Lindseth and Murray (Lindseth and Murray, 2016), a group of young adults were given a high-protein diet, high-carbohydrate diet, high-fat diet and control diet at 2-week "washout'' intervals, during which they consumed a diet of their own preference. The PSQI results demonstrated that, compared to other diets, high levels of fat intake were significantly correlated with better sleep. Furthermore, high levels of carbohydrate intake were found to be significantly correlated with short sleep duration.

Table 2. The mean value, standard deviation and upper-lower limits of the energy and nutrient intake levels of the participants for the two different PSQI categories

Nutritional Element	Sleep Quality				
	Good Sleep Quality (PSQI ≤ 5)		Poor Sleep Quality (PSQI > 5)		
	$\bar{X} \pm SD$	Lower Limit - Upper Limit	$\bar{X} \pm SD$	Lower Limit - Upper Limit	p
Energy (kcal)	1,819.42 ± 779.61	879.60 - 4,067.00	1,701.11 ± 732.25	581.50 - 3,597.30	0.66
Carbohydrate (g)	171.55 ± 102.53	37.30 - 500.60	161.77 ± 68.66	37.40 ± 316.00	0.68
Carbohydrate (%)	38.23 ± 11.61	14.00 - 73.00	39.58 ± 10.45	23.00 - 65.00	0.67
Dietary fiber (g)	21.09 ± 9.61	8.60 - 43.80	18.07 ± 9.78	6.10 - 39.70	0.28
Protein (g)	76.83 ± 39.62	37.30 - 210.10	65.96 ± 28.13	15.80 - 140.10	0.27
Protein (%)	17.54 ± 4.82	6.00 - 28.00	16.04 ± 3.30	11.00 - 24.00	0.16
Plant protein (g)	27.85 ± 14.28	11.50 - 60.10	25.65 ± 12.56	11.50 - 60.10	0.57
Fat (g)	91.76 ± 45.12	25.90 - 198.70	85.02 ± 49.31	16.70 - 196.80	0.62
Fat percentage (%)	43.27 ± 10.20	15.00 - 65.00	44.29 ± 11.67	16.00 - 62.00	0.74
Cholesterol (g)	389.52 ± 260.04	13.50 - 1,066.30	278.80 ± 170.30	43.20 - 699.60	0.08
Saturated fatty acid (g)	28.40 ± 13.67	6.30 - 61.80	28.10 ± 15.22	4.70 - 65.40	0.10
Monounsaturated fatty acid (g)	32.51 ± 12.90	9.50 - 69.50	30.56 ± 17.57	5.90 - 64.70	0.65
Polyunsaturated fatty acid (g)	20.87 ± 19.68	4.10 - 93.40	21.15 ± 15.47	2.60 - 56.90	0.68
Vitamin A (µg)	973.84 ± 552.31	277.40 - 2,236.10	2,261.79 ± 5,087.67	147.00 - 22,929.00	0.52
Vitamin E (mg)	18.57 ± 17.29	4.20 - 88.80	17.45 ± 10.50	1.80 - 40.50	0.66
Vitamin B_1 (mg)	0.77 ± 0.28	0.40 - 1.60	0.70 ± 0.27	0.20 - 1.30	0.41
Vitamin B_2 (mg)	1.28 ± 0.49	0.70 - 2.80	1.56 ± 0.89	0.50 - 5.10	0.10
Niacin (mg)	13.31 ± 8.12	3.10 - 37.00	12.77 ± 6.18	1.90 - 23.50	0.79
Pantothenic acid (mg)	4.32 ± 1.91	2.10 - 11.50	4.31 ± 2.19	1.20 - 11.90	0.81
Folic acid (µg)	283.54 ± 124.63	123.90 - 662.30	252.30 ± 105.16	54.00 - 541.50	0.76
Vitamin B_{12} (µg)	4.38 ± 3.15	0.60 - 14.00	9.98 ± 18.97	0.30 - 87.70	0.96
Vitamin C (mg)	62.02 ± 43.74	9.80 - 186.40	62.22 ± 45.32	2.30 - 84.60	0.99
Calcium (mg)	636.13 ± 302.51	175.10 - 1,752.00	666.46 ± 308.25	133.60 - 1,517.20	0.73
Magnesium (mg)	317.33 ± 181.25	146.30 - 819.40	312.20 ± 174.32	90.00 - 791.00	0.92
Iron (mg)	12.97 ± 5.65	6.50 - 25.90	12.71 ± 6.34	3.00 - 30.80	0.96
Zinc (mg)	11.43 ± 5.29	4.70 - 23.60	10.00 ± 5.18	2.50 - 27.90	0.34
Caffeine (mg)	64.14 ± 57.03	0.00 - 200.00	112.35 ± 75.38	0.00 - 264.00	0.02

Table 3. The mean value, standard deviation and upper-lower limits of the HEI total scores of the participants for the two different PSQI categories

Healthy Eating Index Total Score	Sleep Quality				
	Good sleep quality (PSQI ≤ 5)		Poor sleep quality (PSQI > 5)		
	$\bar{X} \pm SD$	Lower Limit - Upper Limit	$\bar{X} \pm SD$	Lower Limit - Upper Limit	p
Overall diet quality score	51.54 ± 15.07	30.30 - 86.30	49.11 ± 16.03	18.00 - 78.50	0.58

Table 4. The correlation between the HEI sub-factor scores and PSQI total scores of the participants

Healthy Eating Index Factors	Correlation Coefficient
	PSQI Total Score
Total fruits	-0.127 (p = 0.373)
Whole fruits	-0.109 (p = 0.450)
Total vegetables	-0.028 (p = 0.846)
Greens and beans	0.128 (p = 0.377)
Whole grains	-0.288 (p = 0.043)
Dairy	0.194 (p = 0.176)
Total protein foods	-0.072 (p = 0.617)
Seafood and plant proteins	0.018 (p = 0.903)
Fats acids	-0.010 (p = 0.945)
Refined grains	0.038 (p = 0.793)
Sodium	-0.114 (p = 0.430)
Added sugar	-0.169 (p = 0.241)
Saturated fats	-0.031 (p = 0.833)
Overall diet quality score	-0.060 (p = 0.681)

Another study, which was aimed at assessing sleep quality with the PSQI and making a relatively more objective assessment (AARC-APT, 1995) of sleep parameters with polysomnographic (PSG) monitoring records, demonstrated that higher levels of saturated fat intake and lower levels of fiber intake negatively affected sleep depth (St-Onge et al., 2016). The China National Nutrition and Health Survey, based on 3-day food consumption data of 2,828 adults living in Jiangsu Province, found no correlation between sleep duration and protein intake. In this respect, the findings of this study are similar to the findings of our study. The study determined that there was a statistically significant correlation between sleep duration and fat and carbohydrate intake. In the same study, it was ascertained that, the fat-derived energy percentage of individuals sleeping less than 7 hours a day was significantly higher than that of individuals sleeping 7-9 hours daily (Shi et al., 2008). In an experimental study conducted on eight healthy adult men, provided with an isocaloric normal diet, high-carbohydrate/low-fat diet and low-carbohydrate/high-fat diet, it was observed that, compared to the other diets, the high-carbohydrate/low-fat diet shortened the slow wave sleep (SWS) stage. Furthermore, both the high-carbohydrate/low-fat and low-carbohydrate/high-fat diets, and particularly the former, were significantly associated with REM sleep, compared to the normal isocaloric diet (Phillips et al., 1975). The association of sleep with carbohydrate intake has been explained by the amount and quality of dietary carbohydrates, the percentage of daily energy intake from carbohydrates, and alterations in the glycemic load. Alterations in the glycemic load partly account for differences in sleep quality and the waking frequency after the onset of sleep (Vlahoyiannis et al., 2021). While most of the studies referred to above have shown that sleep quality is not correlated with protein intake, some other studies have suggested that sleep quality is not associated with carbohydrate and fat intake either. In the present study, sleep quality was determined not to be correlated with dietary carbohydrate, protein and fat intake levels. No correlation having been determined between these parameters could be attributed to the study being a short-term investigation or to the quality and type of dietary carbohydrates, protein and fats, rather than their amounts and percentages, inducing different effects on sleep.

The Cross-sectional Bavarian Food Consumption Survey II, which assessed the data of 1,050 inhabitants aged 13-81 years and aimed to analyze the correlation of diet with sleep duration, sleep midpoint and sleep quality in the German Bavarian population, demonstrated results similar to ours, suggesting sleep quality not be correlated with energy, carbohydrate, protein and fat intake (Kleiser et al., 2017).

In a study carried out on 3,304 young women living in Japan, a different sleep assessment method was performed, based on sleeping and waking periods, and the sleep midpoint was determined. The sleep midpoint was found to display a significantly negative correlation with cholesterol, potassium, calcium, magnesium, iron, zinc, vitamin A, vitamin D, thiamin, riboflavin and vitamin B intake levels, and a positive correlation with the energy percentage from fat. It was ascertained that participants with a late sleep midpoint tended to delay main meals and skip meals (Sato-Mito et al., 2011). In the present study, no statistically significant correlation was determined between the levels of macro and micronutrients, including vitamin A, dietary fiber, calcium, magnesium, and sleep quality.

Researchers have shown that morning tiredness leads to the consumption of high levels of caffeine, which disturbs the regular sleep pattern the following night and triggers the occurrence of the vicious caffeine cycle (O'Callaghan et al., 2018). Such a caffeine cycle not occurring, and individuals not being affected by caffeine have been explained by several theories suggesting individuals becoming addicted to caffeine, caffeine intake not taking place at late night hours, and individuals showing genetically low sensitivity to caffeine. These theories maintain their validity today (Hindmarch et al., 2000; Rétey et al., 2007). In an experimental study conducted on three periods, including a placebo, caffeinated coffee consumption and terminated caffeinated coffee consumption

period, it was observed that the morning and afternoon coffee consumption of regular drinkers (3 × 150 mg/daily) had no effect on the sleeping pattern of these individuals (Weibel et al., 2021).

In another experimental study aimed at the comparative assessment of the effects of caffeinated beverages and water on cognitive performance and sleep quality, it was ascertained that caffeinated beverages showed a strong dose-dependent negative effect on sleep onset, sleep duration and sleep quality. Furthermore, the highest level of sleep disturbance having been observed in the individuals with the lowest level of caffeine intake demonstrated that the effects of caffeine on sleep are related to the accustomed consumption levels of individuals (Hindmarch et al., 2000). In the present study, although the mean caffeine intake levels of the groups with good and poor sleep quality were not very high, it was observed that individuals suffering from poor sleep quality had significantly higher caffeine intake levels, which suggested that caffeine intake adversely affected sleep quality.

Different studies having reported varying results on the correlation of energy and nutrients with sleep has eventually led researchers to use diet quality scales, which enable the joint evaluation of dietary components. In a previous cross-sectional study investigating the association of food consumption and nutrient intake with sleep quality in 1,548 participants, the researchers made use of the PSQI, China Healthy Diet Index and Food Frequency Questionnaire. While the results showed that the total energy intake was negatively correlated with sleep quality, no statistically significant correlation was detected between other macronutrients, micronutrients and sleep quality. This study is similar to our study findings in that it did not detect a statistically significant correlation between macro and micronutrients and sleep quality. The overall diet quality score was significantly higher in the group of individuals with good sleep quality. Among the dietary quality index components investigated, fruits, fish, shellfish and mollusks were determined to show a significant positive correlation with sleep quality (Wu et al., 2019). In another study, in which the PSQI was used for the assessment of the sleep quality of a total of 3,129 middle-aged female workers with an age ranging from 34 to 65 years, it was observed that poor sleep quality was associated with high confectionary and noodle consumption levels and low vegetable and fish consumption levels (Katagiri et al., 2014). A study that aimed to evaluate the relationship between diet and sleep quality in 868 Spanish university students found that those with an unhealthy diet were more likely to have poor sleep quality. At the same time, an unbalanced intake of vegetables, fruits, dairy products, meat, legumes, sweets and sugary soft drinks was associated with poorer sleep quality (Ramón-Arbués et al., 2022). In another study investigating the effects of fatty fish consumption on the sleep quality of adults, weekly mean fish portion consumption levels and PSQI scores were evaluated. The mean weekly fatty fish consumption level was determined to be 9 ± 6 portions (1 portion = 140 g) and 187 (28%) persons were ascertained to suffer from poor sleep quality. Individuals with good sleep quality were determined to consume a higher level of fatty fish (Del Brutto et al., 2016). Another cross-sectional study, which was based on the use of the data of the United Kingdom (UK) Biobank (UKB) and covered 502,494 middle-aged adults, demonstrated that healthy nutrition, sleep and mental health were positively correlated with each other. It was determined that sleep was positively affected by the consumption of high levels of fruits, vegetables, fish, water and fiber, and negatively affected by the consumption of processed meat and milk (Hepsomali and Groeger, 2021). In all the studies referred to above, fish consumption was found to be positively correlated with good sleep quality. On the other hand, in the present study, sleep quality showed no correlation with the "seafood and plant proteins" score, under which fish consumption was evaluated. This could be due to HEI-2015, the diet quality index used in the present study, not enabling the assessment of fish consumption alone. Furthermore, another possible explanation could be fish consumption having not been noted as part of the daily food consumption records due to the frequency of fish consumption being low. Different from the previous studies referred to above, the present study demonstrated that, among the diet quality components investigated, the wholegrain score had a statistically significant negative correlation with the overall sleep quality score. Thus, it was determined that wholegrain consumption positively affected sleep quality. Literature reports suggest that wholegrain consumption is inversely proportional to the occurrence of noninfectious diseases, including cardiovascular diseases, type II diabetes and some types of cancer. Owing to all these healthful effects, wholegrain consumption may have improved sleep quality in the long term. Furthermore, wholegrains are rich in fiber, and as already highlighted, there are reports indicating the association of high fiber content with good sleep quality. Therefore, due to their rich fiber content, wholegrains could be associated with good sleep quality (St-Onge et al., 2016; Hepsomali and Groeger, 2021).

Recent studies indicate that, in elderly people, children and adolescents, good sleep quality and adequate sleep duration are associated with healthy eating habits and good diet quality (Štefan et al., 2018; Kracht et al., 2019). Likewise, a different study that aimed to determine the relationship between diet and sleep quality in 211 adults, HEI was used to determine diet quality and PSQI was used to determine sleep quality, and it was concluded that sleep quality increased as diet quality increased (Behbahani et al., 2022). Different results have been obtained for adults, such that the Cross-sectional Bavarian Food Consumption Survey revealed no statistically significant correlation between PSQI scores and food groups (Kleiser et al., 2017). Likewise, another study conducted on 1,165 persons aged 19-64 years, using the Recommended Food Score (RFS) and PSQI, revealed no statistically significant correlation between sleep quality and diet quality (Hur et al., 2021). Similarly, in another study aiming to evaluate the relationship between sleep duration and diet quality in 739 Iranian adults, diet quality was assessed

using HEI-2015 and estimated sleep duration was not associated with compliance with HEI-2015 (Yazdani et al., 2023). The present study, in which the same sleep quality scales were used and similar results were obtained, also showed that the groups with good and poor sleep quality did not significantly differ for overall sleep quality, such that overall sleep quality and the PSQI total score were not correlated with each other.

CONCLUSION

It is concluded that reducing caffeine and increasing wholegrain consumption could significantly contribute to improving sleep quality. Different studies having shown different diet quality components to be correlated with sleep quality could be attributed to the use of different diet and sleep quality scales, short-term recall-based consumption records falling short in determining the nutritional status, different diet quality indices being based on different consumption amounts, and consumption limits varying in different cultures. Genetic, cultural and age differences may also affect the sleep pattern of individuals. There is a need for high-participant, high-quality cohort and randomized controlled studies to better understand the relationship between sleep and diet quality and determine the underlying mechanism of any possible correlation.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no actual, potential, or perceived conflict of interest for this article.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

The Bursa Uludag University Ethics Committee approved the study during meeting number 2020-19/11.

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The effects of pruning time on attributes of phenology yield and quality in some table grapes cultivars

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Abstract

This research aimed to determine the effects of winter pruning time (early and late winter pruning) on the phenology, yield, and quality of cvs. Samancı Seedless and Alphonse Lavallée (*Vitis vinifera* L.). This research was planned in a completely randomised design with three replications. Winter pruning was performed when the upper buds were on canes at the dormant stage (BBCH 00) and the 2-leaf stage (BBCH 12). Bursting of bottom buds on canes was delayed as 6 days for cv. Samancı Seedless and 5 days for cv. Alphonse Lavallée when compared to control. However, maturation time and veraison time were not affected. Additionally, the yield and quality of early- and late-pruned vines did not show significant differences. It was recommended that late winter pruning could be performed when the upper buds were at the 2-leaf stage (BBCH 12) to prevent late spring frost damage for both cultivars.

Keywords: Table grapes, Late pruning, Pruning time, Spring frost, Yield, Quality

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INTRODUCTION

The viticulture area in Türkiye is approximately 378,000 hectares. Türkiye is followed by Spain, France, Italy, and China, ranking fifth. The total fresh grape production of Türkiye is about 3.4 million tons (FAO, 2023). In terms of total fresh grape production, Turkiye is the seventh-largest producer after China, Italy, France, the USA, Spain, and India (FAO, 2023). Table grapes accounted for 52.7%, raisins for 36.4%, and must and wine for 10.9% of the total grape production consumed (TurkSTAT, 2024). The total table grape exportation from Türkiye is approximately 146,000 tons. Among the world's countries in terms of table grape exportation, Türkiye ranks 12th, following Peru, Chile, China, Italy, the Netherlands, South Africa, India, the USA, Mexico, and Egypt (FAO, 2023).

Late spring frosts in vineyards in Türkiye have been resulted in significant damages (Uzun, 2004). Similarly, Italian vineyards have also been affected by late spring frosts approximately once every four years during the last 30-year period. The warm weather at the end of March, followed by a cooling period in April, caused damage to vineyards in Italy in 2021 (Giordano et al., 2022). Spring frosts significantly decreased the yield of vines. Spring frosts occur after buds burst or shoots reach about 5 to 10 cm in length when the temperature drops to -1°C. Direct and indirect measures have been used to prevent spring frosts. Some of the direct measures include fogging between night and early morning by firing straws, irrigating if the soil has been tilled, and shortening tall weeds, among others. Some of the indirect measures include site selection, proper cultivar selection, and delayed pruning (McCarthy et al., 1992; Çelik et al., 1998; Throught et al., 1999).

Research on the effects of different pruning times (in November and February) on table grapes was conducted. A 1- to 4-day delay in the budburst date and a 1- to 2-day delay in flowering were observed. Instead of February, the bud-bursting time of basal buds or the dates of the end of March were suggested for late pruning (Celik et al.,

2020). Different pruning times (July and August) effects on budburst time and ongoing phenology were investigated in cv. Cabernet Sauvignon. Budburst dates were observed after September 16. Late pruning resulted in delays of 4.3 days for budburst, 5.0 days for flowering, and 4.1 days for veraison. The total soluble solids (TSS) of late-pruned grapes at the ripening stage have been found to be 0.91% less than those of the control (Martin and Dunn, 2000).

Effects of 10 different pruning times on productivity, vegetative development, and phenology of cv. Trebbiano Toscano were examined. While budburst and flowering time differences were significant, veraison time differences were reduced. However, pruning times did not affect yield (Barbagallo et al., 1993). Similarly, Sotes et al. (1992) found that pruning times affected the yield of cvs. Airen and Tempranillo nonsignificantly.

Cv. Sangiovese grown in pots and vineyards were applied pruning at dormant season (BBCH 00) and at 2 or 3 unfolded leaf stage of distal buds of scions (BBCH 12, 13) (late pruning) and at 7 or 8 unfolded leaf stage of distal buds of scions (very late pruning) (BBCH-17,18). Budburst of basal buds was delayed by 2 and 4 weeks for late and very late pruning applications, respectively. Ripening in pot plants did not change between dormant pruning and late pruning. Yet, ripening in very late pruning is delayed as 6 days. When the three-year average was considered, ripening in vineyards was delayed, and phenolic compounds increased with late-pruned vines. Researchers suggested late pruning applications because bud bursting of basal buds was postponed by two weeks, resulting in decreased berry sugar accumulation and increased berry phenolics without significant yield decreases (Gatti et al., 2016; Palliotti et al., 2017).

One of the other studies examines the effects of pruning time on cv. Merlot. The results showed similarities with cv. Sangiovese. Late and very late pruning resulted in late bud bursting of basal buds, which decreased yield, delayed maturation, and increased phenolic compounds; however, it did not alter anthocyanidins (Allegro, 2019).

Chardonnay is an early bud-bursting cultivar. Another study was conducted in Brazil on Chardonnay. The effects of dormant pruning as a control (25 August, 2010) and late pruning at different times (31 August, 8 September, and 15 September, 2010) on delaying budburst and yield were investigated. The late pruning on 31 August was suggested because of the one-week delay in bud burst without yield reduction due to spring frosts (Brighenti et al., 2017).

The other study about cv. Sauvignon Blanc, which was cane-pruned, was examined to determine the effects of four different pruning times, ranging from harvest to budburst. The upper budburst time for pruning resulted in a slight change in the budburst of basal buds (Trought et al., 2011).

Poni et al. (2022) interpreted 21 research results on cane and spur-pruned vines. According to their interpretations, after the upper buds reached the 2- and 3-unfolded leaf stage, pruning resulted in a 15- to 20-day delay in the budburst of basal buds, with reduced yield loss and delayed ripeness during a calmer period.

In light of the studies mentioned above, this research objective is to determine the effects of late pruning (at the 2- or 3-unfolded leaf stage) on phenology, yield, and quality of Alphonse Lavallée and Samancı Seedless cultivars. Spring frosts can be avoided by postponing bud burst for one or two weeks, which results in no or minimal yield and quality reduction.

MATERIALS AND METHODS

Materials

This research used cvs. Alphonse Lavallée and Samancı Seedless (Beyaz Şam × Perlette), (Ergönül et al., 2018) (*Vitis vinifera* L.) vines were grafted onto 1103 P. The vines were 8 years old. The soil was sandy loam. Vines lined from south to north. cv. Alphonse Lavallée trained bilateral cordon and pruned as a spur with two buds; a bud load of 20 buds per vine was applied. cv. Samancı Seedless vines were trained to a bilateral cordon and pruned as semi-long canes with a 40-bud vine bud load. For both cvs, a half pergola training system was applied, with vines spaced 3.5 m intra-row and 2 m inter-row.

Methods

In this research, pruning time (PT) applications were made using early pruning (EP) and late pruning (LP). While EP was applied to the dormant season of buds (BBCH 00 stage) (Lorenz et al., 1995) on 15 February 2020, LP was applied to two unfolded leaf stages of tip buds (BBCH 12 stage) on 14 April 2020 (Fig. 1 and Fig. 2).

The other cultural applications, such as irrigation and fertilisation, were performed similarly to application parcels. Cvs. Alphonse Lavallée and Samancı Seedless were harvested on August 15, 2020, and August 22, 2020, respectively.

Experiment Plan and Statistical Analysis

The experiment was planned with a completely randomised design with three replications (Gomez and Gomez, 1984). The applications are EP and LP. The R package program was used, and variance analyses and LSD tests ($p \leq 0.05$) were carried out for mean separations (Kaya, 2019; De Mendiburu, 2020; R Core Team, 2021).

Examining Characters

Phenological observations, including budburst, full bloom, veraison, and maturation dates, were recorded. Fresh grape characters, including yield (kg vine^{-1}), cluster number (number vine$^{-1)}$, cluster width (mm), and length (mm), were determined. According to Roessler and Amerine (1958), berry samples were collected from each

parcel one day prior to harvest. The 100-gram berry weight, berry width and length (in millimetres), berry firmness (in grams), chroma, and hue colour values (McGuire, 1992) and leaf chlorophyll values were measured. Total soluble solids (°Brix), titration acidity (g/100 mL), and maturity index were determined from the grape musts.



Figure 1. LP vines at two unfolded leaf stages of cv. Samancı seedless



Figure 2. LP vines at two unfolded leaf stages of cv. Alphonse Lavallée

Total phenolic substances

The total phenolic substances were determined using gallic acid as a standard by the Folin-Ciocalteu method (Singleton and Rossi, 1965). 2.37 mL distilled water, 30 µL extract, and 150 µL Folin-Ciocalteu reagent were added to falcon tubes and left in dark medium for 8 minutes. After that, 0.45 mL saturated sodium carbonate was added, vortexed and held in the tubes at 40 °C for 30 minutes. For the blind, a similar procedure was applied with distilled water instead of extract. Absorbance values of the samples were read by a spectrophotometer at 750 nm, compared with the blind. Gallic acid standard calibration curves were constructed using a similar procedure with gallic acid solutions (50-500 mg/L). The results were expressed as mg GAE per 100 g of fresh grape weight.

Antioxidant capacity

Antioxidant capacity analyses were applied to Blois et al. (1958). 0.1 mL sample extracts diluted at suitable rates and standards were mixed with 2.9 mL 0.1 mM DPPH (2.2-diphenyl-1-picrylhydrazyl) solutions soluted in ethanol and held in darkness for 30 minutes. Mix solution absorbance values read in 517 nm in a spectrophotometer with comparing ethanol. The results were clarified as units in µmol Trolox 100 g^{-1} fresh grape weight.

RESULTS

In cvs. Samancı Seedless and Alphonse Lavallée, EP and LP applied vines phenologies were given in Table 1. In Samancı Seedless and Alphonse Lavallée, considering basal bud bursting time, LP caused a six-day and five-day delay in bud bursting compared to EP respectively. When considering flowering time and veraison time, LP caused only one or 0-day delays, respectively. Véraison's date is July 15, 2020. Similarly, PT did not affect the maturation time. Maturation time is on August 22, 2020, for cv. Samancı Seedless, dated 15 August 2020, for cv. Alphonse Lavallée (Table1).

Table 1. The effects of EP and LP on phenological periods in cvs. Samancı Seedless and Alphonse Lavallée.

	Samancı Seedless		**Alphonse Lavallée**	
	EP*	**LP****	**EP**	**LP**
Bud bursting	08.04.2020	14.04.2020	03.04. 2020	08.04.2020
Full bloom flowering	15.05.2020	16.05.2020	17.05.2020	18.05.2020
Veraison	15.07.2020	15.07.2020	15.07.2020	15.07.2020
Maturation	22.08.2020	22.08.2020	15.08.2020	15.08.2020

***EP:**early pruning**; ** LP:** late pruning

The effects of PT on fresh grape yield on cv. Samancı Seedless was not statistically significant. Yield values ranged between 14.09 and 14.92 kg $vine^{-1}$. Similarly, the effects of PT on fresh grape yield on cv. Alphonse Lavallée was not statistically significant. Yield values ranged between 11.15 and 13.33 kg $vine^{-1}$. For both cvs., PT did not affect cluster weight, cluster number, cluster width, and length (Table 2).

Table 2. The effects of EP and LP on yield and cluster characters in cvs Samancı Seedless and Alphonse Lavallée.

	Samancı Seedless			Alphonse Lavallée		
	EP*	**LP****	**LSD*****	**EP**	**LP**	**LSD**
Yield (kg vine^{-1})	14.92	14.09	n.s.	13.33	11.15	n.s.
Cluster weight (g)	439.8	377.5	n.s.	385.9	386.6	n.s.
Cluster number	33.8	37.3	n.s.	34.3	28.8	n.s.
Cluster width (mm)	166.6	155.5	n.s.	137.7	132.8	n.s.
Cluster length (mm)	188.9	191.7	n.s.	211.3	200.7	n.s.

***EP:**early pruning**; ****LP:** late pruning
*****Means followed by different letters were significantly different at $p \leq 0.05$ using LSD test
n.s. = not significant; s: =significant

In cv. Samancı Seedless, PT did not affect 100 berry weight. 100 berry weight ranged from 354.7 g to 364.3 g. Similarly, in Alphonse Lavallée cv., PT did not affect 100 berry weight. 100 berry weight ranged from 767.4 g to 768.7 g. For both cvs., PT did not affect berry width, berry length and berry firmness (Table 3).

Table 3. The effects of EP and LP on beery characters in cvs. Samancı Seedless and Alphonse Lavallée.

	Samancı Seedleess			Alphonse Lavallée		
	EP*	**LP****	**LSD*****	**EP**	**LP**	**LSD**
100 Berry weight (g)	364.3	354.7	n.s.	767.4	768.7	n.s.
Berry width (mm)	16.7	16.8	n.s.	23.2	23,0	n.s.
Berry length (mm)	20.9	20.8	n.s.	22.7	22.2	n.s.
Berry firmness (g)	493.6	521	n.s.	375.4	369.1	n.s.

***EP:**early pruning**; ****LP:** late pruning
*****Means followed by different letters were significantly different at $p \leq 0.05$ using LSD test
n.s. = not significant; s: =significant

In cv. Samancı Seedlees, PT did not affect total soluble solids (TSS). TSS ranged from 16.7 to 16.9 °Brix. Similarly, in Alphonse Lavallée, PT did not affect TSS. TSS ranged from 18.0 to 18.1 °Brix. For both cvs., PT did not affect titration acidity (g/100mL) and maturation index (Table 4).

Table 4. The effects of EP and LP on maturation characters in cvs. Samancı Seedless and Alphonse Lavallée.

	Samancı Seedless			Alphonse Lavallée		
	EP*	**LP****	**LSD*****	**EP**	**LP**	**LSD**
TSS (°Brix)	16.7	16.9	n.s.	18.0	18.1	n.s.
Titration acidity (g/100 mL)	0.33	0.37	n.s.	0.5	0.5	n.s.
Maturation index	52.3	46.2	n.s.	37.2	36.8	n.s.

***EP:**early pruning**; ****LP:** late pruning
*****Means followed by different letters were significantly different at $p \leq 0.05$ using LSD test
n.s. = not significant; s: =significant

In cv. Samancı Seedless, PT did not effect Hue value. Hue values ranged from 92.3 to 93.2. Similarly, in cv. Alphonse Lavallée, PT, did not affect the Hue colour value, which ranged from 337.2 to 337.8. For both cvs., PT did not affect chroma and leaf chlorophyll values (Table 5).

Table 5. The effects of EP and LP on berry colour and leaf chlorophyll in cvs. Samancı Seedless and Alphonse Lavallée.

	Samancı Seedless			Alphonse Lavallée		
	EP*	**LP****	**LSD*****	**EP**	**LP**	**LSD**
Hue color value	92.3	93.2	n.s.	337.8	337.2	n.s.
Chroma color value	34.2	34.6	n.s.	0.81	1.08	n.s.
Leaf chlorophyll value	0.189	0.186	n.s.	0.178	0.181	n.s.

***EP:**early pruning**; ****LP:** late pruning
*****Means followed by different letters were significantly different at $p \leq 0.05$ using LSD test
n.s. = not significant; s: =significant

In cv. Samancı Seedless, PT did not affect total phenolic substances (TFS). TFS ranged from 83.3 to 100.29 GAE 100 g^{-1} fresh grape. Similarly, in cv. Alphonse Lavallée, PT did not effect on TFS. TFS ranged from 337.2 to 337.8 GAE 100 g^{-1} fresh grape. For both cvs., PT did not affect antioxidant activity (AA) (Table 6).

Table 6. The effects of EP and LP on some biochemical characters in cvs. Samancı Seedless and Alphonse Lavallée.

	Samancı Seedless			**Alphonse Lavallée**		
	EP*	**LP****	**LSD*****	**EP**	**LP**	**LSD**
TFS (mg GAE/100g)	83.3	100.29	n.s.	354.92	378.55	n.s.
AA (µmol TE/100 g)	255.35	331.21	n.s.	870.6	880.52	n.s.

***EP:**early pruning**; **LP:** late pruning
***Means followed by different letters were significantly different at $p \leq 0.05$ using LSD test
n.s. = not significant; s: =significant

DISCUSSION

PT affected only budburst time, as observed in phenological studies. LP, when compared to EP, delayed the bud bursting of basal buds for 6 days in cv. Samancı Seedless and for 5 days in cv. Alphonse Lavalée. For both cvs, when compared to EP, LP caused only a one-day delay in full flowering, while it did not affect veraison and maturation times. PT did not affect yield and quality characters for both cvs. In one of the researches on cv. Trebbiano Toscano, PT, affected budburst time and flowering but did not affect veraison time, yield, and must quality (Barbagallo et al., 1993). In another study, the effects of different winter pruning times, including the green tip leaf stage (BBCH 08 stage), were examined. It was found that there are no differences in yield and quality parameters between pruning times (Ferrara et al., 2022). These findings were compatible with our research. In another study, control (dormant pruning), LP (pruning at two unfolded leaf stages on tip bud shoots), and Very LP (pruning at seven unfolded leaf stages on tip bud shoots) were applied to cv. Sangiovese (*V. vinifera* L.) grown in large pots. LP and VLP caused a 17- and 31-day delay in bud bursting of basal buds, respectively, compared with the control. While maturation time was not affected by LP, 6-day delaying maturation occurred by very LP. In addition, the longer PT was delayed, the greater the decrease in yield and cluster weight became. Therefore, LP, as the optimal pruning time compared to the very LP, was stated (Gatti et al., 2016). In this research, LP resulted in a 5- or 6-day delay in basal bud bursting while maintaining yield and quality at a similar level. In general, these results were consistent with those of Gatti et al. (2016). Similar research applied at Sangivose cv. (*V.vinifera* L.) grown in the vineyards. LP and very LP caused a delay in bud bursting at basal buds at 2 weeks and 4 weeks, resulting in yield decreases of 23% and 43%, respectively. Apical dominance suppressed the basal buds, keeping them dormant at a 20% level in LP. The increase in dormant buds resulted in yield decreases (Palliotti, 2017). In our research, LP caused only 5 or 6 days of delay in bud bursting of basal buds, and no yield decreases were observed. The reason for the differences in the results of both studies can be attributed to cultivar and training differences. In our research, a half pergola system was used, and shoots grew half erect. It could be caused by basal buds being less suppressed by apical dominancy. In long cane-pruned cv. Sauvignon Blanc, when LP is delayed closer to upper bud bursting time, basal bud bursting is delayed by a few days (Trought et al., 2011). Another study also indicates that LP can be applicable when upper buds reach the three-leaf stage for both cane- and spur-pruned Pinot Noir vines (Gatti et al., 2019). However, Poni (2022) suggested that an erect cane position is effective for delaying budburst in cane-pruned vines with late pruning applications.

In one study conducted in Brazil with the early bud-bursting cultivar Chardonnay, PT effects were examined. Upper budburst time was taken as a control on 25 August. LP applications were carried out on 31 August, 8 September, and 15 September as spur pruning. When compared to the control, the 31 August pruning resulted in a one-week delay of bud bursting of basal buds without yield reductions. This result is compatible with our research. Pruning later than August 31 caused later bud bursting of basal buds, but resulted in significant yield reductions (Brighenti et al., 2017).

In other research, LP effects during the dormant period or budburst time were investigated. PT effects from October to March in cv. Trebbiano Toscano was examined. Budburst and flowering times were delayed, but veraison and maturation times remained unchanged. Additionally, yield and quality remained unchanged (Barbagallo et al., 1993). In cv. Cabernet Sauvignon, LP applied closer timing to budburst; budburst, flowering, veraison, and maturation were delayed by an average of five days (Martin and Dunn, 2000). In cvs. Gelin, Bornova Misketi, and Alphonse Lavallée, LP applied in March, compared to the control applied in November, continuing the dormant period. LP caused 4-day, 3-day, and 1-day delays in bud bursting, respectively. It was suggested that a 1-week delay in bud bursting could be achieved by waiting for upper bud shoots to reach 2 or 3 unfolded leaf stages (Çelik et al., 2020).

Our research successfully achieved a 5- and 6-day delay in basal bud bursting without yield or quality reduction for cvs. Samancı Seedless and Alphonse Lavallée. Poni et al. (2022) also suggested that the optimal application time for LP was when upper bud shoots had 2 or 3 unfolded leaf stages, without yield or quality reductions.

CONCLUSION

For cvs. Samancı Seedless and Alphonse Lavallée, LP applications at upper bud shoots with 2 or 3 unfolded leaf stages caused a 5-6 day delay in basal bud bursting without yield and quality reductions. It is suggested to use LP application on upper bud shoots at 2 or 3 unfolded leaf stages for protection against spring frosts. To delay basal bud bursting time from one week to two weeks by using LP, it is recommended to use vines with vertical shoot position training systems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author contribution

This manuscript was taken part of the second author's master's thesis, and the first author was the supervisor of the master's thesis. The first author planned the experiment and helped the data analysis and manuscript writing oganisation. The second author set the experiment, collected data and wrote the results.

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Molecular identification and phylogenetic profiling of keratinolytic bacteria isolated from goose and chicken farms in a cold region of Türkiye

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Abstract

The aim of this study was to investigate bacterial diversity in certain goose and poultry farms in Kars, Türkiye a cold climate zone. Feather waste samples rich in organic compounds were collectedfrom multiple farm locations and evaluated for bacterial diversity. A total of 27 bacteria were isolated. 16S rRNA gene sequencing, morphological, physiological, biochemical and molecular techniques were used. Isolates included *Escherichia coli*, *Ralstonia picketti*, *Pantoea agglomerans*, *Kosakonia cowanii*, *Bacillus licheniformis*, *Bacillus zhangzhouensis*, *Bacillus pumilus*, *Exiguobacterium sibiricum*, *Exiguobacterium artemiae*, *Macrococcus caseolyticus*, *Mammaliicoccus vitulinus*, *Mammaliicoccus sciuri* and *Rothia nasisuis*. Most of the isolates are positive for characteristics such as spore formation, motility and catalase activity. These isolates also showed adaptation to nutrient-rich and varying temperature parameters. Molecular identification results confirmed the results of phylogenetic analyses, showing strong bootstrap support. The identification of resistant bacteria in these keratin-rich regions plays an important role in the control of poultry waste management. These bacteria, which are desirable for biotechnological applications, are also importance in enzyme studies. This study provides valuable information on the functional diversity of microbial communities in goose and poultry farming. It draws attention to the importance of microbial enzymes in the disposal of keratin wastes.

Keywords: 16S rRNA, Sequence, Microorganisms, Keratinase, Phylogenetic, Poultry and goose farm microbiota

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INTRODUCTION

Poultry farming is a primary means of meeting the global demand for animal protein (Josephson, 2020). Goose and chicken breeding, which come to mind first in poultry farming, contribute to the rural economy in terms of both meat and egg trade (Gilbert, Xiao, & Robinson, 2017). Kars and its surroundings provide a favourable environment for goose breeding due to its geographical conditions and cold climate. Microbial organisms are one of the most important factors affecting production efficiency in goose and poultry farms (Akhtar, Shafiq, & Ali, 2022). Poultry environments are home to many bacteria (Xu et al., 2022). These bacteria can include both pathogenic and beneficial species. Determination of the diversity of bacteria is very important in animal breeding (Wang & Jin, 2019). Bacteria can be found in large quantities in the gut microbiota of poultry and in the poultry house. Beneficial bacteria contribute to the correct functioning of the digestive system of poultry (Rychlik, 2020). Pathogenic bacteria, on the other hand, can cause diseases leading to poultry mortality in a short time. Especially bacteria such as *Salmonella*, *E. coli* and *Staphylococcus aureus* are among the most undesirable pathogens in poultry farming (Geletu, Usmael, & Ibrahim, 2022). For this reason, determination of bacterial diversity is very valuable for the continuity of animal husbandry, especially in rural areas, both for the protection of animal health and the prevention of zoonotic diseases.

In recent years, molecular biology techniques have been frequently preferred in the determination of microbial diversity because of more precise results. 16S rRNA gene sequencing and metagenomic analyses are among the most widely used methods by researchers (Clarridge Iii, 2004). In addition to these, biochemical tests are also used effectively in the identification of bacteria. These techniques can reveal detailed analyses on bacterial diversity. Identification of bacteria isolated from goose and poultry farms in and around Kars will provide a scientific contribution to the livestock farming activities in the region. It will be inevitable to take necessary precautions against pathogens that may be present in these environments and to get more effective results in farm management.

Bacteria with keratinolytic activity play an important role in the biological transformation of keratin-rich organic materials, especially poultry waste. These bacteria are able to biodegrade environmentally harmful wastes by hydrolyzing difficult-to-degrade proteins such as keratin with keratinase enzymes and in this process, valuable by-products (amino acids, peptides) are formed. Thanks to these properties, they are used in various industrial applications such as recycling of agricultural wastes, feed additive production and biological dehairing in the leather industry. They also offer important biotechnological opportunities to reduce environmental pollution caused by the indiscriminate release of keratin wastes into nature and to make waste management more sustainable (Akhter, Wal Marzan, Akter, & Shimizu, 2020; Vikash, Kamini, Ponesakki, & Anandasadagopan, 2025). In recent years, keratinolytic bacteria have emerged as sustainable and environmentally friendly biotechnological tools, especially for the biodegradation of poultry feather waste. The potential of these bacteria to convert organic wastes into valuable by-products through keratinase production has important applications in industrial biotechnology and environmental bioremediation (Gerlicz, Sypka, Jodłowska, & Białkowska, 2024; Moktip et al., 2025).

This study targeted the microbial diversity isolated from goose and chicken farms in Kars and its surroundings. The obtained findings are intended to contribute to the measures to be taken in the protection of animal health, targeted antibiotic selection and the direction in which farm hygiene should be progressed and improved. In addition, the results of this study can help to understand the microbial risks and what measures can be taken against these risks.

MATERIALS AND METHODS

Biological Samples

The biological material of this study comprises bacteria isolated from feather waste and environmental samples collected from goose and chicken farms in Kars during the breeding season. The spatial distribution of the sampling sites is illustrated in Figure 1. Samples were meticulously gathered from areas with dense feather waste and organic materials in the vicinity of farmyards. Sterile 2-litre glass jars sealed with aluminium foil to prevent exposure to sunlight were used for sample collection. Coordinates of each sampling site were recorded for documentation and reproducibility. In each location, samples were collected at consistent intervals, ensuring representation from different sections of the farms. A portable digital device (Model No. AZ 8685, Taiwan) was used to measure the temperature and pH of the samples at the time of collection. The probe was inserted 5 cm into the substrate. Immediately following collection, samples were either kept at +4°C for processing or transferred into sterile tubes and subjected to serial dilution (10^{-1} to 10^{-6}) with physiological saline (NaCl 0.85 g per 100 mL distilled water) for the purpose of bacterial isolation (Ulucay, Gormez, & Ozic, 2022b).

A 0.1 mL aliquot from each dilution was plated on Luria Bertani (LB) agar (Sigma-Aldrich), and the plates were incubated for 24 to 72 hours at temperatures ranging from 35 to 45 ºC. The colonies with distinct morphologies were selected for further study. Feather waste, which is abundant in the sampled farms, was the main source of keratin-degrading bacteria, showing potential for keratinase enzyme production, an important step for industrial applications of these isolates (Gerlicz et al., 2024).

Identification of bacteria isolates

Morphological Characteristics

Examinations for morphological features and motility patterns are carried out on a light microscope (BS-2030T, Bestscope) (Silva-de la Fuente et al., 2021). Gram staining and endospore imaging were performed to determine cell wall characteristics and spore formation, while motility was assessed using standard wet mount techniques described in the literature (Turenne, Snyder, & Alexander, 2015).

Motility

The motility tests, cell morphology, and endospore production of freshly cultured (24-h-old) bacterial samples were observed by microscopy (Bestscope BLM-280 LCD, China). These observations were useful for preliminary classification and differentiation of bacterial groups prior to molecular identification (Ulucay, Gormez, & Ozic, 2022a)

Gram and Endospore Staining

Using a sterile loop, colonies were transferred to one side of a clean microscope slide and resuspended in physiological saline. The slides were air-dried and heat-fixed, then immersed in crystal violet solution for 2 minutes, followed by a water rinse. Lugol's iodine solution was applied for 1 minute to enhance staining, and after another rinse and drying step, 96% alcohol was used to decolorize for 15-20 seconds. After a final water rinse and drying, fuchsin dye was added for 30 seconds to complete the staining. Dried slides were soaked in blotting paper

before being observed by mounting under an immersion lens on a microscope using immersion oil. Bacteria stained blue or purple were identified as gram-positive, while those with a pink color were classified as gram-negative (Ulucay et al., 2022b).

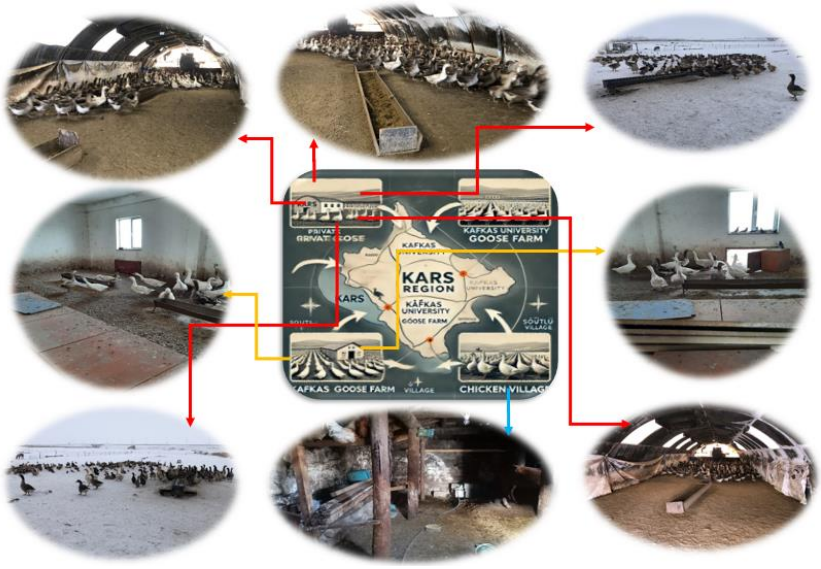


Figure 1. Areas highlighted with red arrows indicate the Kars Private Goose Farm, those marked with yellow arrows represent the Kafkas University Goose and Chicken Farm, and the areas identified by blue arrows depict the Söğütlü Village Goose and Chicken Farm.

Endospore staining was performed on overnight cultures grown on LB agar. Colonies were subsequently transferred to glass microscope slides using a sterile loop. Slides with dried preparation were stained with carbol fuschsin for 5 minutes then rinsed and treated with 10% nitric acid for 10 seconds. Methylene blue was then reacted against the counter cells for 2 minutes, washed, and allow to dry by air. The images were set for observation with an immersion oil under the microscope (Beveridge, Lawrence, & Murray, 2007).

Physiological Properties

The physiological characteristics of the isolates, including their responses to varying temperatures, pH levels, and sodium chloride (NaCl) concentrations, were examined across different growth stages under aerobic conditions (Oztas Gulmus & Gormez, 2020). Incubation temperatures ranged from 36°C to 45°C, and growth was monitored at pH levels from 4.0 to 12.0 in increments of 1.0. To assess the effect of NaCl concentration, the growth medium was supplemented with NaCl up to 10% (w/v). Optical density was the method used for quantifying bacterial growth to measure by spectrophotometer (GENESYS, Thermo Scientific) at 600 nm (Md Sidek et al., 2018).

Genomic DNA Isolation

Genomic DNA analyses of bacteria were carried out by modifying the classical phenol: chloroform: isoamylalcohol method. Genomic DNA samples were stored at -80°C until the 16S rRNA locales were dissected (Sambrook, 1989).

Sequencing of 16S rRNA

Subjected to a modified classical protocol with phenol, chloroform, and isoamyl alcohol, total genomic DNA was extracted for further molecular identification using 16S rRNA sequencing (Nikunjkumar, 2012). To amplify the 16S rRNA gene, we used the thermal cycler (Gene Pro, Bioer Technology Co., Ltd, TC-E-96G) as described before (Meena & Baranwal, 2016). The amplification of the region of interest in DNA was accomplished with universal primers: the forward primer 27F and the reverse primer 1385R (Ulucay et al., 2022b). The reactions for PCR were carried out in a total volume of 25 µL which included 10X PCR buffer (Applied Biosystems, Roche), 25 µM magnesium chloride, 25 mM dNTPs, 25 pmol of each primer, and 5 units/µL of Taq DNA polymerase (Applied Biosystems; Table 1). The PCR amplifying cycle was done a total of 40 times, one would have been run through ordinary denaturation, annealing at 55°C, and extension steps, with finally, a final extension lasting for 10 minutes at 72°C.

A 1% agarose gel was utilized in the electrophoretic process for PCR product analysis. The purified PCR products were cloned into vectors and subsequently sequenced. The resultant sequence data were analyzed against GenBank and deposited in the NCBI GenBank sequence database.

Table 1. Basic PCR Components

Component	Quantities
Taq Polymerase (5U/µL)	0,25 µL
10x Buffer PCR	2,5 µL
$MgCl_2$ (25 mM)	2 µL
Primer 27 F (25pmol/ µL)	2,5 µL
Primer 1385 R (25pmol/ µL)	2,5 µL
DNA	1 µL
dNTP (25mM)	1,5 µL
ddH_2O	12,75 µL

Phylogenetic

Phylogenetic status among the identified isolates was inferred using the Neighbour-Joining algorithm implemented in MEGA11 software (Tamura, Stecher, & Kumar, 2021). Type strains and outgroups were included in the analysis to ensure the appropriate taxonomic configuration of each isolate based on 16S rRNA gene sequences. The robustness of the resulting phylogenetic tree was assessed through 1000 bootstrap replicates with branch support values indicating the frequency of clustering between resampled datasets (Felsenstein, 1992). Evolutionary distances between sequences were calculated using the p-distance model, allowing the assessment of genetic divergence between lineages. The final tree structure was organised to reflect both evolutionary closeness and taxonomic resolution (Nei & Kumar, 2000).

RESULTS AND DISCUSSION

Isolate Screening and Classification

A total of 27 bacterial isolates were identified from goose and poultry farms in Kars city centre and Söğütlü village. In the initial isolation stages, the ability of bacteria to grow under aerobic conditions was investigated.

The isolates able to grow at high temperatures (50-60 °C) were selected. Colonies with different morphological characteristics were selected and grown on feather meal agar and casein containing media. Preliminary screening showed that some isolates produced extracellular keratinase activity in the medium, causing clear zones to appear in the medium. The diameter of these zones varied, reflecting differences in enzymatic diffusion and degradation potential.

The isolates were evaluated for gram staining, endospore formation, motility, catalase and oxidase activities and tolerance to varying NaCl concentrations and pH ranges (Figure 2 and Table 2). The majority of the bacterial isolates obtained were gram-negative, motile and catalase-positive. Strains belonging to the genus *Bacillus* generally showed sporulation capacity. 16S rRNA gene amplification and sequencing techniques were used for the identification of the isolates. The sequences obtained as a result of sequencing were analyzed using BLAST in the NCBI database and taxonomic comparison was made. The genera identified included *Bacillus*, *Kosakonia*, *Exiguobacterium*, *Pantoea*, *Ralstonia*, *Escherichia*, *Rothia*, *Macrococcus* and *Mammaliicoccus*. Phylogenetic analysis grouped these isolates into distinct clusters with high bootstrap support, highlighting their evolutionary relationships (Figure 5). These findings provide the identification of bacterial cultures with keratinolytic potential in poultry raised under cold climate conditions in Kars region. The predominance of both spore-forming and facultatively anaerobic species underlines the adaptability and resistance of these isolates under harsh ecological conditions.

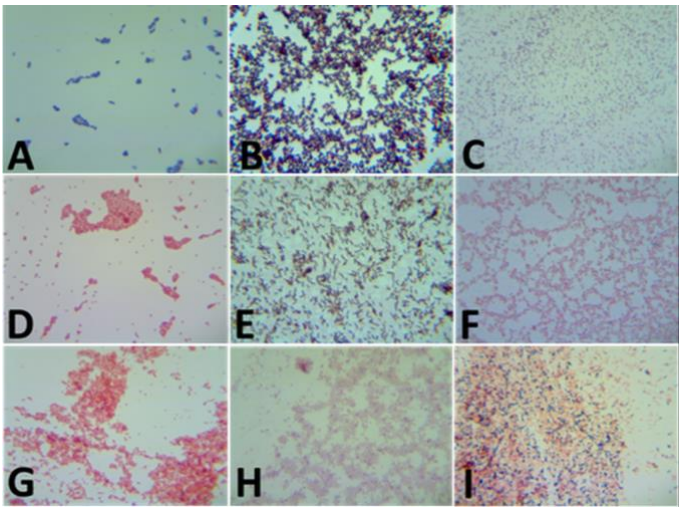


Figure 2. Gram staining of the isolates. **A:** A4, **B:** A5, **C:** A8, **D:** B3, **E:** C10, **F:** C12, **G:** D2, **H:** D5 and **I:** D6

Table 2. Phenotypic and molecular characterization of bacterial isolates recovered from goose and poultry farm samples.

Isolate Code	GenBank ID	Identification	Location	Temp./ T_{opt} (°C)	pH	NaCl (%)	Anaerobic growth	Chain	Movement	Endospore	Gram Test
A1	PQ632386	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A2	PQ632387	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A4	PQ632388	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A6	PQ632389	*Macrococcus caseolyticus*	Center	36-95/55	5-7	≤ 7	+	-	-	-	+
A8	PQ632390	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
B1	PQ632391	*Mammaliicoccus vitulinus*	Kafkas	36-95/55	5-7	≤ 7	+	-	-	-	+
B2	PQ632392	*Escherichia coli*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B3	PQ632393	*Rothia nasisuis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	-	+
B4	PQ632394	*Mammaliicoccus sciuri*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
B5	PQ632395	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B6	PQ632396	*Escherichia coli*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B8	PQ632397	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C1	PQ632398	*Bacillus zhangzhouensis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
C2	PQ632399	*Exiguobacterium sibiricum*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
C3	PQ632400	*Bacillus pumilus*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
C4	PQ632401	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C5	PQ632402	*Kosakonia cowanii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C8	PQ632403	*Kosakonia cowanii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C9	PQ632404	*Exiguobacterium artemiae*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
C10	PQ632405	*Mammaliicoccus vitulinus*	Kafkas	36-95/55	5-7	≤ 7	+	-	-	-	+
C11	PQ632406	*Ralstonia* sp.	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C12	PQ632407	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C13	PQ632408	*Bacillus licheniformis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
D2	PQ632409	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D4	PQ632410	*Ralstonia pickettii*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D5	PQ632411	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D6	PQ632412	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-

16S rRNA Gene Regions of Isolates

Genomic DNA from bacterial isolates was visualised by electrophoresis gel verification (Figure 3). DNA bands that appeared clear were considered indicative of a quality DNA isolate for further analyses. Repeated isolation studies were performed for genomic DNAs that could not be fully visualised. All universally agreed upon primers, including those targeting conserved regions, were enlisted to magnify the 16S RNA gene. Agarose gel electrophoresis (Figure 4) confirmed about 1300 to 1500 base pairs. The gel purified products were subjected to sequencing analysis. The amplicons obtained were compared with the NCBI database with the use of BLASTn to identify the similarity percentages and name the isolates. Isolates successfully identified at the genus or species level were assigned accession numbers through the NCBI system (Table 2). Many isolates are commonly found in organic-rich environments. These isolates showed high sequence similarity to *E. coli*, *R. pickettii* and *P. agglomerans*. In addition to these common species, several thermotolerant or endospore-forming genera such as *Bacillus* and *Exiguobacterium* have been identified. GenBank accession numbers and corresponding isolate codes are presented in Table 2.

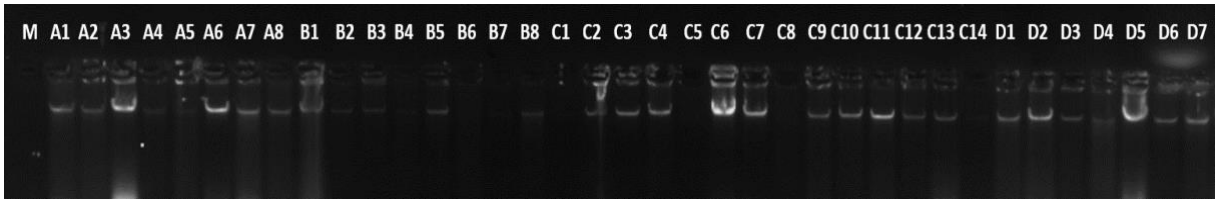


Figure 3. Genomic DNA extraction profiles of bacterial isolates recovered from goose and poultry farm environments. M: DNA ladder; lanes A1–D7 represent isolate codes.

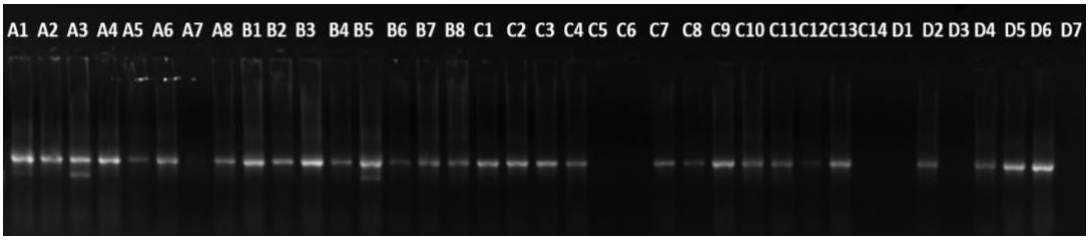


Figure 4. PCR amplification of 16S rRNA gene regions using universal primers. Amplicons are approximately 1500 bp. M: DNA ladder; lanes A1–D7 correspond to bacterial isolates. (GenBank PQ632386 - PQ632412)

Taxonomic Distribution of the Isolates

The bar chart shown in Figure 5 displays the relative abundance of the identified genera. Among the above-mentioned anaerobes belong facultative anaerobes like *E. coli* and *R. pickettii*, while also a group of well-represented sporulating genera such as *Bacillus* and *Exiguobacterium*. Findings like these indicate adaptation by microbes to environments that are high in nutrients and thermally variable, such as farms.

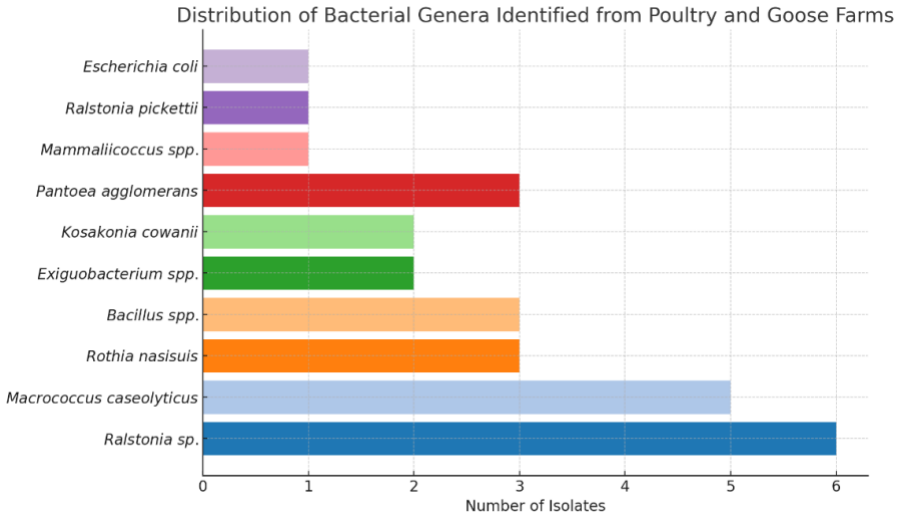


Figure 5. Distribution of bacterial genera identified from goose and poultry farm environments. The bar chart shows the number of isolates corresponding to each genus based on 16S rRNA gene sequencing results.

16s-Based Phylogeny

A phylogenetic tree was produced by the Neighbour-Joining method using the 16S rRNA gene sequences of the 27 bacteria isolated from the poultry area. The phylogenetic tree was rooted with *Bacteroides fragilis* as the outgroup and reference strains were included for the ingroup (Figure 6).

The study identified a total of 9 major clades, each of them strongly supported by bootstrap values, and revealed different relationships in terms of evolution among the isolates. One of the major clusters contained *E. coli* strains A1, A2, A4, B2, A8, B6 and grouped it closely to *E. coli* strain U 5/41 with bootstrap values falling within 66-97. This uniformity supports the reliability of their identification. Another clustered clade which demonstrates a quite close phylogenetic affinity between members of the *Enterobacteriaceae* is that with *K. cowanii* (C5, C8) and *P. agglomerans* (D5, D6); whereas, *R. pickettii* isolates (B5, B8, C4, C12, D4), which together with *R. pickettii* RAL01, formed a coherent cluster that was supported by bootstrap values ranging between 55 and 96, indicating a very strong monophyletic origin.

Spore-forming isolates such as *B. pumilus*, *B. lichenformis* and *B. zhangzhouensis* were clearly separated from other gram-positive bacteria such as *E.sibiricum*, *M. vitulinus* and *M. caseolyticus*. Interestingly, *Exiguobacterium* isolates (C2, C9) closely clustered with *E. artemiae*, suggesting possible environmental adaptation to stress-resistant niches.

The overall structure of the tree illustrates the results of molecular identification and ecological variation among the isolates with isolate consistency. The presence of distinct races, such as facultative anaerobes, thermotolerance bacilli and environmental opportunists, highlights the wide variety of bacterial taxa that thrive in habitats dependent on geese and poultry livestock.

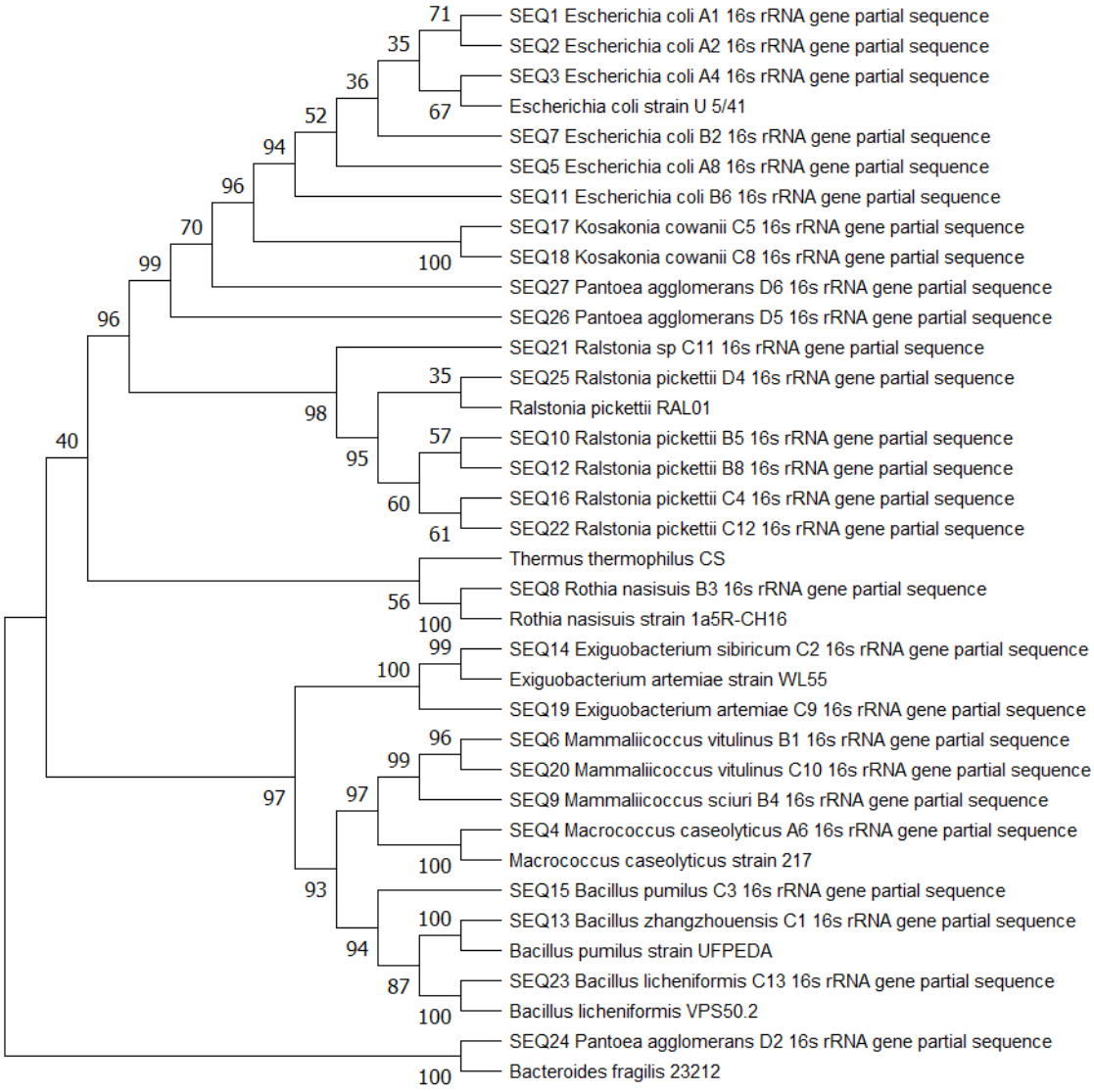


Figure 6. A molecular phylogenetic tree based on 16S rRNA was constructed by the neighbor-joining method with 1000 bootstrap replicates using *Bacteroides fragilis* 23212 as the outgroup.

This study provides information on microbial bacterial diversity obtained from goose and chicken farms in Kars, one of the coldest provinces of Turkey. On the basis of the results of these morphological, biochemical, and molecular characterizations, including the 16S rRNA gene sequence analysis, 27 isolates belonging to the genera *Escherichia*, *Ralstonia*, *Pantoea*, *Kosakonia*, *Exiguobacterium*, *Bacillus*, *Rothia*, *Macrococcus* and *Mammaliicoccus* were identified. These outcomes yield at least the essential results in the context of bacterial diversity encountered in agricultural ecosystems characterized by the accumulation of organic waste.

Species such as *E. coli*, *R.pickettii* and *P. agglomerans* in the study area, which is contaminated with faeces and rich in organic compounds, are consistent with other reported studies (Althouse & Lu, 2005; Charnock, 2004; Mokoena, 2013; Väisänen, Weber, Bennasar, Rainey, & Busse, 1998). *E. coli*, a facultative anaerobe frequently encountered in the gut microbiota of poultry, has been reported similarly in previous studies (Adil & Magray, 2012; Peng et al., 2021). However, the presence of this bacterium in external environmental samples in the study area increases the risk of contamination. Due to their antibiotic resistance, the isolates provide an excellent opportunity for the spread of potential zoonoses (Dafale, Srivastava, & Purohit, 2020). *R. pickettii* can naturally resist many antibiotics; therefore, it is frequently isolated from soil and water-associated environments. This species is also known as an opportunistic pathogen (Ryan & Adley, 2013).

The frequent isolation of *R. pickettii* during this study points to its ability to readily adapt to the nutrient-rich feather waste. Furthermore, even though it is another definition of a species found on plant surfaces, *P. agglomerans* can also adapt to poultry settings (Dutkiewicz, Mackiewicz, Lemieszek, Golec, & Milanowski, 2016; Holmström et al., 2025). In humans and animals, *P. agglomerans* can cause infections (Yu et al., 2025). In addition to their pathogenic properties, these genera contribute to the nutrient cycle and are involved in the hydrolysis of keratin.

Thermotolerant and spore-forming bacteria such as *B. licheniformis*, *B. pumilus* and *E. sibiricum* usually exist in protein-rich environments. The habitats of poultry are also favourable environments for these species. *Bacillus* species that can synthesize enzymes like keratinase and protease have drawn attention in the area of protein microbial hydrolysis (Areche et al., 2025; de Oliveira Sousa et al., 2025; Vikash et al., 2025). For instance, *B. licheniformis* degrades biological wastes that contain keratin. It also converts keratin wastes into hydrolysates rich in amino acids (Tadevosyan, Margaryan, & Panosyan, 2025). Similarly, *Exiguobacterium* species capable of keratin hydrolysis can adapt to both cold and hot environments. This adaptability makes them important for industrial biotechnology (Galván, Alonso-Reyes, & Albarracín, 2025).

The phylogenetic analysis results grouped the isolates with reference strains at the species level with high bootstrap support values and taxonomically correctly positioned. *Enterobacteriaceae* family members such as *K. cowanii* and *P. agglomerans* had similarities in phylogenetics (Berinson et al., 2020). Example separation of *Bacillus* species from gram-positive cocci (*Macrococcus*, *Mammaliicoccus*) demonstrates selection pressures in the environment leading to evolutionary divergence. When observed ecologically, factors such as temperature, oxygen and abundant keratinous nutrient media influence microbial diversity and adaptation. Similar occurrences of keratinolytic bacteria have been observed in environments like compost, feather waste, chicken manure, and slaughterhouses (Ningthoujam et al., 2018).

The findings suggest potential for future industrial applications. From the perspective of environmental sustainability and economic benefit, the transformation of keratinous wastes (mainly feathers) by microorganisms assumes great significance. Traditional disposal methods (like incineration or dumping) cause environmental devastation and create costs. However, using local microbes that are able to produce keratinase can help in ameliorating these processes (Akhter et al., 2020). The presence of rare species such as *M. caseolyticus* and *M. vitulinus*, whose keratinolytic abilities have not been clearly revealed, also makes the study interesting. Comparative genomic analyses show that these genera are active in antimicrobial peptide production.

CONCLUSION

This study revealed that keratinolytic bacteria isolated from goose and poultry farms have an important potential in the biological transformation of feather waste. Especially spore-forming and thermotolerant species were found to be effective in these processes. These bacteria are valuable biocatalysts for sustainable waste management and environmentally friendly biotechnological applications. In future studies, it is recommended to investigate the keratinase production potential of these isolates in detail and to carry out studies for industrial scale applications.

Future studies should focus on examining keratinase activity in more detail and investigating how it can be used more advantageously in industrial areas.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text and Table are original and that they have not been published before

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Assessing the effects of farming on water quality of fish farms in Ghana: the case study of Sunyani Municipality

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Abstract

This research examines the effects of farming activities on water quality of fish farms located in the Sunyani Municipality of Ghana. Six fish farms were selected for the study, including three fish farms close to farming activities (WFA) and another three far from farming activities (WOFA). Water samples were obtained monthly between May and September 2023, and analyzed for physicochemical parameters. The results showed that concentrations of nitrate, nitrite, total suspended solids (TSS), and total dissolved solids (TDS) were significantly higher in the farms without farming activities (with averages of 43.28 ± 2.83 mg/L for nitrate, 0.16 ± 0.05 mg/L for nitrite, 187.60 ± 24.30 mg/L for TSS, and 148.70 ± 12.90 mg/L for TDS) compared to farms linked to farming activities (18.40 ± 2.83 mg/L for nitrate, 0.05 ± 0.01 mg/L for nitrite, 103.60 ± 15.90 mg/L for TSS, and 113.60 ± 11.30 mg/L for TDS). Principal Component Analysis (PCA) revealed that pH and sulfate had positive coefficients (0.55 and 0.63, respectively) in farms with farming activities, whereas nitrate had a positive coefficient (0.52) in farms without farming activities. The high levels of water quality parameters in the farms without farming activities were attributed to internal pond mechanisms, while farming activities were observed to significantly influence sulfate concentrations in the fish farms. In conclusion, the study demonstrates that agricultural runoff and nutrient leaching directly increased sulphate concentrations in fish farms, disrupting water chemistry and posing risks to aquatic ecosystems and fish health. Based on the findings, structured water testing schedule can help detect changes in key parameters such as pH, nitrate and ammonia levels should be formulated and implemented. Also, a 100-meter buffer zone between fish farms and farming areas to prevent contamination should be established.

Keywords: Farming Activities, Water Quality, Fish Farms, Aquaculture, Sunyani, Ghana

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INTRODUCTION

The increasing global demand for fish has positioned aquaculture as a vital strategy to supplement dwindling capture fisheries and ensure food security (Agbeko et al., 2019a). In sub-Saharan Africa, aquaculture is largely conducted in earthen ponds, which are generally less intensive and technologically advanced than systems used in Asia, Europe, and the Americas (Frimpong et al., 2014).

Aquaculture production in Ghana has grown from 5,590 tonnes in 2008 to 89,375.48 tonnes in 2021 due to government interventions to the sector by providing free vaccines to fish farms that were affected. Tilapia remains the most popular cultured fish in Ghana, followed by catfish (GSS, 2023). Since 2018, Ghana has emerged as a leading producer of farmed tilapia in sub-Saharan Africa, second only to Egypt in the broader African context (Ragasa et al., 2022). While industrial aquaculture has attracted substantial research and investment, small-scale aquaculture, especially earthen pond farming remains a dominant practice across many regions of Ghana (Kassam

& Dorward, 2017). Pond aquaculture faces multiple constraints including limited technical expertise, high operational costs, and, notably, declining water quality (Ragasa et al., 2022). Water quality plays a critical role in fish health, influencing growth rates, disease resistance, and mortality (Agbeko et al., 2019b). Poor water conditions, often resulting from nutrient buildup, phytoplankton imbalances, and microbial activity, can trigger eutrophication and increase fish vulnerability to pathogens (Otoo et al., 2019).

In the Sunyani Municipality, the proximity of small-scale fish farms to vegetable farms, especially along the banks of the Tano River and its tributaries intensifies this water quality challenges (Obiri et al., 2021). Farmers cultivate crops such as tomatoes, pepper, and cabbage using fertilizers and organic manure, which, through surface runoff and seepage, enter fish ponds and increase nutrient loading (Rashmi et al., 2020). This encourages phytoplankton blooms and supports the growth of harmful microorganisms (Balasuriya et al., 2022). Additionally, microbial breakdown of uneaten fish feed and faeces deplete oxygen and further release substances that destabilize pond ecosystems (Mramba & Kahindi, 2023). These conditions differ from those in other regions, where water sources may be less affected by adjacent farming or where integrated aquaculture-agriculture systems are more systematically managed.

While several studies in Ghana have investigated the impact of fish farming on downstream water bodies (e.g., Saah et al., 2021; Otoo et al., 2019), no study on impact of agricultural activities on water quality parameters within fish ponds has been conducted in Ghana. Absence of such crucial study renders any management measures towards sustainable pond aquaculture in Ghana ineffective. This study therefore seeks to fill this critical gap by assessing the impact of agricultural activities on the water quality parameters of earthen fish ponds in the Sunyani Municipality, Ghana**.** The finding from this study will contribute to the sustainable management of small-scale aquaculture in Ghana, providing insights for policy interventions, fish farmer training, and integrated land-water use strategies.

MATERIALS AND METHODS

Study Area

The study was performed in Sunyani Municipality which is the capital of the Bono Region, Ghana. The study area is situated between latitudes 7°20′ and 7°05′ N and longitudes 2°10′ and 2°30′ W, covering an area of 506.7 km² and has a population size of 136,022 (Appiah et al., 2018; GSS, 2020). The study area was selected due to the presence of high number of active fish farms (Anokyewaa & Asiedu, 2019).

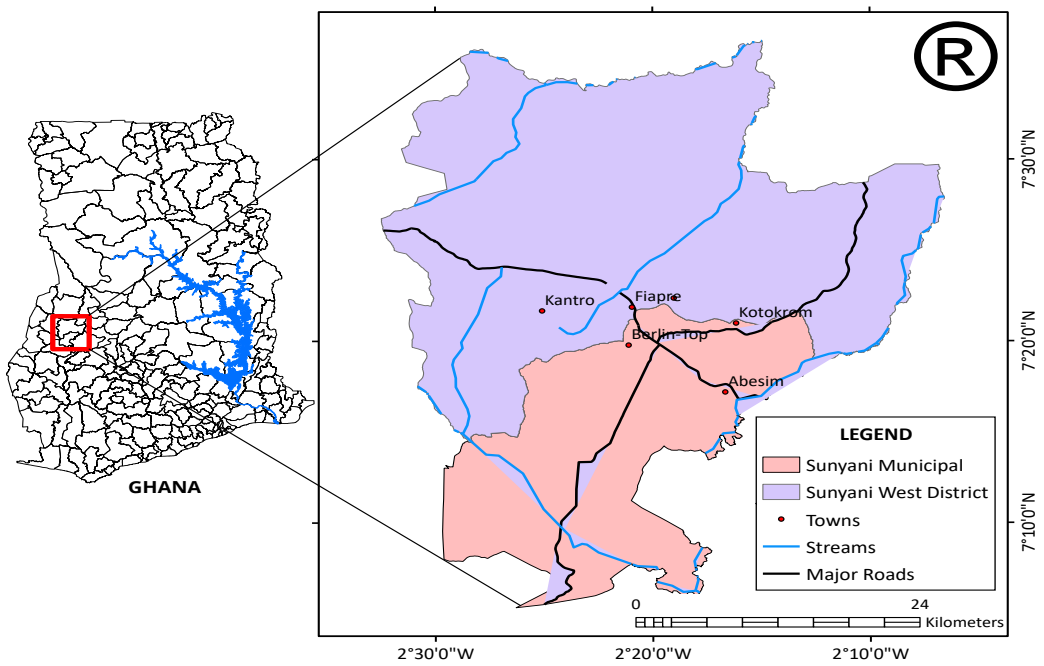


Figure 1. Map showing the study area (Ghana, 2024)

Data Collection

The study was carried out between May and September 2023, coinciding with significant rainy season in the Municipality. This sampling period was chosen because rainfall during these months has a substantial impact on surface runoff, which can transport fertilizers, pesticides, and other agricultural inputs into neighboring water bodies, including fish ponds. As a result, this season offers a significant opportunity to analyze the influence of adjacent farming activities on water quality parameters. The study chose six earthen fish ponds: three within 100

meters of active vegetable farms (e.g., tomatoes, pepper, cabbage), and three more than 500 meters away from any agricultural activity. These classifications were used to distinguish between ponds that were possibly exposed to agricultural runoff and those that had no external influence. Five water samples were obtained monthly from each pond, totaling 25 samples per pond and 150 samples throughout the study period. To guarantee consistency, samples were collected at various points across the pond (center, inlet, outflow, and two midpoints) at depths of 20-30 cm. Water quality measurements were evaluated *in-situ* between 8:00 a.m. and 12:00 p.m. to reduce temperature variability. A multi-parameter probe (Hach HQ40d), calibrated in the laboratory based on standard procedure was utilized to assess dissolved oxygen (DO), pH, temperature, conductivity, salinity, total dissolved solids (TDS), and total suspended solids (TSS). Turbidity was measured separately with a Hach 2100Q turbidity meter. All equipment was calibrated prior to each sampling session in accordance with manufacturer specifications. The detection limits and accuracy ranges for the instruments were: DO: 0.01-20 mg/L ±0.1 mg/L; pH: 0-14 ±0.02 pH units. Turbidity: 0-1000 NTU, ±2%

Data Analysis

Water samples were collected in clean polyethylene bottles, immediately stored in iceboxes, and transported within 4 hours to the Ghana Water Company Laboratory. Samples were preserved according to APHA (1998) standards. Chemical parameters including ammonia, nitrites, and nitrates, were analyzed using a UV-VIS spectrophotometer at wavelengths of 543 nm and 635 nm, depending on the parameter. Laboratory and field data were compiled and analyzed using Microsoft Excel (2019) and PAST (version 4.13). A Shapiro-Wilk test was performed to assess the normality of data distributions. Only parameters meeting the assumption of normality ($p > 0.05$) were subjected to independent samples T-test to compare means between ponds near and far from agricultural areas. Non-parametric Mann-Whitney U test was used to examine parameters that violated normality assumptions ($p < 0.05$). Additionally, Principal Component Analysis (PCA) was used to discover underlying patterns and correlations between the water quality measures in the two pond groups. Water quality values were compared to Ghana Standards Authority (GSA) and World Health Organization (WHO) aquaculture water quality criteria to assess the impact on fish health and pond sustainability.

RESULTS AND DISCUSSION

The concentrations of water quality parameters from two types of fish farms including fish farms with farming activities and fish farms without farming activities. For parameters such as ammonia, turbidity, and sulphate, the mean concentration was higher in fish farms with farming activities (Table 1). Conversely, the mean concentration of the remaining nutrients was greater in fish farms without farming activities (Table 1). Significant differences were recorded for the mean concentrations of nitrite, nitrate, sulphate, calcium, Total Suspended Solids, Total Dissolved Solids, pH, and ammonia between fish farms with farming activities and fish farms without farming activities, with a p-value < 0.05 (Table 1).

Table 1. Mean concentration of water quality parameters between fish farms with farming (WFA) and without farming activities (WOFA)

Name	Fish farms with farming (n = 8 farms) Mean ± standard error	Fish farms without farming (n = 8 farms) Mean ± standard error	p-value
Dissolved oxygen (mg/L)	1.90 ±0.84	2.33 ± 0.19	0.61
pH	6.71 ± 0.07	7.63 ± 0.18	0.00*
Ammonium (mg/L)	0.25 ± 0.04	0.04 ± 0.01	0.00*
Nitrite (mg/L)	0.05 ± 0.01	0.16 ± 0.05	0.02*
Nitrate (mg/L)	18.40 ± 2.83	43.28 ± 2.83	0.01*
Turbidity (NTU)	23.17±2.91	18.44 ± 1.72	0.17
Temperature (°C)	26.39 ± 0.20	26.43 ± 0.24	0.91
Sulphate (mg/L)	19.56 ± 2.86	11.38 ± 2.41	0.05*
Total Suspended Solids (mg/L)	103.60 ± 15.90	187.60 ± 24.30	0.01*
Total Dissolved Solids (mg/L)	113.60 ± 11.30	148.70 ± 12.90	0.05*
Conductivity (µS/cm)	222.10 ± 22.70	258.10 ± 20.90	0.25

NB: Parameters with asterisk (*) indicate significant difference.

Nutrients play a vital role in aquatic ecosystems as they facilitate metabolic activities (Okomoda 2011). The observed significant difference in nitrate, pH and nitrate concentration between the two categories of fish farms could be linked to algal blooms, low vegetation cover, internal mechanisms of the pond, as well as pH and alkalinity (Saha & Paul 2023; Singh et al. 2022; Naeem et al. 2022). Additionally, Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) were significantly higher in fish farms without farming activities. The high concentration of TSS and TDS could facilitate the intrusion of pollutants into fish ponds (Rahman et al. 2021). In addition, high concentrations of TSS and TDS may reduce the concentration of naturally occurring dissolved

oxygen, potentially leading to increased fish kills. Conversely, ammonia concentrations was higher in fish farms with farming activities, which may be due to inadequate abundance of nitrifying bacteria and restricted interactions with the external environment (Nayak & Nanda, 2021).

The concentrations of nitrate, ammonia, TSS, and TDS observed in the study surpassed the levels recommended by World Health Organization (WHO, 2003). Also, mean sulphate concentration from the study was outside the ideal range suggested by Umeh et al. (2020) for growth, maintenance, and enhanced fish production. Elevated water quality parameters could contribute to high mortality of farmed fish if proper management measures are not developed and enforced (Wanja et al. 2020). Furthermore, high levels of ammonia accumulation in fish farms could have negative influence on thealth status of farmed fish (Hargreaves & Tucker 2004). However, average pH and nitrite levels recorded in the study fell within the recommended range by WHO (2003). This suggests that these water quality parameters are suitable for farming fishes of commercial importance.

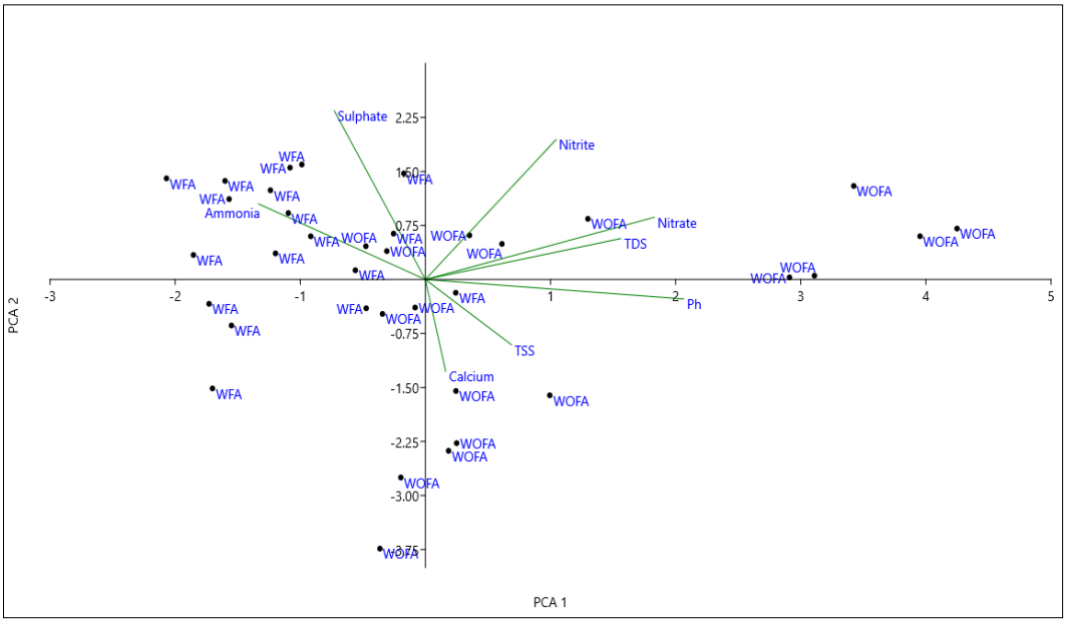


Figure 2. PCA output of significant water quality parameters and fish farms with and without farming activities.

From the PCA output, axes 1 and 2 collectively accounted for more than 50% of the variation (see Figure 2). Based on PCA 1, pH with a coefficient of 0.55, increased in fish farms with farming activities. Conversely, nitrate having a coefficient of 0.52, increased in fish farms without farming activities (Figure 2). In PCA 2, sulphate increase in fish farms with farming activities, having a coefficient of 0.63 (Figure 2). Positive correlation implied that increase in one variable translates into increase in the other variable or vice versa while a negative corelation suggested as one parameter increases, the other variable declines or vice versa. The concentration of pH showed a positive correlation with fish farms close to farming activities. A study by Wanja et al. (2020) indicated that farming activities tend to increase pH concentration owning to rising nutrient input from waste and feed. Additionally, positive correlation of sulphate in fish farms with farming activities may be linked to the existence of sulphate-containing organic fertilizers and waste runoff (Numbere et al., 2023). According to Vehanen et al. (2022), sulphate in fish farms increase may be due to drainage of acid sulphate soils and oxidation of metal sulphides. On the other hand, nitrates exhibited a positive correlation with fish farms without farming activities. This finding was in variance to study by Hejazy (2023), who reported rising nitrate concentrations in fish farms with farming activities. The variation may be reliant on the absence of industrial effluent and the distance from farming activities (Sahare, 2014).

CONCLUSION

This study sheds light on the impact of farming activities on water quality parameters of fish farms in the Sunyani Municipality, Ghana. Findings from the study showed that farming activities significantly affects the concentration of sulphate in fish farms close to farm lands. This suggests that runoff and nutrient leaching from farming areas contribute to alterations in water qaulity parameters, affecting aquatic ecosystems and fish health. However, changes in the concentration of ammonia, nitrates, TSS and TDS of fish farms far from farming activities are mostly due to the internal mechanisms of the ponds rather than contribution from farming activities. As part of recommendations from this study, it is crucial to continuously monitor water quality parameters, especially nitrate, pH and ammonia because increase in these parameters may result in euphrophication and high fish kills. Also, implementing policies that establish buffer zones between fish farms and farmlands is essential, mostly 100

m interval between fish farms and agricultural lands. Such policies should be formulated after proper consultations with stakeholders to ensure feasibility and effectiveness of the policies.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflicts of interest to declare.

Author contribution

All authors contributed to the design and conceptualization of study. The first draft of the manuscript was done by Mohammed, A.M. and Amponsah, S.K.K, while İncoom, A.B.W reviewed the manuscript. All authors approved the final manuscript.

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The rootstock potential of Citron *(Citrullus amarus)* for watermelon

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Abstract

Nowadays, global warming, abiotic and biotic stress factors pose significant challenges to the planet. Based on the increase in the population, the crop production of most agriculturally, economically, and nutritionally important crops as well as watermelon should be increased to their maximum. Grafting is a horticultural method by which the plant tissues are connected to enable the plants to continue growing together. It is usable technique to develop several horticultural traits in watermelon production such as yield and fruit quality and quantity. The main purpose of this study was to analyze the rootstock potential of citron (*Citrullus amarus)* genotypes for watermelon in terms of total yield, fruit characteristics and fruit quality (sugar and carotenoid contents). This study was conducted in the open field under low tunnel conditions within two successive years in Adana, Türkiye. The 11 *Citrullus amarus* genotypes (Kar 234, Kar 324, Kar 326, Kar 327, Kar 328, Kar 351, Kar 374, Kar 375, Kar 376, G 38, and G 40) were used as rootstocks and Crimson Tide F_1 was scion. Commercial rootstocks: Maximus a *Cucurbita* hybrid rootstock (*Cucurbita maxima* × *Cucurbita moschata*) and Argentario (*Lagenaria siceraria*) were utilized for comparison, and non-grafted Crimson Tide was considered as a control group. According to the results, plants grafted onto *C. amarus* genotypes produced the highest total yield compared to the control group, but lower than to the plants grafted onto commercial rootstocks. All fruit measurement values of plants grafted onto commercial rootstocks resulted higher or similar than plants grafted onto *C. amarus*. It was determined that using *C. amarus* rootstocks positively affected fruit sugar content, however the amount of total lycopene and carotenoid content varied among genotypes. Kar 328, Kar 234 and Kar 351 were found to be promising genotypes as rootstock in terms of total soluble solids, total lycopene, total sugar, and total carotenoid contents respectively. In conclusion citron genotypes could be applied as an alternative rootstock for watermelon grafting program.

Keywords: Watermelon, Genetic resources, Grafting, Lycopene, Fruit quality

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INTRODUCTION

Grafting is an alternative and useful method to solve problems associated with soil-borne diseases caused by intensive cultivation in limited agricultural areas (Adiguzel et al., 2022). Grafting is a vegetative growth technique that involves combining the rootstock (plant's underground root portion, with the scion (shoot part) (Goldschmidt, 2014). Besides, it is widespread in Japan and Korea at the same time other Asian and European countries (China, Spain, Italy, Greece, and Israel) where vegetables have edible fruit, are being cultivated intensively (Davis et al., 2008; Maurya et al., 2019). The first purpose of vegetable grafting is to provide resistance to diseases sourced from soil (Edelstein et al., 1999; Han et al., 2003; Yetişir et al., 2003; Lopez-Galarza et al., 2004; Yetişir et al., 2007; Keinath and Hassell, 2014; Toporek and Keinath, 2020; Aydin et al., 2022) and to increase yield (Passam, 2003; Yetişir and Sarı, 2003, Miguel et al., 2004; Davis et al., 2008; Yetişir et al., 2010). In addition, grafting is

also used for enhancing tolerance and resistance to low and high temperatures (Rivero et al., 2003; Hu et al., 2006) to promote resistance/tolerance to the salinity, drought and flooding (Colla et al., 2006; Yetişir et al., 2006; Sakata et al., 2007; Colla et al., 2010), to induce water and nutrients uptake and efficient use in plant (Rouphael et al., 2008; Colla et al., 2011; Ozmen et al., 2015; Huang et al., 2016). Recently, grafting technique has been also applied for increasing yield and quality in seed production of seeded and seedless watermelons (Kombo and Sari, 2019; Aras, 2020; Hussein and Sari, 2020).

Cucurbitaceae as well as watermelons are important vegetable species grown nutritionally and economically in the worldwide. Türkiye is ranked the world's 3rd watermelon producer after China and India, with 3 147 921 tonnes in 640 700 da area (FAO, 2023). Watermelon has been cultivated in many regions of Türkiye, however, the serious yield losses occured due to soil-borne diseases, especially from *Fusarium* wilt, and to one after another cropping (Yetisir and Sari, 2003; Karaca et al., 2012). Grafting vegetable crops was introduced to Türkiye in the late 20th century (Sari et al., 1998) and nowadays watermelon and tomato are the primary vegetable crops in which grafted seedlings are used in Türkiye. Most of the watermelons are grafted mainly on bottle gourd, pumpkin and interspecific hybrid rootstocks (Davis et al., 2008; King et al., 2010; Karaağaç and Balkaya, 2013; Kong et al., 2014). The use of different rootstock has been indicated to affect watermelon plant vigor and yield (Yetisir and Sari, 2003). Watermelon grafting on *Lagenaria* or *Cucurbita* increases vigor, fruit size, and crop yield (Alexopoulos et al., 2007; Davis et al., 2008). Nevertheless, in certain cases grafting onto *Cucurbita* rootstocks may have a negative effect on fruit quality, such as less crispiness, harder fruit flesh with white strands, fruit deformation and changes in fruit size (Cohen et al., 2014). Frequently, it stated that grafting affects the fruit's morphological traits (size, shape, color), firmness, texture, flavor components (sugar, organic acids, and aroma), and compounds related to human health (minerals, vitamins, and carotenoids) (Rouphael et al., 2010; Edelstein et al., 2014).

Moreover, various studies have been demonstrated that grafting possess negative and postive impacts on the fruit quality (Yetişir and Sari, 2003; Lopez-Galarza et al., 2004; Davis and Perkins-Veazie, 2005-2006; Liu et al., 2006; Perkins-Veazie et al., 2008; Proietti et al., 2008; Bekhradi et al., 2011; Turhan et al., 2012; Çandır et al., 2013; Guler et al., 2014; Petropoulos et al., 2014; Soteriou et al., 2014; Aydin et al., 2022). The difference in grafting responses may be due to different agronomic practices and environments, genetics, scions, rootstocks, scion-rootstock combinations, growing conditions and the stage of fruit maturity (Yetisir and Sari, 2003; Davis et al., 2008; Rouphael et al., 2010). In addition to these, one way to compromise the quality of grafted watermelon varieties may be to use rootstocks that deviate from the *Citrullus* genes (Aydin et al., 2022). Yet, it has been mentioned that grafting watermelon onto wild type of watermelon rootstocks prevents the occurrence of *Fusarium* wilt (Davis et al., 2008) and it may overcome the decrease in fruit quality that resulting from the use of *Cucurbita* rootstocks (Huh et al., 2002; Cohen et al., 2014; Edelstein et al., 2014; Thies et al., 2015).

Citrullus amarus (previously expressed as "citron", "citron melon", and "preserving melon") known as *Citrullus lanatus* var. *citroides*. The wild and local citron forms exist in South Africa although it is known to be cultivated (Laghetti and Hammer, 2007; Chomicki et al., 2020). Its rind is used to make pickles, jam and fruits that are fed to livestocks (Wehner, 2008), its leaves are edible as green leafy vegetables. The citron's flesh is boiled in water, and mixing with maize meal to cook porridge, its name is "Kgodu", in South Africa and Botswana (Bultosa et al., 2020). Ripe citron seeds are dried in the sun, roasted and consumed as a snack (Mandizvo et al., 2021). Citron is also used in the jam and confectionery in France. Accessions of citron are precious rootstocks for grafting watermelon providing high grafting compatibility and resistance to root-knot nematodes (Thies et al., 2010; Thies et al., 2012; Thies et al., 2015). Fredes et al. (2016) also reported that *C. amarus* as hopeful alternative to the use of *Cucurbita* rootstocks which increases resistance against nematodes. Moreover, drought is one of the most important factors that lead to the limitation of agricultural production in regions with arid and semi-arid areas such as our country. One of the permanent methods to be applied in today's breeding to minimize the negative effects of drought stress is the development of drought-tolerant varieties or rootstocks and their utilization in agricultural production (Atakul, 2024). Citron can show high level of tolerance to drought thanks to grow mainly in arid and semi-arid regions. Because of that, citron expresses more tolerant character to drought than sweet watermelon and commercial hybrid rootstocks (Mandizvo et al., 2022; Metin et al., 2024).

The objective of this study was to investigate the effect of *C. amarus* genotypes rootstocks on Crimson Tide F_1 watermelon's total yield, fruit characteristics and fruit quality parameters such as sugar and carotenoid contents.

MATERIALS AND METHODS

Genetic material and field experiment

This study was carried out at the experimental field of the Department of Horticulture, Faculty of Agriculture, University of Cukurova, Adana-Türkiye (latitude 37°1'48.63"N, longitude 35°22'3.74" E, altitude 56 m) for two successive years.. The 11 *Citrullus amarus* genotypes (Kar 234, Kar 324, Kar 326, Kar 327, Kar 328, Kar 351, Kar 374, Kar 375, Kar 376, G 38, G 40) from watermelon genetic resources collection of Cukurova University, Faculty of Agriculture, Department of Horticulture, Türkiye were used as rootstock. The code, varieties and origins of the genotypes used in this study are given in Table 1. The rootstock potential of these *C. amarus* germplasms

was compared with two commercial rootstocks [Maximus a *Cucurbita* hybrid rootstock (*Cucurbita maxima* × *Cucurbita moschata*) and Argentario (*Lagenaria siceraria*)]. Crimson Tide (CT) F_1, a commercial hybrid variety was used as a scion in all treatments and non-grafted Crimson Tide F_1 was used as control group.

Table 1. The code, varieties and origins of the genotypes

Code	Variety	Origin
Kar 234 (PI 296 341)	*Citrullus amarus*	Seminis
Kar 324 (PI 270563)	*Citrullus amarus*	USDA (USA)
Kar 326 (PI 295843)	*Citrullus amarus*	USDA (USA)
Kar 327 (PI 295843)	*Citrullus amarus*	USDA (USA)
Kar 328 (PI 482342)	*Citrullus amarus*	USDA (USA)
Kar 351	*Citrullus amarus*	INRA / Avignon / France
Kar 374 (PI 271769)	*Citrullus amarus*	Republic of South Korea
Kar 375 (PI 189225)	*Citrullus amarus*	Republic of South Korea
Kar 376 (PI 299379)	*Citrullus amarus*	Republic of South Korea
G 38	*Citrullus amarus*	Hungary
G 40	*Citrullus amarus*	Hungary
Kar 38 x Kar 234	*C. amarus* x *Citrullus amarus*	Çukurova University / Türkiye
Argentario	*Lagenaria siceraira*	Syngenta
Maximus	*Cucurbita maxima* x *Cucurbita moschata*	Antalya Tarim Company
Crimson Tide	*Citrullus lanatus* var. *lanatus*	Syngenta

Seed sowing, grafting applications and maintenance of the seedlings were carried out at Antalya Tarim Productive, Consultant and Marketing Co. in Antalya, Türkiye. Grafting was perfomed as described by Hassell et al., (2008). Grafted and control seedlings were planted under low tunnels conditions at a spacing of 2 m × 0.5 m in Adana, Türkiye (Figure 1). The field investigation was arranged in a complete randomized block design with four replications containing 10 plants for each rootstock-scion combination and control. Plants were drip irrigated and fertigated regularly, and weeds, insects and diseases were kept under control whenever the symptoms appeared, regularly. Fertilizers were implemented regularly at a rate of 200 kg P_2O_5 ha^{-1}, 180 kg N ha^{-1}, and 180 kg K_2O ha^{-1} (Yetisir and Sari, 2003). Phosphorus fertilizer was applied as an entire base fertilizer; nitrogen and potassium were given in 3 applications and the first application was applied as base fertilizer, the remaining applied at 3-week intervals with irrigation. All tunnels were held up one month after transplanting in both years.



Figure 1. Planting stages (setting tunnel wires, planting seedlings, closing the tunnels, appearance of the land after planting and closing the tunnels)

Harvest and fruit analysis

Mature fruits that have completely dried stipules and tendrils were harvested on the 11th of July in the first year and on the 25th of June in the second year (Figure 2). During harvest, all fruits were weighed in each plot by using a weighing balance to determine total fruit yield (kg/m^2). For pomological analysis, three fruits from each replication were selected. Fruit diameter (cm), and fruit length (cm) were measured by using a ruler. Fruit weight was measured using weighing balance (g). Data of the rind thickness of fruit (mm) was received by using a digital caliper (Mitutoyo CD-15D), and a digital refractometer (ATAGO Pocket PAL-3l) was used to determine the fruit total soluble solids content (%).



Figure 2. Harvested grafted fruits

Liquid chromatography analysis of sugar content

The extraction of samples was performed the methods reported by Kumar et al. (2012). A HPLC system (Agilent 1260 HPLC) consisted of a refractive index detector (G1362A RID), and a pump system was used for sugar amount analysis. The analytical conditions were 0.5 mL/min flow, eluent 0.09 mol/L H_2SO_4 with 6% acetonitrile (v/v). Sugar amounts were determined on an HPX-87H Aminex column (Bio-Rad - 300 × 7.8 mm), and fixed at 55°C. To detect the relationship between the concentration and peak area, a calibration curve was created using standards (Fredes et al., 2016). Sugar content analyses were performed only on samples from the second year of the study.

Extraction and analysis of carotenoids

For the analysis of carotenoids, samples were prepared through ethanol-hexane (5:4, v/v) as defined by Cucu et al. (2012). The homogeny extract was sonicated for 5 minutes after that centrifuged at 4500 rpm at 4°C for 15 minutes. The supernatant part was transferred into a falcon and the extraction process was duplicated. For LC-MS/MS analysis, two supernatants were mixed, evaporated, and resolved in 1 mL B mobile phase (methanol: Water: MTBE, 90:5:5, v/v/v). An Agilent 6430 LC-MS/MS spectrometer (integrated with an electrospray

ionization source) was used for the analysis of carotenoids. Carotenoids were isolated and identified in accordance with the method described by Lv et al. (2015). A C30 column was used for the isolation of carotenoids (250 x 4.6 mm i.d., 5 µm, YMC). Carotenoid analyses were performed only on samples from the second year of the study.

Statistical analysis

The experiment was established as complete randomized block design with four replications, containing 10 plants for each combination, and the data were analyzed using JMP (v8.00) statistical software. ANOVA was performed to detect the effects of the years and rootstocks on exanimated features. To examine differences among the different groups, the least significant difference test was performed.

RESULTS AND DISCUSSION

Total fruit yield and average values of measured fruit parameters of grafted and non-grafted watermelons in two successive years are presented in Table 2 and Table 3. Analysis of variance indicated that grafted plants produced higher yield compared to non-grafted control and significant difference was found between rootstock and years. The highest yield was obtained from Argentario/CT (14.8 kg/m^2) and Maximus/CT (14.6 kg/m^2) while the lowest (5.84 kg/m^2) from non-grafted Crimson Tide. Citron (*Citrullus amarus*) genotypes performed better over non-grafted Crimson Tide but lower than those of two commercial rootstocks. Among the ten Citron rootstocks, total yield ranged between 9.01 kg/m^2 (Kar 375) and 11.20 kg/m^2 (G 38/CT). In the case of years first resulted higher than second. The reason for this situation was the difference in rainfall between the two years. Total rainfall during the season was 561 mm in 2012 and 272 mm in 2013.

Results of this present study indicated that grafting provides significant increase in total fruit yield inconsistent with several research reports (Yetisir et al., 2003; Yetisir and Sari, 2003; Colla et al., 2006; Alan et al., 2007; Alexopoulos et al., 2007; Cushman and Huan, 2008; Davis et al., 2008; Karaca et al., 2012; Mohamed et al., 2014; Alan et al., 2017). Davis et al. (2008) reported that even scion variety determines the mature stage size and yield in grafted plants, and rootstock types have significant effects. In our study, we have found higher yields when watermelons grafted onto commercial *Lagenaria* and *Cucurbita* interspecific hybrid (*Cucurbita maxima* × *Cucurbita moschata*) rootstocks compared to Citron (*Citrullus amarus*) rootstocks.

Despite the wide usage of bottle gourd and *Cucurbita* interspecific hybrids as a rootstock for watermelon, few studies (Thies et al., 2010; Edelstein et al., 2014; Thies et al., 2015; Fredes et al., 2016; Nkoana et al., 2022) have investigated the rootstock potential of Citron (*C. amarus*) and its effects on quality and yield. We demonstrated that Citron rootstocks provide an increase in fruit yield compared with non-grafted control plants. Similar to our findings Thies et al. (2010), Thies et al. (2012), and Thies et al. (2015) reported an increase in yield and declared that accessions of *C. amarus* are valuable and efficient rootstocks for watermelons grown in soils infested with nematodes. Also citron can be easily grown in arid regions due to its tolerance to abiotic and biotic stress conditions and can be used as a rootstock for cultivated watermelon to increase fruit quality (Nkoana et al., 2022). In previous studies, 2 different citron genotypes applied as rootstocks were determined as superior rootstocks in arid conditions and were found to have the characteristics that can be utilized as commercial rootstocks for watermelon (Yavuz et al., 2020).

Grafting did not alter the fruit weight statistically and the effect of the rootstock was found to be non-significant. The mean fruit weight ranged between 5176 g (non-grafted control) and 6974 g (Argentario/CT). In the agreement with our study, Petropoulos et al. (2014) observed no difference in mean fruit weight of two hybrid watermelon cultivars grafted onto *Cucurbita* interspecific hybrid and *Lagenaria* sp. rootstock. Similarly, in the study of Cushman and Huan (2008), fruit weight of four triploid watermelons was not affected by grafting onto five rootstock genotypes. However, Fredes et al. (2016) reported that both citron and *Cucurbita* hybrids have been improved fruit weight considerably compared to both non-grafted and self-grafted watermelons. Accordingly, Edelstein et al. (2014) described when the use of *Cucurbita* rootstocks as a rootstock for watermelon, plants produced heavier fruits than those from grafted on watermelon accessions. This situation can be explained as follows: Plants grafted onto strong rootstocks absorb more water and ions than non-grafted plants, and carry these water and ions to the scion (Aydin et al., 2022).

The content of TSS in watermelon is a prominent factor for consumers. We obtained statistical differences among the rootstock and years in terms of TSS. Both the highest and lowest TSS was observed in grafted watermelons. Watermelons grafted onto citron rootstock Kar 328/CT had the highest TSS (10.6%) while Maximus/CT combination had the lowest (8.93%) value. Other citron genotypes produced similar TSS content compared to non-grafted Crimson Tide. In agreement with our study, Fredes et al. (2016) reported the citron was the main rootstock material that notably increased (10.2 Brix) TSS compared to self-grafted (9.3 Brix) and non-grafted (9.6 Brix) watermelons. Several studies (Miguel et al., 2004; Liu et al., 2006; Yetisir et al., 2007; Huitron-Ramirez et al., 2009; Karaca et al., 2012; Turhan et al., 2012) indicated that total soluble solids of watermelon is highly affected by grafting, however increased, decreased or unchanged TSS contents are mainly due to the type of rootstock (Yetisir et al., 2003). Changes could be also attributed to different production environments, rootstock/scion combinations used and harvest periods (Davis et al., 2008).

Table 2. Total fruit yield, fruit weight and TSS of watermelons grafted on different rootstocks

Rootstock/ scion combination	Total fruit yield (kg/m²)		Mean of rootstock	Fruit weight (g)		Mean of Rootstock	TSS (%)		Mean of rootstock
	Year			Year			Year		
	First	Second		First	Second		First	Second	
Kar 234/CT	11.0	9.4	*10.2 BC*	7236	4687	*5962*	10.0	9.4	*9.74 BCD*
Kar 324/CT	10.7	11.8	*11.2 B*	7548	4772	*6160*	10.7	10.1	*10.4 AB*
Kar 326/CT	11.7	10.2	*10.9 B*	6789	3651	*5220*	10.0	9.2	*9.60 CDE*
Kar 327/CT	10.0	10.2	*10.1 BC*	6756	5216	*5986*	10.0	8.9	*9.45 CDE*
Kar 328/CT	11.3	8.5	*9.92 BC*	6802	5623	*6212*	10.9	10.3	*10.6 A*
Kar 351/CT	10.3	9.6	*9.93 BC*	6747	4978	*5863*	9.96	8.6	*9.29 CDE*
Kar 374/CT	11.5	9.9	*10.7 BC*	6914	4818	*5866*	9.8	9.2	*9.49 CDE*
Kar 375/CT	8.7	9.4	*9.01 C*	6531	4620	*5576*	10.4	9.7	*10.0 ABC*
Kar 376/CT	10.9	9.7	*10.3 BC*	7815	4694	*6255*	10.7	9.2	*9.98 A-D*
G 38/CT	12.0	10.3	*11.2 B*	7885	4294	*6090*	10.7	9.4	*10.0 A-D*
G 40/CT	10.4	9.9	*10.2 BC*	7270	4777	*6024*	10.2	9.8	*10.0 A-D*
Argentario/CT	15.3	14.2	*14.8 A*	8049	5899	*6974*	9.8	8.7	*9.26 DE*
Maximus/CT	15.2	14.1	*14.6 A*	7997	5508	*6752*	9.7	8.2	*8.93 E*
Crimson Tide	6.3	5.4	*5.84 D*	6738	3614	*5176*	10.8	9.1	*9.95 A-D*
Mean of year	11.08 A	10.2 B		7220 A	4797 B		10.3 A	9.28 B	
LSD_{year} = 0.65**				LSD_{year}*** = 385.3			LSD_{year}*** = 0.30		
$LSD_{rootstock}$* = 1.73**				$LSD_{rootstock}$ = N.S.			$LSD_{rootstock}$** = 0.78		
$LSD_{year\ x\ rootstock}$ = N.S.				$LSD_{year\ x\ rootstock}$ = N.S.			$LSD_{year\ x\ rootstock}$ = N. S.		

Differences between the means of rootstock were shown with different letters
N. S: Not Significant, ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05

Table 3. Fruit length, diameter and rind thickness of watermelon grafted on different rootstocks

Rootstock scion combination	Fruit length (cm)		Mean of rootstock	Fruit diameter (cm)		Mean of rootstock	Fruit rind thickness (mm)		Mean of rootstock
	Year			Year			Year		
	First	Second		First	Second		First	Second	
Kar 234/CT	27.8	22.2	*25.0 BCD*	22.0	19.1	*20.6 BC*	11.6	12.6	12.1
Kar 324/CT	28.8	23.5	*26.2 AB*	22.2	19.3	*20.8 BC*	11.9	10.3	11.1
Kar 326/CT	27.3	20.4	*23.8 D*	21.8	18.3	*20.1 BC*	11.6	12.2	11.9
Kar 327/CT	27.1	24.3	*25.7 ABC*	21.7	19.7	*20.7 BC*	11.7	12.9	12.4
Kar 328/CT	26.5	24.1	*25.3 A-D*	21.9	20.2	*21.1 BC*	13.0	12.9	12.9
Kar 351/CT	26.9	22.9	*24.9 BCD*	21.4	19.1	*20.2 BC*	11.1	11.5	11.3
Kar 374/CT	28.3	22.6	*25.5 A-D*	21.0	19.3	*20.1 BC*	12.0	12.3	12.1
Kar 375/CT	27.3	22.8	*25.0 BCD*	21.4	18.9	*20.2 BC*	11.9	12.6	12.2
Kar 376/CT	29.6	22.9	*26.3 AB*	22.4	19.4	*20.9 BC*	12.2	12.5	12.3
G 38/CT	29.1	20.8	*24.9 BCD*	22.8	18.2	*20.5 BC*	12.4	11.6	12.0
G 40/CT	27.7	23.5	*25.6 A-D*	21.4	19.3	*20.3 BC*	11.6	11.5	11.5
Argentario/CT	28.4	25.0	*26.7 AB*	23.7	20.8	*22.2 A*	13.1	14.4	13.8
Maximus/CT	28.9	25.1	*26.9 A*	22.4	19.9	*21.2 AB*	13.4	11.6	12.5
Crimson Tide	26.7	21.7	*24.1 CD*	21.9	18.2	*20.1 C*	12.6	12.1	12.4
Mean of year	27.9 A	22.9 B		22.0 A	19.3 B		12.1	12.2	
LSD_{year}* = 0.68**				LSD_{year}*** = 0.42			LSD_{year}= N.S.		
$LSD_{rootstock}$* = 1.80				$LSD_{rootstock}$* = 1.12			$LSD_{rootstock}$= N.S.		
$LSD_{year\ x\ rootstock}$ = N.S.				$LSD_{year\ x\ rootstock}$ = N.S.			$LSD_{year\ x\ rootstock}$ = N.S.		

Differences between the means of rootstock were shown with different letters
N.S.: Not Significant, ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05

The significance of scion and rootstock interaction stated by Edelstein et al. (2014) has been indicated that grafting onto the same rootstocks decreases TSS in mini watermelon scion while increases in midi watermelon scion.

The findings of variance analysis showed that fruit length and diameter were significantly different among the rootstocks (Table 3). Regarding fruit length watermelons grafted onto Maximus had the longest fruits (26.9 cm) while citron rootstock Kar 326 (23.8 cm) had the shortest. Non-grafted Crimson Tide exhibited similar values to most of the citron rootstocks except Kar 324 and Kar 376. Based on the fruit diameter, larger fruits were obtained from plants grafted onto *Lageneria* rootstock Argentario (22.2 cm) compared to all citron rootstocks and non-grafted Crimson Tide. *Cucurbita* hybrid rootstock Maximus had higher values than non-grafted Crimson Tide but similar to citron rootstocks. Consistent with our results Fredes et al. (2016) demonstrated obtaining wider and longer fruits, without remarkable fruit shape variations using both citron and the two F_1 *Cucurbita* hybrids as a

rootstock. Similarly, in the study of Yetisir et al. (2003), larger fruits were obtained from plants grafted onto *Lagenaria* than from non-grafted plants. Turhan et al. (2012) found that fruits were larger in plants grafted onto hybrid *Cucurbita* rootstocks Dynamo and RS- 841 as well.

No significant difference was found in rind thickness when grafted watermelons compared to non-grafted watermelons. The values ranged between 11.1 mm (Kar 324/CT) and 13.8 mm (Argentario/CT). Accordingly, Petropoulos et al. (2014) observed no difference in fruit rind thickness between grafted and non-grafted plants. However, in the study of Fredes et al. (2016) fruits with thicker rinds were obtained from plants grafted on both Citron melon and *Cucurbita* rootstocks than non-grafted and self-grafted plants. Also, various studies have proven that adverse effects like thicker fruit rind have been recorded in watermelon plants grafted on *Cucurbita* and *Lagenaria* rootstocks compared to non-grafted watermelons (Alexopoulos et al., 2007; Cushman and Huan, 2008; Turhan et al., 2012).

As a sugar component glucose, sucrose, and fructose were determined in examined extracts. Total amounts of sugar were between 75.54 g/kg (Maximus/CT) and 105.74 g/kg (Kar 234/CT) (Table 4). Most citron rootstocks had higher values than *Cucurbita* and *Lagenaria* rootstocks and non-grafted Crimson Tide. The main parts of carbohydrates in extracts are the three simple sugars: Glucose, sucrose, and fructose (Fig. 3). They represent many parts of the TSS of watermelon. The total amount of sugars detected was fructose>glucose>sucrose, respectively. According to Elmstrom and Davis (1981), cultivars or maturity that outcome from high fructose concentrations have a charming character since fructose has a greater effect on sweetness than sucrose. As reported in previous studies, also in our study, fructose was detected in the largest amounts and there was a significant difference between rootstocks and control. The highest content was found in Kar 234/CT and Kar 374/CT (57.99 g/kg and 55.86 g/kg respectively) and the lowest in Maximus/CT (40.00 g/kg). Glucose is the second most abundant sugar and significant differences were obtained between rootstocks. Its concentration ranged from 31.85 g/kg (Maximus/CT) to 43.43 g/kg (Kar 234/CT). Regarding sucrose, it constitutes about 4% of total sugar in all combinations.

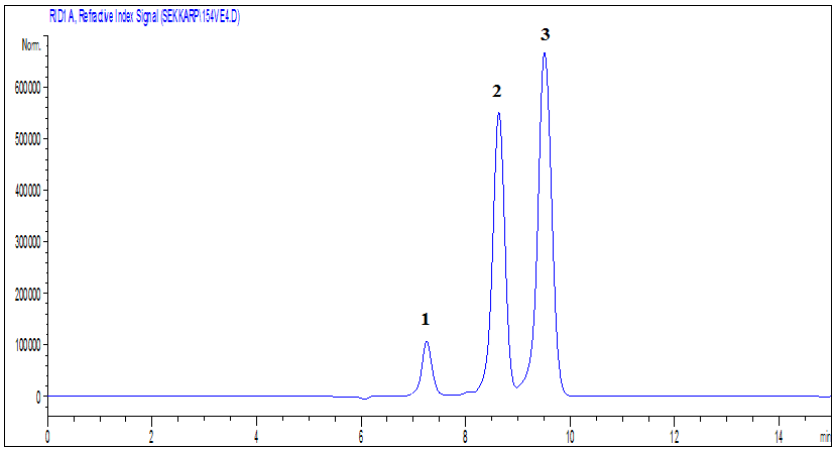


Figure 3. HPLC chromatogram of sugars in Kar 234/CT (Peaks: 1. Sucrose; 2. Glucose; 3. Fructose)

Table 4. Sugar concentration of watermelon fruits grafted on different rootstocks

Rootstock/Scion combinations	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	Total sugars (g/kg)
Kar 234/CT	4.32	43.43 a	57.99 a	105.74 a
Kar 324/CT	3.74	42.33 ab	55.14 ab	101.21 ab
Kar 326/CT	4.31	38.33 cd	47.64 e	90.28 de
Kar 327/CT	3.89	37.75 cde	51.27 cd	92.91 cde
Kar 328/CT	3.75	39.43 bcd	52.36 bc	95.55 bcd
Kar 351/CT	4.25	35.10 efg	51.49 cd	90.84 de
Kar 374/CT	3.99	39.91 bc	55.86 a	99.76 ab
Kar 375/CT	4.89	39.63 bcd	55.78 ab	100.30 ab
Kar 376/CT	3.79	39.45 bcd	55.22 ab	98.46 bc
G 38/CT	3.87	36.51 def	48.16 de	88.54 ef
G 40/CT	3.46	32.91 gh	45.74 e	82.11 fg
Argentario/CT	3.94	33.81 fgh	45.76 e	83.51 f
Maximus/CT	3.68	31.85 h	40.00 f	75.54 h
Crimson Tide	3.53	32.49 gh	40.80 f	76.82 gh
LSD	N.S.	3.15 ***	3.43***	6.48***

Differences between the means were shown with different letters
N.S.: Not Significant***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05

The total sugar ingredients, like sucrose, fructose, and glucose, are responsible for the watermelon sweetness (Yativ et al., 2010). Bianchi et al. (2018) reported that sucrose and glucose amount to 20-40% and fructose to 30-50% of total sugars in mature watermelons. Grafting often affects fruit attributes such as the content of sugars, acids, aroma volatiles, carotenoids, vitamins, and minerals (Rouphael et al., 2010). Our results indicated that grafting affects total sugars, glucose, and fructose content, however, changes in the grafted watermelons can be attributed to the type of the rootstock. We obtained mostly higher values from Citron rootstocks compared to *Cucurbita* hybrid and *Lagenaria* rootstock and non-grafted control. However, Fredes et al. (2016) reported that the Citron melon had no dramatic impact on sugars in comparison non grafted watermelons excluding a rise in glucose substance which also appeared in the *Cucurbita* rootstocks.

A total of five carotenoids were determined and quantified in watermelons, including cryptoxanthin, phytofluene, β-carotene, *cis*-lycopene and *trans*-lycopene. The total content of carotenoids in watermelon determined from different rootstock/scion combinations ranged from 41.60 (Kar 374/CT) to 102.01 (Kar 351/CT) mg/kg. The major carotenoid was *trans*-lycopene as it calculated for the largest part of the total carotenoid. The highest level of *trans*-lycopene was detected in Kar 351/CT (78.06 mg/kg), followed by non-grafted Crimson Tide (75.80 mg/kg) and Kar 376/CT (73.95 mg/kg) (Table 5).

Among the main carotenoids in watermelon, are lycopene and β-carotene (Bianchi et al., 2018), or lycopene is known to be higher than many other fruits and vegetables (Tadmor et al., 2005). Several studies (Davis and Perkins Veazie, 2005; Alan et al., 2007; Proietti et al., 2008; Turhan et al., 2012; Çandır et al., 2013; Petropolulos, 2014; Fredes et al., 2016; Kyricau et al., 2017) examined the effects of grafting on lycopene content, which is is the prevalent carotenoid and one of most important quality criteria in red-fleshed watermelons. However, these studies reported variable results in the lycopene content of fruits when grafted and non-grafted watermelons were compared. Nevertheless, in our case, the highest lycopene content was found both in fruits of plants grafted onto Kar 351 which is a Citron rootstock, and non-grafted control. Besides, the lowest value was also observed in a Citron rootstock (Kar 375). The amounts of β-carotene and lycopene were evaluated in mature watermelons according to stated by Perkins-Veazie et al. (2006) (35-112 mg/kg and 0.9-10.2 mg/kg, respectively).

Table 5. Carotenoid concentration (mg/kg) of watermelon fruits grafted on different rootstocks

Rootstock scion combinations	Cryptoxanthin	Phytofluene	*β*-carotene	*cis*-lycopene	*trans*-lycopene	Total lycopene	Total carotenoid
Kar 234/CT	0.54 a	1.19 b	4.68 a	11.75 g	52.86 d	64.61 fg	71.02 hi
Kar 324/CT	0.48 b	1.05 c	2.04 cd	20.26 a	60.34 c	80.59 cd	84.16 cd
Kar 326/CT	0.41 c	0.89 d	1.35 def	17.11 b	52.08 d	69.19 ef	71.83 gh
Kar 327/CT	0.34 d	0.74 ef	0.83 f	14.66 de	66.22 b	80.88 cd	82.79 cde
Kar 328/CT	0.35 d	0.93 cd	4.10 a	13.65 ef	68.23 b	81.88 c	87.26 c
Kar 351/CT	0.32 de	0.85 def	1.98 cde	20.80 a	78.06 a	98.86 a	102.01 a
Kar 374/CT	0.15 h	0.40 ı	0.92 f	8.45 ı	31.69 e	40.14 h	41.60 j
Kar 375/CT	0.21 fg	0.58 gh	1.34 ef	8.04 ı	35.60 e	43.64 h	45.77 j
Kar 376/CT	0.53 ab	1.44 a	3.34 b	16.08 c	73.95 a	90.03 b	95.35 b
G 38/CT	0.17 gh	0.45 hı	4.47 a	17.13 b	54.96 cd	72.09 e	77.18 efg
G 40/CT	0.49 ab	1.33 ab	1.24 f	9.86 h	52.64 d	62.50 g	65.56 i
Argentario/CT	0.22 fg	0.59 gh	2.56 c	13.48 f	59.82 c	73.30 e	76.68 fgh
Maximus/CT	0.26 ef	0.71 fg	2.46 c	15.05 d	59.99 c	75.04 de	78.48 def
Crimson Tide	0.32 d	0.88 de	3.37 b	17.38 b	75.80 a	93.18 ab	97.75 ab
LSD	0.06 ***	0.14***	0.69***	1.02***	5.59***	5.87***	5.85***

Differences between the means were shown with different letters
N.S.: Not Significant***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05

CONCLUSION

The results of this study showed that grafting supplies a radical increase in total fruit yield. Plants grafted onto *C. amarus* rootstocks had higher total yield in comparison to non-grafted plants, but lower than the commercial *Lagenaria* and *Cucurbita* hybrid rootstocks. All fruit characteristics from plants grafted onto commercial rootstocks yielded higher or comparable results to those from plants grafted onto *C. amarus* genotypes. Besides, it was determined that *C. amarus* rootstocks improved fruit sugar content, however the total lycopene and carotenoid content varied among genotypes. Regarding total sugar, total soluble solids, total lycopene, and total carotenoid levels, Kar 328, Kar 234, and Kar 351 were determined to be the most promising of the citron genotypes utilized as watermelon rootstock. Overall, our results indicated that Citron (*Citrullus amarus*) genotypes can be applied as an alternative rootstock and have a high potential for watermelon grafting.

Compliance with Ethical Standards

Peer-review
Externally peer-reviewed.
Author contribution
The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript.
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Performance evaluation of a small scale palm fruit biomass-fired autoclave boiler

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Abstract

Different fuel mixture (empty fruit bunch, fiber and shell from palm kernel) at different ratios were used to fire an existing boiler to determine the most efficient fuel mixture composition for an improved performance of the boiler. This was carried out to determine the effects of the different types of fuel mixture (at different proportions) on the steam temperature, pressure, rate of steam production and boiler efficiency of the boiler. It was discovered that fuel mixture with higher percentage of fibre cum palm-kernel shells produced the best results for high combustion rate and steam generation. The result of the steam temperature, pressure and enthalpy results for all the fuel combinations ranges between 141 to 161°C, 3.7 to 6.34 bar, and 27.351 to 27.591 MJ kg^{-1}, respectively. Fuel combination ratio 1:8:1 produced the best result in terms of rate of steam generated while the fuel mixture ratio 1:2:7 produced the highest value for boiler efficiency (74.2%). The boiler consumed more fuel with combinations that have high percentage of fibre content (2:7:1) due to the physical and combustion properties of fibres but fuel combinations with higher shell content in the mixture (1:1:8) burns longer in the combustion chamber than mixtures with higher percentages of other fuel. The fuel mixtures produced high temperatures with ranges of 602 to 738°C for processing operation which indicated the suitability of these fuels as good potentials for boilers. These results indicates that preparation of the waste product from palm oil processing as fuel to fire the boiler have the capacity to supply the energy required in the mill in order to boost the extraction efficiency and oil quality of the small scale palm fruit processors upon incorporation into their process line.

Keywords: Small-scale boiler, Biomass-fired boiler, Fuel combinations, Boiler efficiency, Steam production, Efficiency

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INTRODUCTION

The production of palm oil can be grouped (according to their throughput and degree of complexity) into traditional methods, small-scale mechanical units, medium-scale mills and large industrial mills. Of all the methods of production, steam remains a vital resource which can either be produced manually or with the use of steam generators, otherwise called boilers (Jack *et al*., 2024). The importance of boilers in the industry has led to the development of series of models of boilers which has also been scaled down to the different methods of palm oil production. FAO (2002) classified a plant capacity that can process about 2 tons of fresh palm fruits in an hour, 3-8 tonnes per hour and more than 10 tonnes per hour as small scale, medium-scale and large-scale respectively. Regardless of this classification, the basic unit operations which involves fruit loosening, fruit sterilization, digestion, oil extraction, clarification and packaging (Salako *et al.*, 2014) requires the function of a

boiler for smooth running of the plant and desired output (Winarto and Sari, 2024). A boiler generates steam required through the chemical energy stored in the fuel (Labibah *et al.*, 2024; Mohammed *et al.,* 2021) which must have sufficient calorific value for improved efficiency of the boiler (Agustiar *et al.,* 2022). Erivianto and Dani (2024) reported that the moisture content of palm oil waste used as fuel materials for boilers is a major factor that affects the calorific value of the waste (fuel) which in turn add economic values to the system if the waste are properly managed. The major component of a boiler system is the combustion chamber and the evaporator. The combustion chamber produces the heat energy while the evaporator produces the steam which is channelled to the required area for palm oil processing (Nugroho et al., 2021).

For quality palm oil production, ('Quality' is entirely subjective and depends on the demands) there must be a low content of free fatty, low content of products of oxidation and readily removed colour for increasing commercial values of the products. To achieve this, bunch sterilization immediately after harvest, effective clarification and drying of the crude oil after extraction are important but remains a major mirage at the small and medium scale of palm fruit processing and the role of steam generation cannot be underestimated (Maulana *et al.,* 2016)

The extraction efficiency and quality of oil produced by small and medium scale processors (who are major key players in the Nigerian palm oil industry) are very low while majority of the wastes generated from these mills tends to be a waste and a huge source of pollution in the industry (Syukry, 2016). Drop in temperature of the fruit mash due to the time lag from one processing stage to the other has been identified as the major factor affecting the extraction efficiency and quality of the oil produced. Timely processing of fresh palm fruit bunch can only be possible with the inclusion of boiler in the process line. Also, indiscriminate dumping of palm fruit co-product around the mill constitutes serious environmental hazard and therefore has to be effectively utilised by ploughing it back into the mill (Agustiar *et al.,* 2022; Owolarafe, 2015).

In Nigeria, boilers being utilised by large scale plant (mostly imported) are usually expensive and beyond the reach of small scale processors. In order to bridge the gap, a small scale boiler that utilizes the palm fruit wastes as fuel to supply the steam required at high pressure to sterilise the fresh fruit bunch (FFB) thereby achieving substantial fruit recovery, high oil extraction efficiency as well as ensuring production of high quality oil is urgently required (Winarto and Sari, 2024; Salako *et al.,* 2014). These boilers are designed to utilize the biomass generated from palm oil processing as a means of fuel as Indonesia significantly identify the potential of biomass as a viable source of fuel for boilers (Yus *et al.,* 2018; Sam. 2016). Steam boilers today ranges in size from those required to heat a small-size home to the very large ones used in large scale processing industries. The fundamental requirement for a boiler system is how to ensure the smooth and continuous energy flow which may vary in capacity and pressure of the steam produced (Muzaki and Mursadin, 2019). To satisfy this requirement, it is desirable that the rate and quality of steam generation be properly controlled so that the production and consumption of steam energy can be maintained in equilibrum at all times (Mahlia *et al.*, 2003). Figure 1 shows the boiler developed in previous study to enhance the productivity of the processors but its performance needs to be optimised.

MATERIALS AND METHODS

Composition of Fuel for Boiler

Palm fruit biomass was combined differently to determine the best proportion for optimum boiler performance. Table 1 below shows the different combinations of the biomass that were considered in this study to provide an insight into the effect of utilizing these co-products on the qualitative and quantitative properties of the steam produced, boiler efficiencies, flue gas temperature profile, rate of fuel consumption amongst other variables. 36 different combination proportions were used which more consideration on the traditional methods of combinations by end users or processors.

Boiler Evaluation Procedure

The fuel materials were combined in 36 different proportions to determine the effects of the properties of steam (temperature, pressure, enthalpy, entropy and specific volume) on the boiler efficiency, rate of steam production, rate of the boiler fuel consumption and the core temperature of the furnace while monitoring the temperature profile of the flue gas. The various fuel proportions were fed into the system based on the different burning characteristics of the composition of the fuel material. The theoretical and the excess air required for complete combustion of the various fuel materials were determined and a thermocouple was used in measuring the temperature profile of the steam as well as the flue gas.

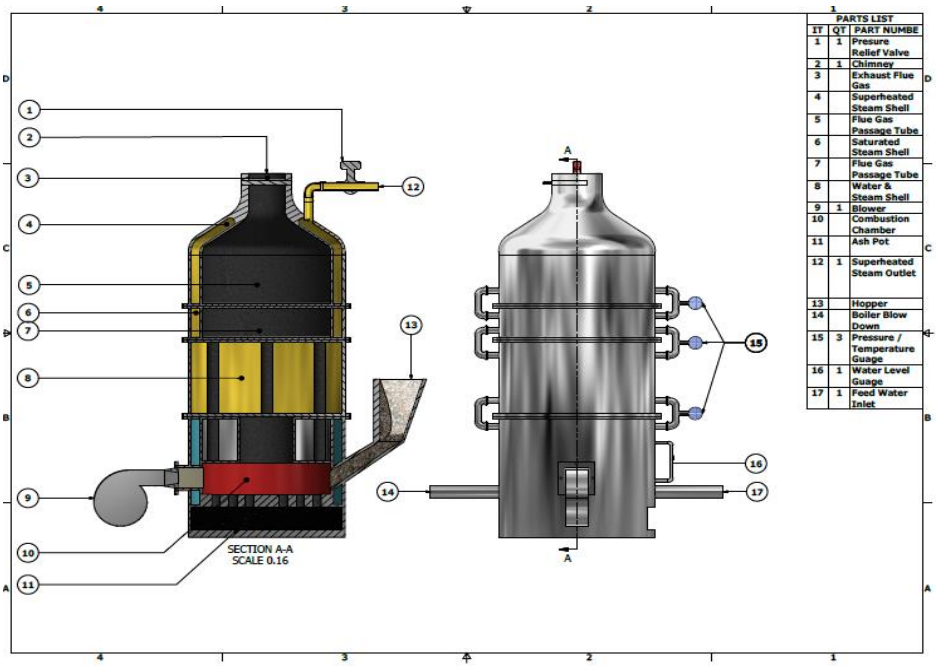


Figure 1. Detailed labelled view of the modified biomass fired boiler

Table 1. Combinations of the palm fruit biomass in different proportions

S/No	Fuel Combinations (EFB:Fiber:Shell)	S/No	Fuel Combinations (EFB:Fiber:Shell)	S/No	Fuel Combinations (EFB:Fiber:Shell)
1	0.1 : 0.1 : 0.8	**13**	0.3 : 0.6 : 0.1	**25**	0.2 : 0.3 : 0.5
2	0.1 : 0.8 : 0.1	**14**	0.6 : 0.1 : 0.3	**26**	0.2 : 0.5 : 0.3
3	0.8 : 0.1 : 0.1	**15**	0.6 : 0.3 : 0.1	**27**	0.3 : 0.2 : 0.5
4	0.1 : 0.2 : 0.7	**16**	0.1 : 0.4 : 0.5	**28**	0.3 : 0.5 : 0.2
5	0.2 : 0.7 : 0.1	**17**	0.1 : 0.5 : 0.4	**29**	0.5 : 0.2 : 0.3
6	0.7 : 0.1 : 0.2	**18**	0.4 : 0.1 : 0.5	**30**	0.5 : 0.3 : 0.2
7	0.1 : 0.7 : 0.2	**19**	0.4 : 0.5 : 0.1	**31**	0.2 : 0.4 : 0.4
8	0.2 : 0.1 : 0.7	**20**	0.5 : 0.1 : 0.4	**32**	0.4 : 0.2 : 0.4
9	0.7 : 0.2 : 0.1	**21**	0.5 : 0.4 : 0.1	**33**	0.4 : 0.4 : 0.2
10	0.1 : 0.3 : 0.6	**22**	0.2 : 0.2 : 0.6	**34**	0.3 : 0.3 : 0.4
11	0.1 : 0.6 : 0.3	**23**	0.2 : 0.6 : 0.2	**35**	0.3 : 0.4 : 0.3
12	0.3 : 0.1 : 0.6	**24**	0.6 : 0.2 : 0.2	**36**	0.4 : 0.3 : 0.3

RESULT AND DISCUSSION

The results of the combination of the fuel samples (EFB, Fibre and Shell) in different proportions on the properties of steam produced, boiler efficiency, rate of steam production and flue gas temperature profile are presented as follows;

Effect of Fuel Combination on Rate of Steam Temperature

Fuel samples produces heat energy in the combustion chamber while the evaporator produces the steam required for the process of palm oil production. It was discovered that the rate of the temperature of the generated steam varies according to the fuel types and composition (Figures 2a – 2f). The highest steam temperature of 161°C was achieved at a fuel combination ratio of 3:5:2, closely followed by fuel combination ratio of 2:6:2 with a steam temperature of 160 °C. The ANOVA result (Table 2) indicated that the heating values of the various fuel compositions have direct effect on the resulting steam temperature as fuel combinations with higher proportion of fibre were observed to produce the best result in terms of steam temperature. This might be due to the percentage of carbon content in the fibre (Fitria *et al*., 2022; Sari *et al*., 2016, 2017). Similarly, the least value for steam temperature of 141 °C was obtained at fuel combination 1:2:7. The effect of the different fuel combinations were observed to significantly ($p < 0.05$) affects the steam temperature profile of the boiler. This result is corroborated by the findings of Salako *et al.* (2014) and also the findings of Oladosu *et al.* (2018). Generally, fuel combination with higher proportion of fiber reaches boiling point faster but burns quickly in the furnace which can result to drop in the temperature if corresponding rate of fuel input into the furnace is not maintained. On the other hand, fuel combination with higher shell proportion burns longer in the furnace therefore the frequency of fuel input is lower compare to fiber and EFB.

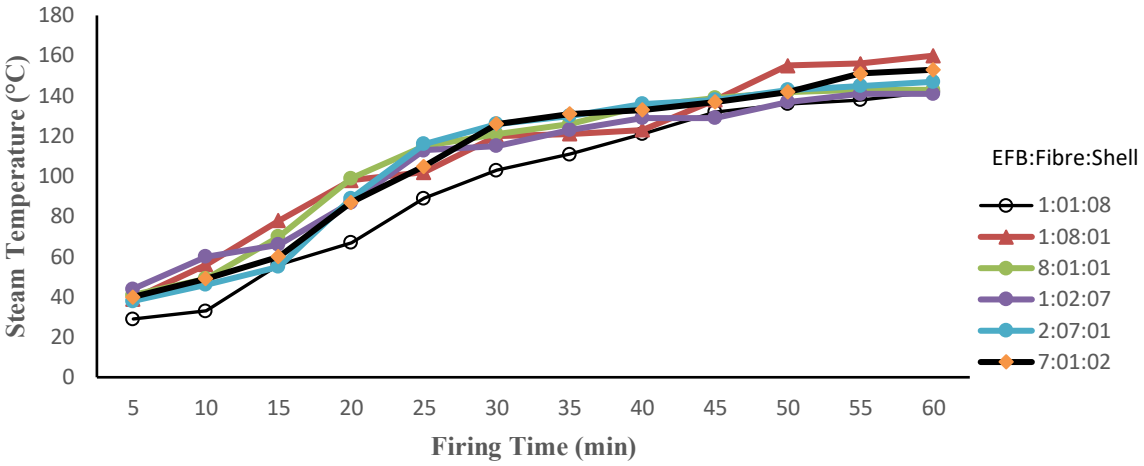


Figure 2a. Effect of Fuel Combination on Steam Temperature

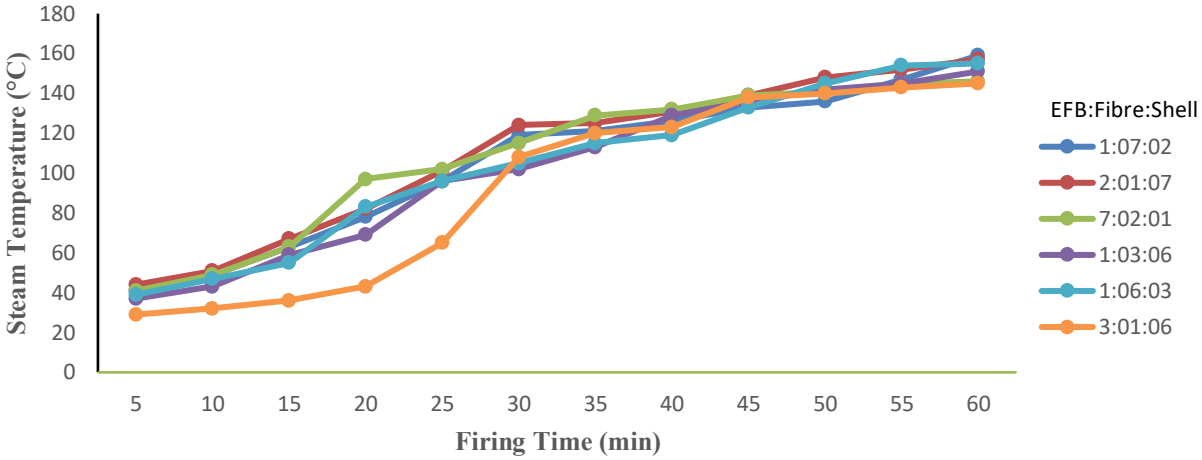


Figure 2b. Effect of Fuel Combination on Steam Temperature

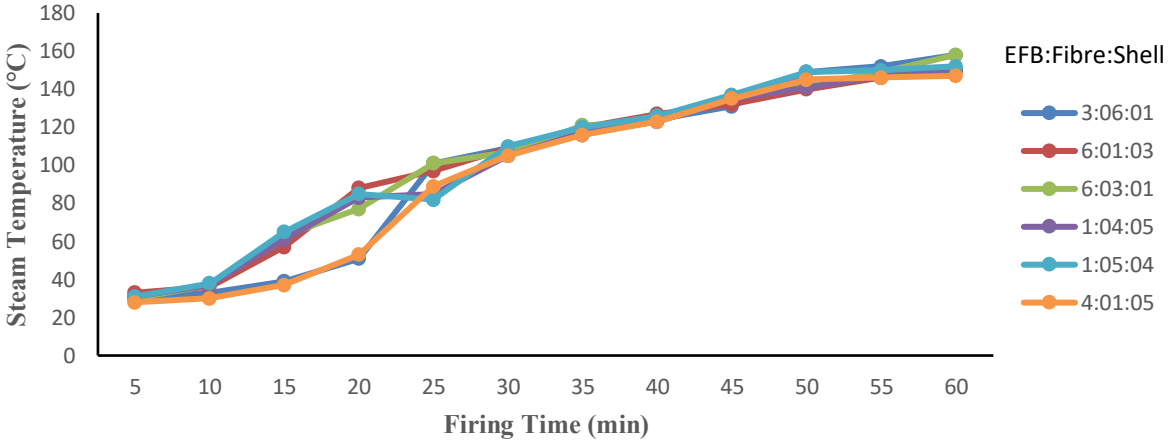


Figure 2c. Effect of Fuel Combination on Steam Temperature (*contd.*)

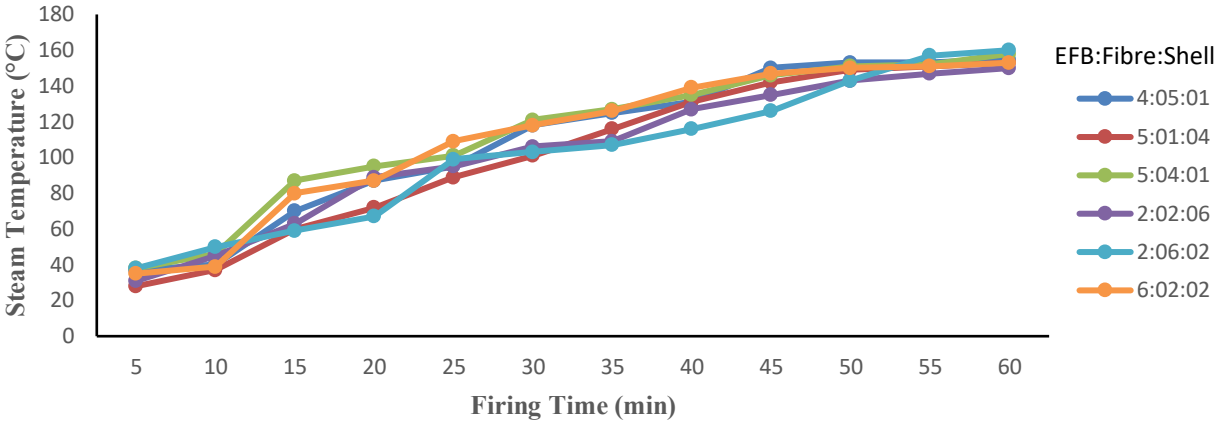


Figure 2d. Effect of Fuel Combination on Steam Temperature (*contd.*)

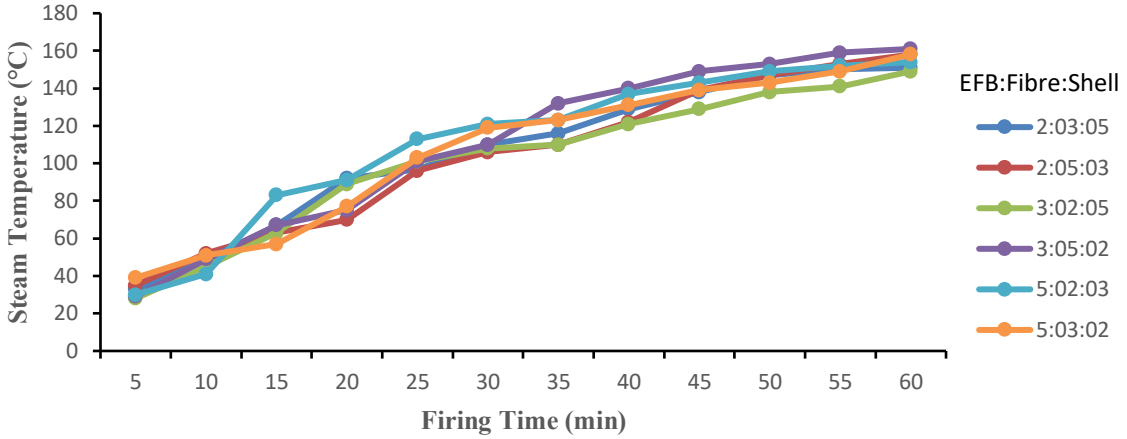


Figure 2e. Effect of Fuel Combination on Steam Temperature (*contd.*)

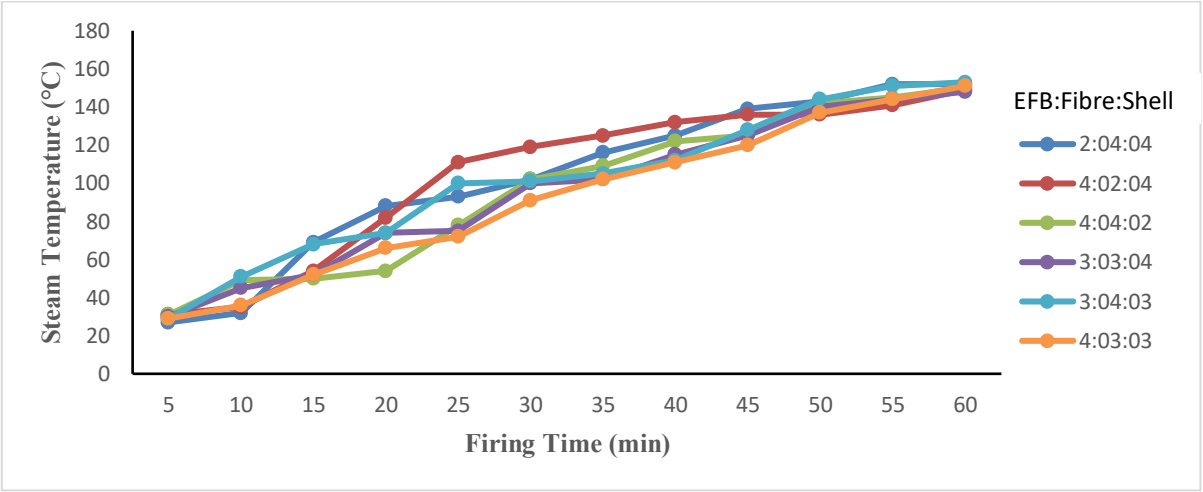


Figure 2f. Effect of Fuel Combination on Steam Temperature (*contd.*)

Table 2. ANOVA Table Showing Effect of fuel combinations on Steam Temperature

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	at P<0.05
FuelType	5	23077.3393	4615.4679	766.86	<.0001	s
Time	11	139432.2446	12675.6586	2106.06	<.0001	s
FuelType×Time	50	35739.7107	714.7942	118.76	<.0001	s
Model	66	187909.6343	2847.1157	473.05	<.0001	s
Error	134	806.5000	6.0187			

Effect of Fuel Combination on the Steam Pressure

For all the fuel samples, the steam pressure was observed to be increasing over time. At 25 to 30 mins, pressure builds up in the boiler was about 100 kPa but later increased rapidly as shown in Fig. 3 (a – f). The vapour pressure turned to have a direct relationship with the rate of fuel consumption at the combustion chamber of the boiler. The pressure of the steam when fired with fuel combination 3:5:2 reached the maximum of 6.34 bar closely followed by fuel combination 2:6:2 with 6.18 bar at 60 minutes firing time. The vapour pressure builds up slowly but more sustained when fired with fuel combination with higher proportion of shell in comparison with fibre and EFB. Similarly, the least value for steam pressure of 3.72 bar was also obtained at fuel combination 1:2:7. The change in fuel combination has significant effect ($p < 0.05$) on the steam pressure, this result follows the trend reported by Oladosu *et al.* (2018) while evaluating the performance of a palm kernel shell-fired boiler, and also supported by the findings of Salako *et al.* (2014).

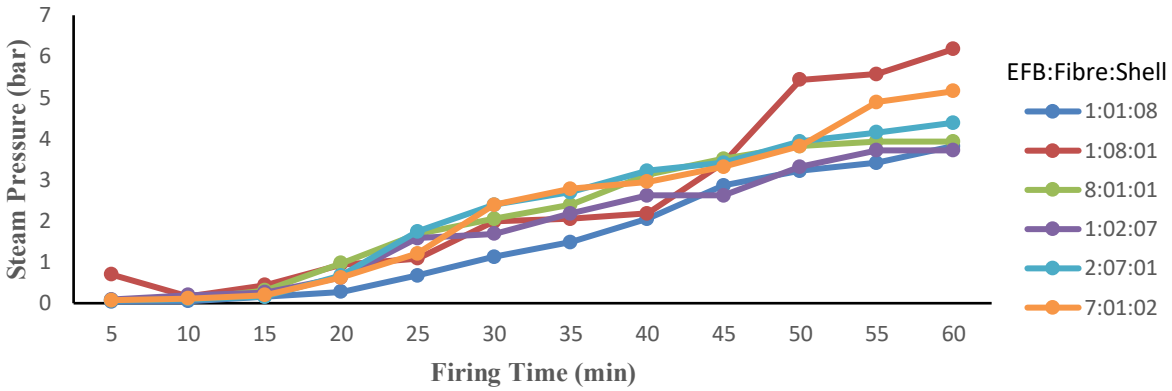


Figure 3a. Effect of Fuel Combination on Steam Pressure

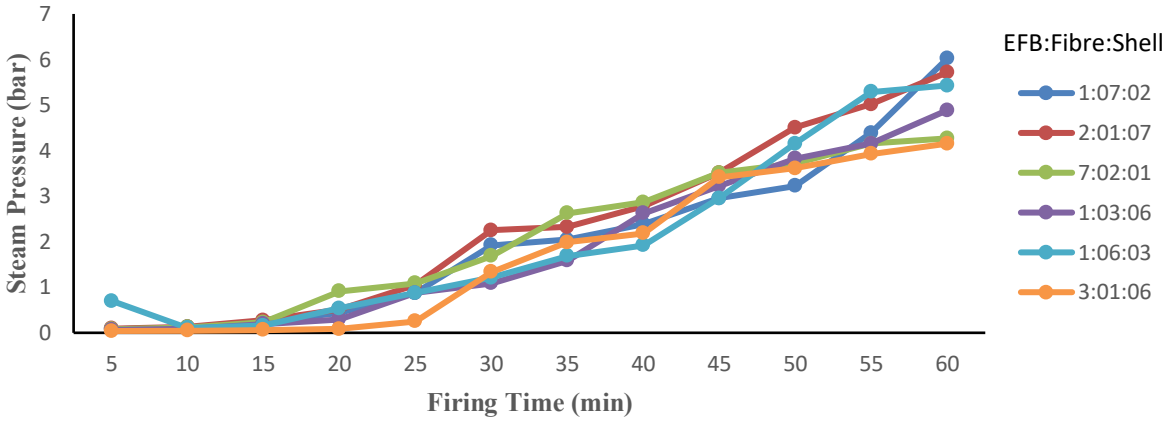


Figure 3b. Effect of Fuel Combination on Steam Pressure (*contd.*)

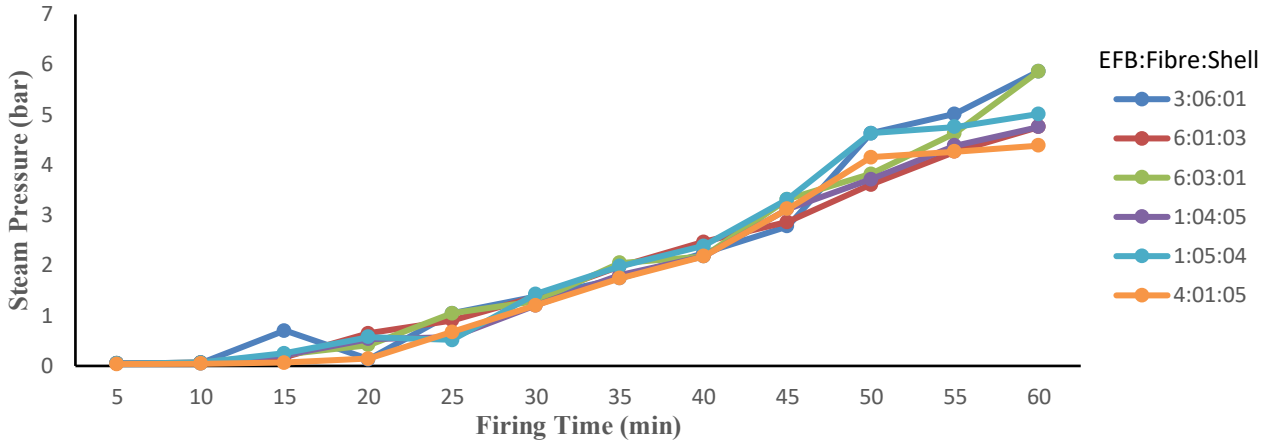


Figure 3c. Effect of Fuel Combination on Steam Pressure (*contd.*)

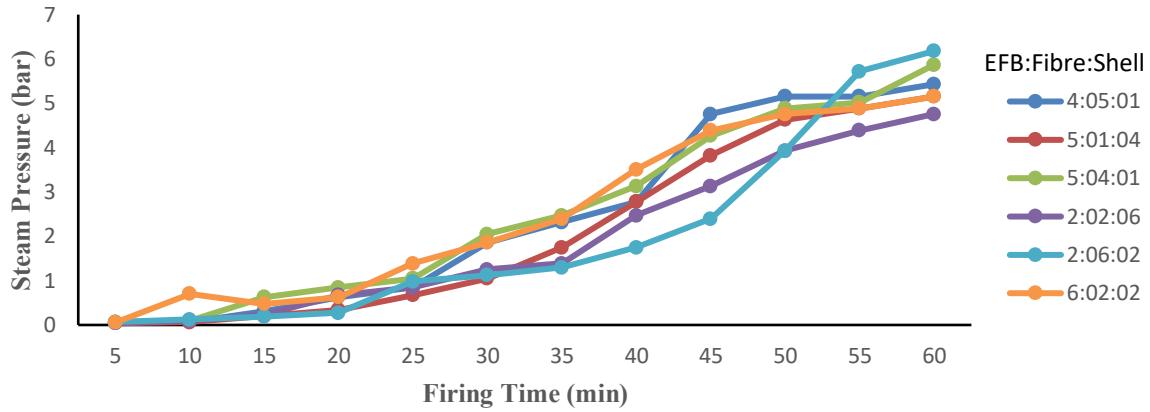


Figure 3d. Effect of Fuel Combination on Steam Pressure (*contd.*)

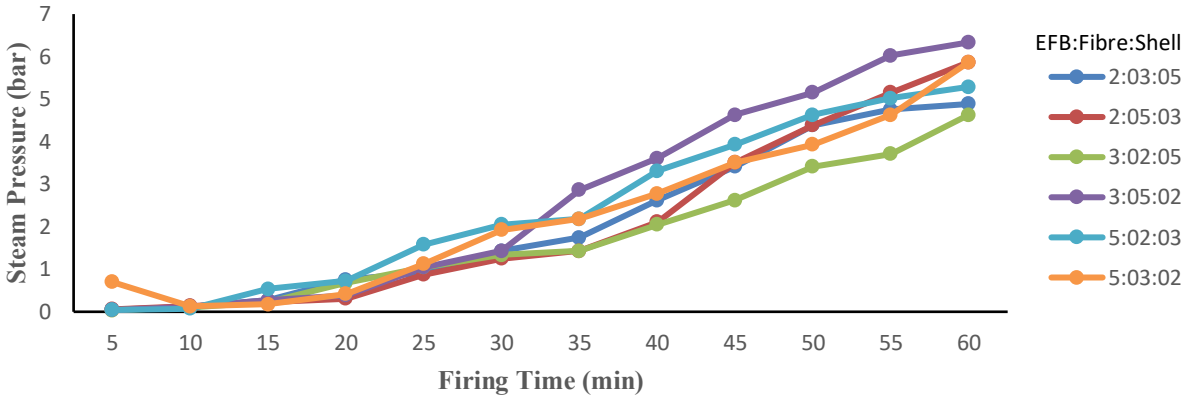


Figure 3e. Effect of Fuel Combination on Steam Pressure (*contd.*)

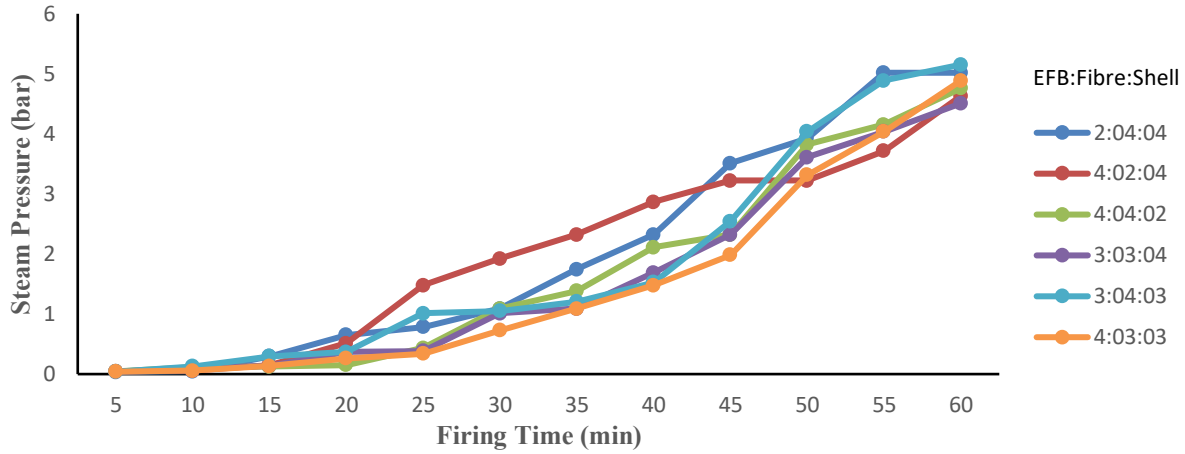


Figure 3f. Effect of Fuel Combination on Steam Pressure (*contd.*)

Table 3. ANOVA Table Showing Effect of fuel combinations on Vapour Pressure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	at P<0.05
FuelType	5	347096.385	69419.277	1549.87	<.0001	s
Time	11	1230371.448	111851.950	2497.24	<.0001	s
FuelType×Time	50	487107.861	9742.157	217.51	<.0001	s
Model	66	1924911.945	29165.333	651.15	<.0001	s
Error	134	6001.894	44.790			

Effect of Fuel Combination on Rate of Steam Enthalpy

The effect of the fuel material on enthalpy of the saturated vapour is as shown in Figure 4 (a – f) and this shows the significance at ($p < 0.05$) according to the ANOVA result (Table 3). The highest steam enthalpy value of 27.591 MJ kg^{-1} was achieved at fuels combination ratio of 3:5:2, while fuel combination ratios of 2:6:2 and 1:8:1 yielded almost the same value (27.580 MJ kg^{-1}). The heating value of the various fuel compositions has a direct effect on the resulting steam enthalpy as in the case of steam temperature and pressure. Fuel combination with higher proportion of fibre was observed to produce the best steam enthalpy value while the least value for steam enthalpy of 2735.1 MJ kg^{-1} was obtained at fuel combination 1:2:7. The result obtained follows the trend reported by Salako *et al.* (2014). This affirms that the combination of these palm fruit biomass (shell, efb and fibre) in different proportions produces viable fuel source or energy needed in the palm oil processing industry.

Effect of Fuel Combination on Rate of Steam Production

Fuel mixture with higher percentage of fibre produced the highest quantity of steam followed by fuel mixture with higher percentage of EFB. This may be due to the combustion property of fibre over the other materials. Over 111 kg of steam were produced per hour at fuel combination ratio of 1:8:1 and 2:7:1 (EFB, Fibre and Shell), while the lowest quantity of steam generation was attained for fuel ratio 1:1:8. The result has a direct correlation with the findings of Salako *et al.* (2014). The indicate that fuel combinations ratios have significant effect on the rate of steam production ($p < 0.05$).

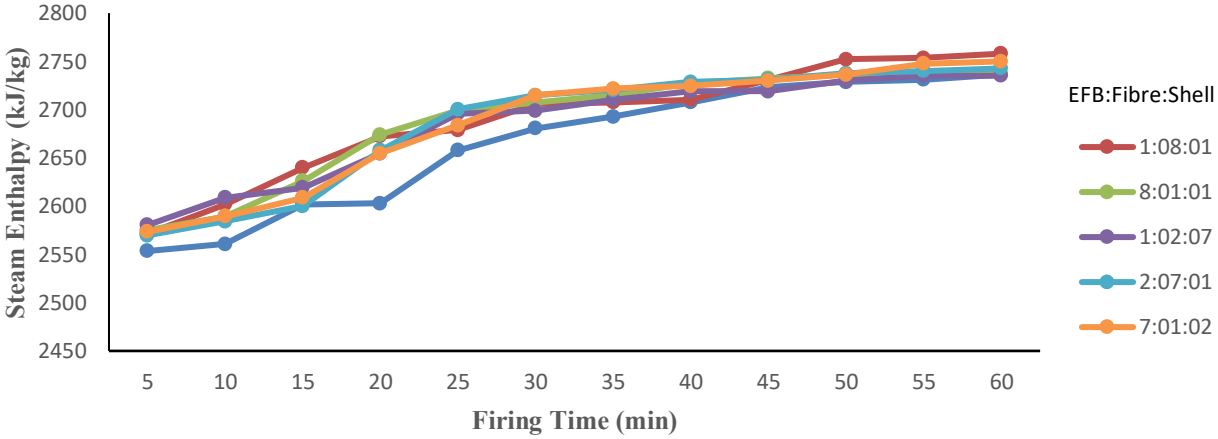


Figure 4a. Effect of Fuel Combination on Steam Enthalpy

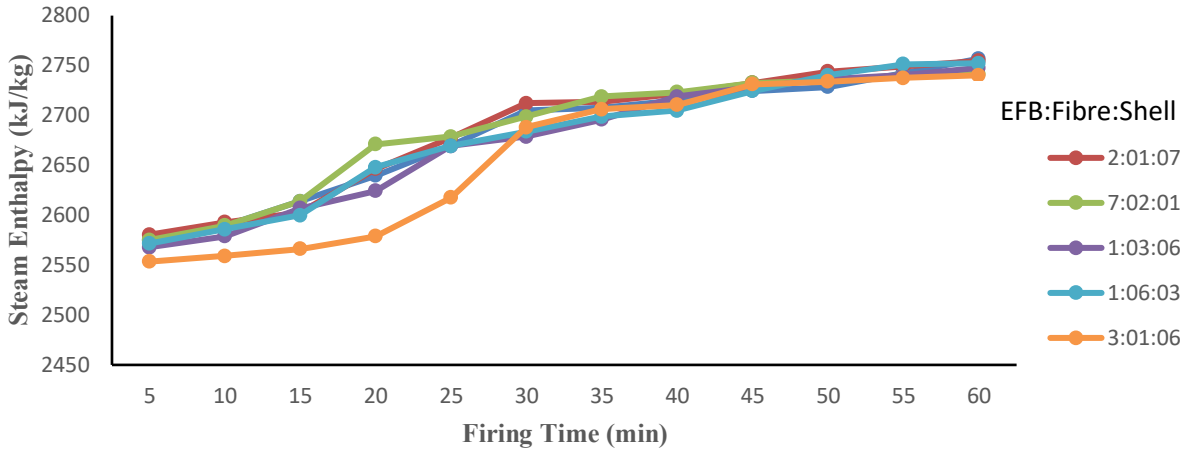


Figure 4b. Effect of Fuel Combination on Steam Enthalpy (*contd.*)

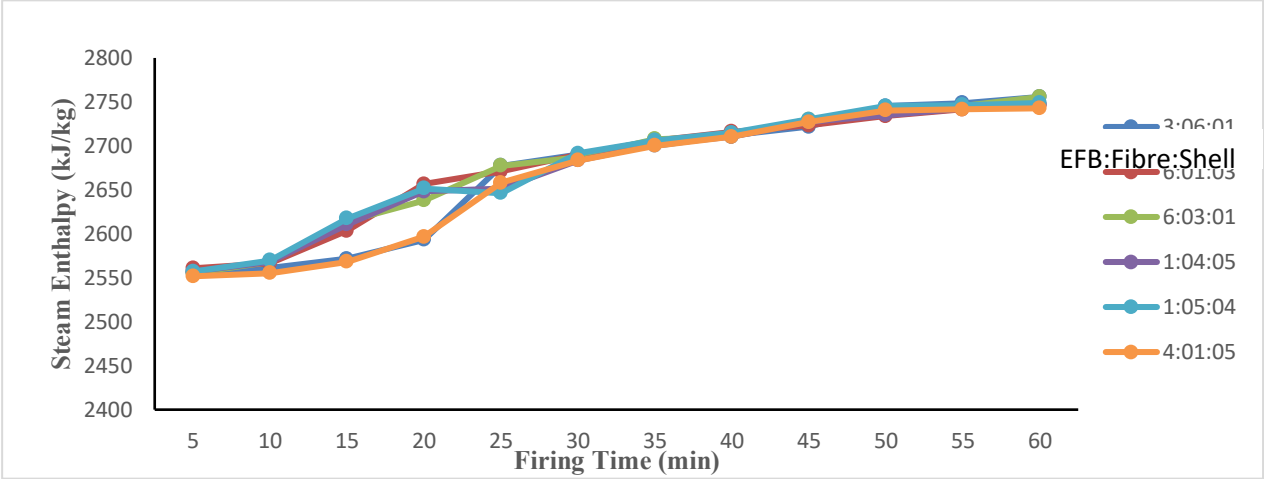


Figure 4c. Effect of Fuel Combination on Steam Enthalpy (*contd.*)

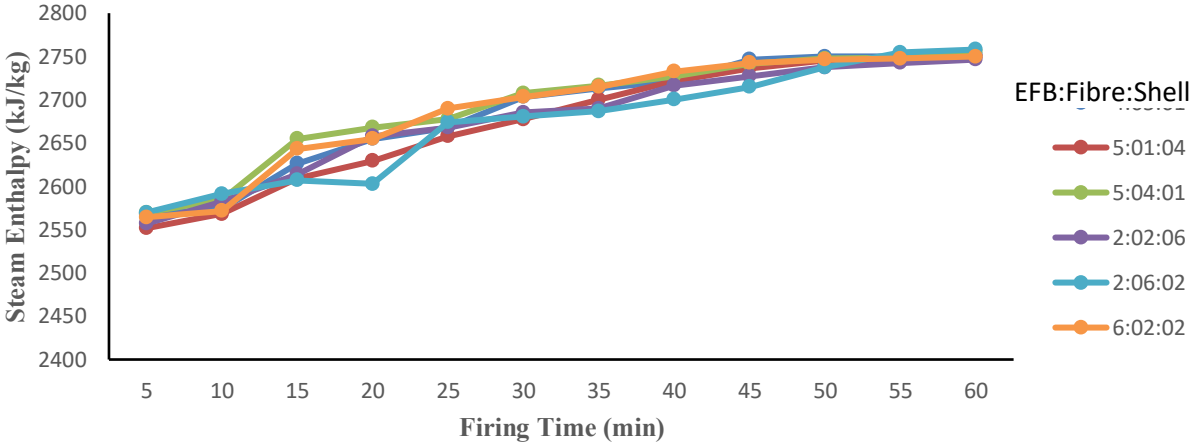


Figure 4d. Effect of Fuel Combination on Steam Enthalpy (*contd.*)

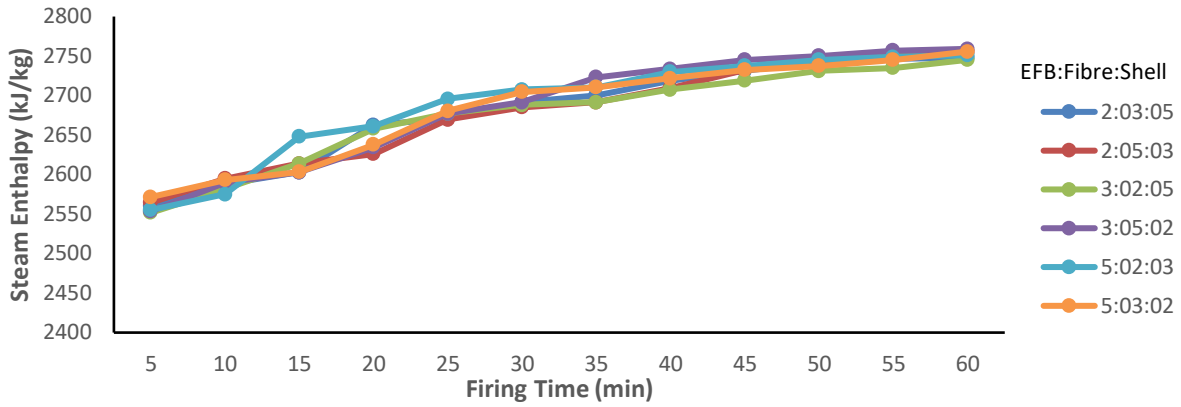


Figure 4e. Effect of Fuel Combination on Steam Enthalpy (*contd.*)

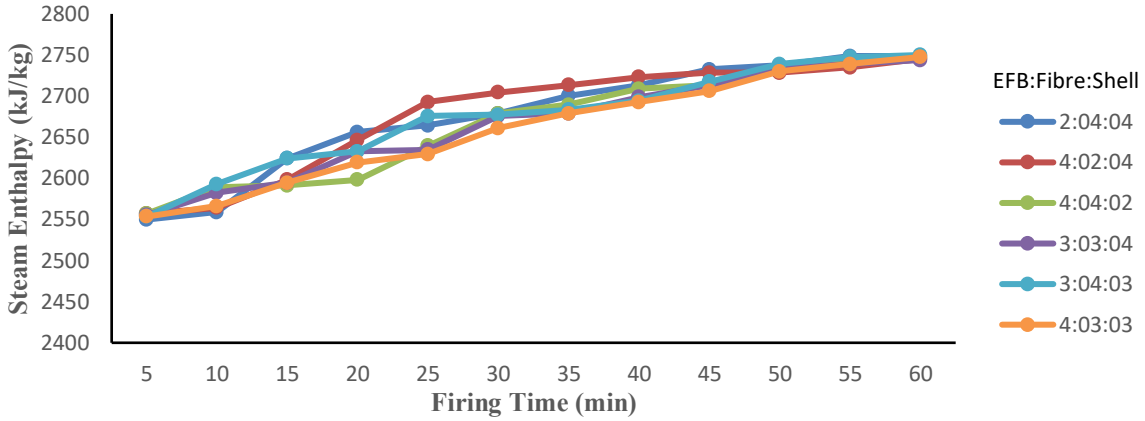


Figure 4f. Effect of Fuel Combination on Steam Enthalpy (*contd.*)

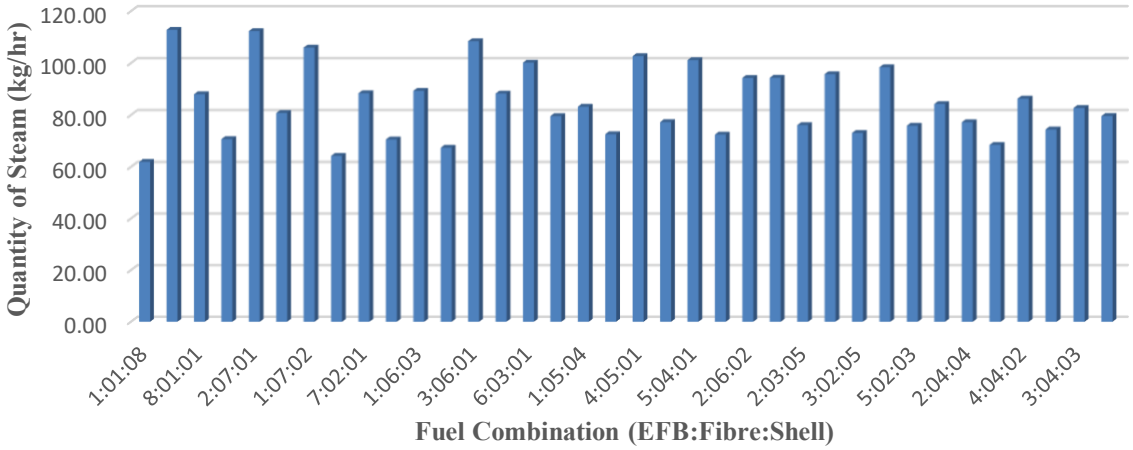


Figure 5. Effect of fuel combination on the rate of steam production

Effect of Fuel Combination on Rate of Boiler Efficiency

The efficiency of the machine varied differently when fired with the various combination of the palm fruit biomass. Fuel mixture with highest percentage of shell composition (1:2:7) produced the highest boiler efficiency (74.2%) while the least efficiency (69.4%) was produced at a fuel combination of 3:5:2. This position fuel materials with high shell composition as a potential when mixed with fibre due to its combustion property. This result is in consonance with the reports of Sivabalan (2013), Oladosu *et al.* (2018) and also Salako *et al.* (2014) who also confirmed that fuel combinations affects has a significant effect on the boiler efficiency.This shows (Figure 6) a better result compared to other fuel materials and position palm fruit biomass as a potential to supply the energy required by a boiler for palm oil production.

Effect of Fuel Combination on the Rate of Fuel Consumption

The rate of fuel combustion is the ratio of the total mass of fuel used in the firing process to the burning time. The different burning characteristics of the composition of the fuel material and the low heating values resulted to the variation in the rate of fuel consumption of the boiler (Hartanto *et al.,* 2020) which also influences the boiler efficiency (Winarto and Sari, 2024).

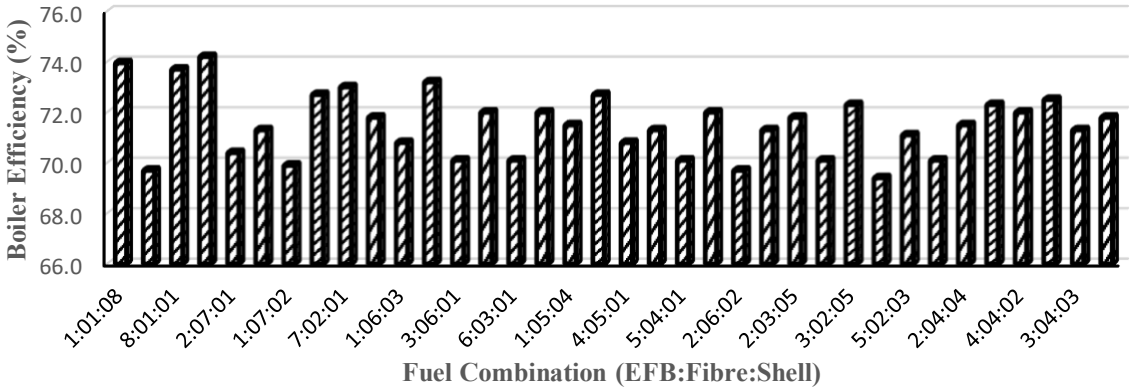


Figure 6. Effect of fuel combination on the boiler efficiency

According to the result, fibre has the highest potential in terms of heat released but shell has the longest burning time, followed by the EFB and fibre in that order. The longer burning time of the uncrushed raw palm shells used for the experiment can be attributed to its physically hard property that requires more time for char combustion to be achieved over the entire particle, while the EFB were dried quartered bunches. This submission is corroborated by Ilmi *et al.* (2013) and also in accordance with Hasibuan and Napitupulu (2013) who affirmed that fuel with low heating values affects the rate of fuel consumption and boiler efficiency. The burning rate of the char depends on both the chemical rate of the carbon-oxygen reaction at the surfaces and the rate of boundary layer and internal diffusion of oxygen. Fuels with higher densities typically has lower oxygen diffusion rate. This surface reaction generates primarily CO, which then reacts with free oxygen or other formed substances outside the particle to form CO_2. As obtained in the result, fuel combination with higher composition of fibre requires larger quantity of fuel in order to maintain steady rate of combustion and heat supply to the heat exchangers. Therefore, as shown in Figure 7, as much as 21 kg of fuel was utilized when fired with a 2:7:1 and a combination of 1:1:8 only requires 12.5 kg of fuel. The result obtained is also in line the findings of Sivabalan, (2013) and Oladosu *et al.* (2018).

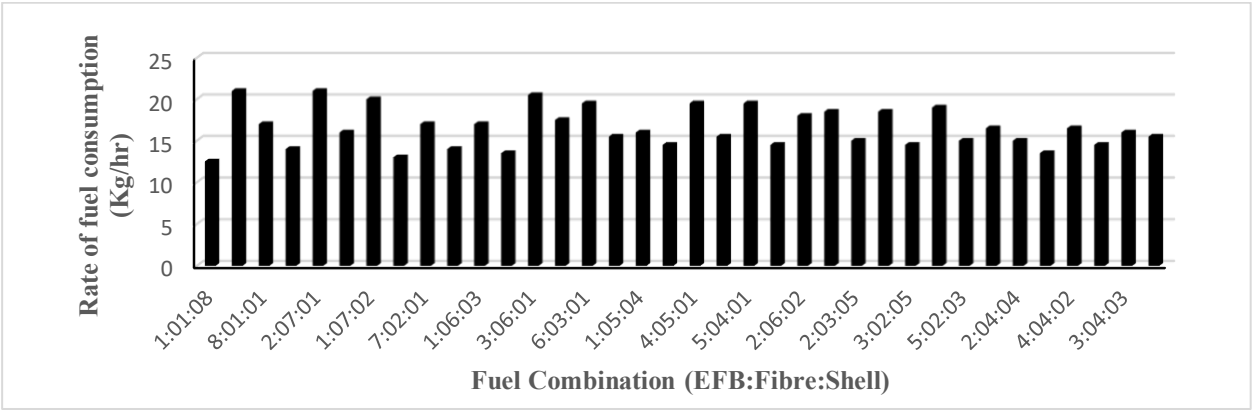


Figure 7. Effect of fuel combination on the boiler rate of fuel consumption

Effect of Fuel Combinations on the Flue Gas Temperature Profile of the Boiler.

Temperatures of the flue gas at all the sections increases with time due to steady in-flow of fuel material and decreases along its flow path (Figure 8). This shows that a rapid cooling rates along the direction of travel of the combustion towards the exhaust. From the high range of temperature obtained (602-738°C), fuel samples with higher concentration of fiber produced the peak result but more fuel were consumed during the process due to the percentage of carbon content in the fibre (Fitria et al., 2022; Sari et al., 2016, 2017) and its physical properties. This result is in line with findings of Najmi *et al.* (2008). The average temperature difference between the saturated tube, superheated tube and the exhaust gas at the chimney was also estimated to be about 15°C.

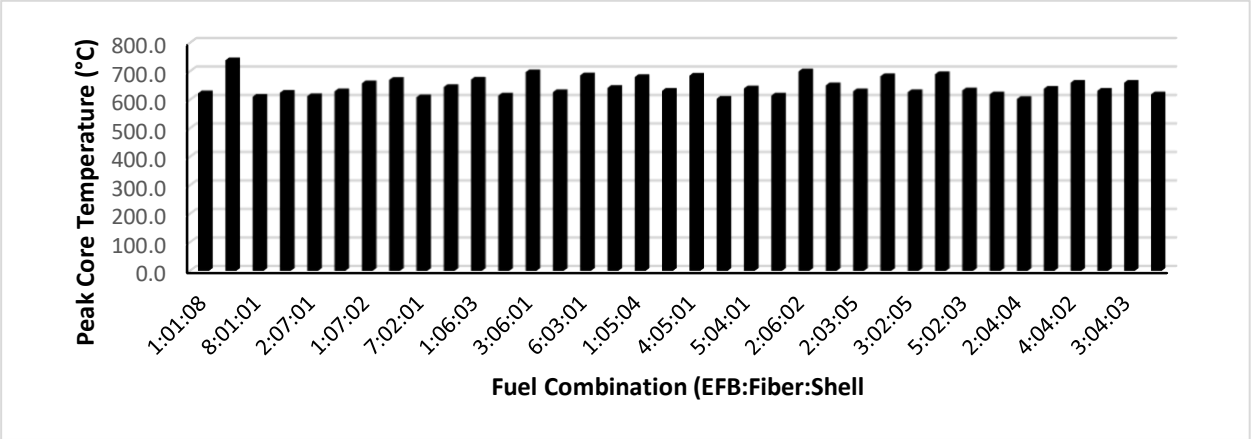


Figure 8. Effect of fuel combination on the peak core temperature of the boiler

CONCLUSION

The mixture of Palm fruit biomass (EFB, Fibre and Shell) is found to be a viable fuel to fire a boiler. These materials can generate required steam by any small or medium scale boiler for various special palm oil processing operations. Fuel mixture of palm kernel shell and fibres are best to produce sufficient energy required by boilers for special palm oil production due to the combustion properties of the fibres which tends to compliments the useful properties of the palm kernel shell. This study signifies the importance of palm oil biomass as fuels for boilers that are designed for palm oil processing. This will add more economic values to the production of palm oil and develop the appetite of more processors to the production of valuable oil from palm kernel.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript.

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Effect of flax rhizobacteria on germination and seedling growth under copper toxicity

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Abstract

Plant growth promoting rhizobacteria (PGPR) supports plant growth by decreasing heavy metal levels in soil through methods that alter metal pathways and convert toxins into safe forms. Five rhizobacterial isolates (FLX-1, FLX-2, FLX-3, FLX-4, and FLX-5) isolated from flax rhizosphere were identified using MALDI-TOF-MS techniques based on morphology, biochemistry, and plant growth-promoting properties. Four isolates (FLX-1, FLX-2, FLX-3, FLX-5) capable of fixing nitrogen, four (FLX-1, FLX-3, FLX-4, FLX-5) dissolving inorganic phosphate, and three (FLX-2, FLX-4, FLX-5) producing IAA and HCN. Additionally, in this study, the effects of 5 different rhizobacteria and copper ($CuSO_4$) doses (0, 5, 10, 15, and 20 mM) on growth and development parameters of flax seeds were determined under fully controlled climate chambers. Moreover, Different rhizobacteria treatments significantly improved growth and development parameters in flax seed compared to the control group. It was observed that increasing copper doses resulted in a significant decrease in parameters such as germination rate, mean germination time, germination power index, root length, and shoot length in bacterial treatments. When evaluating the responses of different rhizobacteria treatments to copper heavy metal in flax seed germination, it was determined that the treatment of *Pseudomonas chlororaphis* FLX-5 yielded better results compared to other treatments and was effective in reducing the negative effects of copper in terms of the tested germination characteristics. In conclusion, the current study suggests that *Pseudomonas chlororaphis* FLX-5 may efficiently be used in tolerating copper toxicity, indicating that further research is needed in this area.

Keywords: Flax, Copper, PGPR, Germination, *Pseudomonas chlororaphis*

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INTRODUCTION

Flax (*Linum usitatissimum* L.) is a dicotyledonous plant belonging to the Linaceae family. It has been one of humanity's oldest industrial crops, having been used for thousands of years. This economically valuable plant is grown in many parts of the world, including Asia, the Eastern Mediterranean, the Middle East, and India, and is known for its high fiber and oilseed content. Out of around 100 species globally, about 38 can be found in Türkiye (Kumral & Şimşek, 2023). The term "flax" in Latin means "extremely useful," and it contains omega-3 fatty acids, phenols, phytoestrogenic lignans, flavonoids, proteins, sterols, antioxidants, and fiber. Natural fibers derived from flax fibers generally have significant potential in the material industry (Omer et al., 2022). About 40% of flaxseed is oil, 28-30% is protein, 6% is mineral matter and ash, and the remaining portion is hull. Due to its alpha-linolenic acids (ALA), short-chain polyunsaturated fatty acids (SC-PUFA), phytoestrogenic lignans (secoisolariciresinol diglucoside or SDG), and other bioactive compounds, flax seed has preventive and therapeutic effects against various diseases such as cardiovascular diseases, diabetes, hyperglycemia, obesity, breast-prostate cancer, osteoporosis, and reproductive problems (Kauser et al., 2024).

Copper (Cu) is recognized as a vital micronutrient that plays a crucial role in the morphological, physiological, and biochemical processes of all plants. However, excessive Cu has negative effects on plant growth and productivity. In many parts of the world, heavy metal accumulation is the main factor limiting the yield of many plants, especially flax. It has been reported that many oilseed crops under heavy metal stress have decreased grain yield and oil yield (Fatnassi et al., 2015; Abdel Latef et al., 2020). The widespread use of certain copper (Cu)-based agricultural chemicals, such as chemical fertilizers, fungicides, herbicides, insecticides, and nematicides, has resulted in the accumulation of copper in the soil at varying concentrations (Schoffer et al., 2020). The use of Cu-containing compounds to control plant diseases has increased in the amount of copper in the soil (Ballabio et al., 2018). In some cases, the treatment of phosphate fertilizers has also significantly elevated Cu concentrations in the soil (Ruyters et al., 2013). High concentrations of copper in the soil can be toxic to certain soil microorganisms and hinder the mineralization of macro-nutrients like phosphorus (P) and nitrogen (N). Since the accumulated Cu in the soil cannot be broken down biologically or chemically, it poses a threat to the environment, food safety, and human health. Thus, copper has become a critical issue in the management of horticulture and agricultural products. The increasing pollution of heavy metals like copper in the soil leads to a decrease in the number and diversity of soil microorganisms, severely disrupting ecological balance. In such cases, only organisms resistant to heavy metals can survive, enabling them to remediate the contaminated soil (Azeez et al., 2015).

Plant growth promoting rhizobacteria (PGPR) is a group of bacteria that actively colonizes plant roots and enhances plant growth and development through various mechanisms such as nitrogen fixation, phosphate solubilization, IAA production, and ACC deaminase production. PGPR reduces heavy metal concentrations in the soil through immobilization, chelation, active removal, biosorption, and bioaccumulation. In recent years, bioremediation of heavy metal-contaminated soil through PGPR has garnered significant attention for developing eco-friendly solutions to remove heavy metals from the ecosystem. Identifying the relationships of these bacteria with plants is extremely important for sustainable agriculture, particularly in determining their potential contributions in environmental stress conditions (Gupta et al., 2024). Excessive levels of heavy metals in contaminated soils diminish microbial activity, soil health, and crop productivity. Consequently, bioremediation is an alternative approach to managing soil pollution. This technique employs specific microorganisms to extract heavy metals from polluted soil or convert highly toxic forms into less toxic ones. Plant growth-promoting rhizobacteria are capable of surviving in soils contaminated with heavy metals, thus their use aids in bioremediation. Although research on metal tolerance of PGPRs has increased recently, studies on copper tolerance are still limited. Many studies with PGPR suggest that heavy metal resistance of local isolates should be investigated in different ecosystems and plant species. This study aims to identify the bacteria in the rhizosphere of *L. usitatissimum* using MALDI TOF MS and to further investigate their plant growth-promoting properties, as well as the effects of different copper levels and five PGPR treatments (FLX-1, FLX-2, FLX-3, FLX-4 and FLX-5) on the seed germination, root and stem growth, and physiological characteristics of flax plants.

MATERIALS AND METHODS

The experiment was conducted in a fully controlled climate room of the Department of Field Crops, Faculty of Agriculture, Ankara University in 2023. The flax seeds (*L. usitatissimum*) used in this study were obtained from the Department of Field Crops of Ankara University Faculty of Agriculture. Copper sulfate ($CuSO_4.5H_2O$, Merck) was used at concentrations of 0, 5, 10, 15 and 20 mM.

Sample collection

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

Isolation of rhizobacteria

Rhizospheric bacteria were isolated from 1 g of dried soil samples by serial dilution method. The soil samples were homogenized in 10 ml of sterile isotonic saline water. Each 1 g of soil sample was mixed in 9 ml of 0.85 % saline (NaCl) sterile water and then homogenized in a shaker for 30 min. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6}. These dilutions were spread on nutrient agar (NA) solidified in Petri dishes and incubated at 28 °C. After incubation, different single colonies were screened in Petri dishes containing NA medium to obtain a pure colony and conserved in stock solution at -85 °C for later use.

Identification of bacterial isolates

Physiological, biochemical tests, Gram staining, and spore formation tests of the bacterial isolates were examined using methods described by Bashan et al. (1993). MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) was used for bacterial identification. Microorganisms were identified by their unique molecular fingerprints by the MALDI Biotyper CA System. Protein profiles of microorganisms' biomolecules (such as protein, peptide, sugar, and polymer) were ionized and then passed through an electric and/or magnetic field in this method. These profile spectra were compared graphically to

reference microorganisms in the system's database to accurately identify them at genus and species level (Sivri & Öksüz, 2019).

Determination of Plant Growth-Promoting Characteristics

Evaluation of Phosphate Solubilizing Bacteria

The bacterial isolates were tested for their ability to solubilize phosphate according to the methods of Pikovskaya (1948) using Pikovskaya agar (PKA) medium (0.2 g L^{-1} NaCl, 10 g L^{-1} glucose, 0.2 g L^{-1} KCl, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.1 g L^{-1} $MgSO_4.7H_2O$, 0.002 g L^{-1} $FeSO_4.7H_2O$, 0.5 g L^{-1} yeast extract, 0.002 g L^{-1} $MnSO_4.H_2O$, and 1000 ml distilled water). The plates were inoculated with bacteria and incubated at 28±2 °C for 5 days. Colony, formed a clear halo zone around them, indicating phosphate solubilization. The experiments were performed in triplicate. The Phosphate solubilization index (PSI) was determined using measurements taken after seven days of growth from a point inoculation on a PKA medium at 28±2 °C (Meena et al., 2015).

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Halo zone diameter.}}{\text{Colony diameter}}$$

Identification of Nitrogen Fixing Isolates

Determination of the nitrogen fixation abilities of the isolates was used according to the protocol specified by Park et al. (2005). The isolates were streaked on NA medium and incubated at 28 ºC for 24 hours. Thereafter incubation, the fresh isolates were incubated for 4 days at 28±2 ºC by inoculating them on Petri dishes having solid Burk's N-free medium (0.05 g L^{-1} Na_2SO_4, 10 g L^{-1} glucose, 0.2 g L^{-1} $CaCl_2$, 0.41 g L^{-1} KH_2PO_4, 0.52 g L^{-1} K_2HPO_4, 0.0025 g L^{-1} Na_2MoO_4. $2H_2O$, 0.1 g L^{-1} $MgSO_4.7H_2O$, 0.005 g L^{-1} $FeSO_4.7H_2O$, and 1.8 g L^{-1} agar 1 L dsH_2O) by line culturing method. These growing isolates on Burk's N-free medium were evaluated as positive.

Evaluation of HCN Producing Isolates

The HCN production assay was carried out according to the method of Bakker and Schippers (1987). The bacteria were inoculated on NA medium and filter papers impregnated with picric acid (0.5% picric acid, 2% sodium carbonate) placed on the edge of the Petri plate. Petri dishes were tightly closed with paraffin and incubated at 28±2 °C for 4 days. At the end of the incubation, yellow colored picric acid-impregnated papers turned brown, which was considered a positive result (Deshwal & Kumar, 2013).

Determination of Isolates Producing Indole-3-Acetic Acid (IAA)

Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce Indole-3-Acetic Acid (IAA). Bacterial cultures were grown for 48 hours at 36±2 °C. These fresh cultures were centrifuged for 30 min at 3000 rpm. The supernatant (2 ml) was combined with 4 ml of the Salkowski reagent (50 ml, 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution) and two drops of orthophosphoric acid. Pink appearances indicated the presence of IAA which was determined spectrophotometrically (Shimadzu UVmini-1240 Spectrophotometer) at 530 nm.

Seed treatment

Flax seeds were placed in 10 % sodium hypochlorite (NaOCl) solution, for 15 minutes. It was followed by five times rinsing with distilled tap water to ensure sterilization. The bacterial strains stored at -85 °C were inoculated on NA medium and incubated at 30±2 °C for 24 h. The pure cultures were grown on Nutrient Broth (NB) medium containing 4 % sugar. The concentration of the prepared bacterial solutions was adjusted to 10^8 cfu/ml with sterile distilled water. The seeds were then treated with bacterial suspensions for 3 h under sterile conditions. The sterile seeds were not treated with bacteria in the control group (Heinonsalo et al., 2004) (Figure 1A).

Germination tests

Petri dishes and whatman paper number 1 were used as germination material. The seeds were germinated by placing them between 2 pieces of sterile whatman paper in each sterile Petri dish. 20 seeds were placed in each Petri dish and treated with 15 ml of respective copper concentrations. Sterile distilled water (0mM) was used as the control treatment. Petri dishes were covered with parafilm to prevent moisture loss. The research was established according to the factorial experimental design in randomized plots with three replicates and 20 seeds in each replicate (Figure 1B). Thereafter, the flax seeds were germinated in dark condition (25 °C and 65% relative humidity) for 14 days. Four days after the seeds were transferred to Petri dishes, an average of 10 ml sterile distilled water was added to each Petri dish for 14 days. The germinated seeds were counted on each day and the seed with a rootlet length of 2 mm was considered as germinated following "International Seed Testing Association (ISTA 1993)"(Ertekin et al., 2020). Germination percentage (%), mean germination time, germination power index, root length and stem length (cm), root and stem fresh weight and root and stem dry weight (mg) were analysed. After the flax seeds were placed in the germination medium, the counts were made every day for 14 days and the germination characters stated below were calculated.

Germination Percentage (GP) (%): Counted every day and calculated by dividing the total number of germinated seeds by the number of seeds taken for germination after 14 days (Pordel et al., 2019).

Mean Germination Time (MGT): Calculated by dividing the number of germinated seeds on the counting day by the number of counting days (Dezfuli et al., 2008).

Germination Power Index (GPI): Calculated by multiplying the sum of the average stem length and average root length by the total germination percentage (Pordel et al., 2019).

The seedlings were taken out of climate cabinets to measure their root and shoot lengths.

Root and Stem Fresh Weight (mg plant^{-1}): The root and stem fresh weight of the plants was determined by weighing them on a precision scale at the end of the germination test.

Root and Stem Dry Weight (mg plant^{-1}): They were dried in an oven at 70 °C for 48 hours and weighed on a precision scale to determine the root and stem dry weight of the plants.

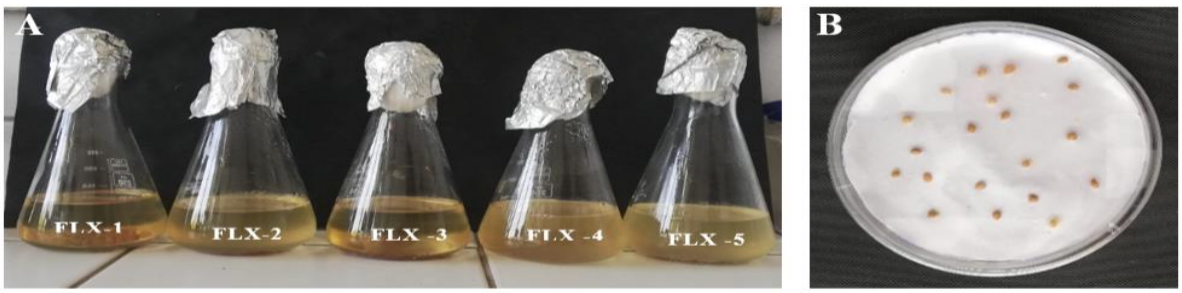


Figure 1. Preparation of bacterial culture in NB medium(A) and application of PGPR-Cu to flax seeds (B)

Statistical Analysis

Statistical analysis of the data was performed using JMP Pro 17.0 statistical software. Normally distributed dependent variables were presented as mean ± Standard Error (X±Sx). The differences in the mean amounts of variables were determined using the Tukey test (Genç & Soysal, 2018).

RESULTS AND DISCUSSION

Identification of isolates

Five bacterial isolates were identified based on biochemical, morphological, and MALDI-TOF MS analysis in the present study. The isolates were characterized as species within the genera *Bacillus*, and *Pseudomonas*. Among all isolates, 2 showed Gram (+) reaction. The catalase test was positive for all isolates, whereas the oxidase test was positive for 4 isolates except for *Bacillus thuringiensis* FLX-1. The sporulation ability of the isolates was examined and the results showed that two isolates (*Bacillus thuringiensis* FLX-1 and *Bacillus mojavensis* FLX-4) produced spores and the other isolates (*Pseudomonas libanensis* FLX-2, *Pseudomonas chlororaphis* FLX-5 and *Pseudomonas boreopolis* FLX-3) did not. Additionally, Motility tests for all other isolates were positive. The morphological and biochemical results of the isolates are presented in Table 1.

Table 1. Morphological and biochemical traits of isolates

Bacterial isolate code	Isolates	Morphological Characteristic				Biochemical Characteristic		
		Gram reaction	KOH 3%	Colony color	Motility	Catalase	Oxidase	Spore forming
FLX-1	*Bacillus thuringiensis*	+*	-	cream	+	+	-	+
FLX-2	*Pseudomonas libanensis*	-	+	white	+	+	+	-
FLX-3	*Pseudomonas boreopolis*	-	+	white	+	+	+	-
FLX-4	*Bacillus mojavensis*	+	-	Milky white	+	+	+	+
FLX-5	*Pseudomonas chlororaphis*	-	+	white	+	+	+	-

Note: * +, positive; –, negative

The MALDI-TOF MS identification method, is based on the detection of intracellular molecules, especially ribosomal proteins at the genus, species, and strain level (Solntceva et al., 2021). It is one of the most reliable tools used to identify microorganism species in fields such as medicine, food, and agriculture due to its high accuracy and rapid results (Stîngu et al., 2008; Vega-Castano et al., 2012). Çelikten and Bozkurt (2018) used the MALDI-TOF method to identify 120 bacteria they isolated from the wheat rhizosphere to investigate plant growth-promoting bacteria. Öksel et al. (2022) used the MALDI-TOF MS method to identify bacteria in wheat rhizospheres. Similarly, Ünlü et al. (2023) used MALDI-TOF MS to identify bacterial strains isolated from the

alfalfa rhizosphere as belonging to the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Acinetobacter*, and *Enterobacter*. In a recent study, Güler, (2024) used the MALDI TOF MS method to identify phosphate solubilizing bacteria isolated from different medicinal aromatic plants.

Plant growth-promoting properties of isolates

The isolates exhibited various plant growth-promoting traits. Four isolates (FLX-1, FLX-2, FLX-3, and FLX-5) demonstrated nitrogen-fixing ability, four (FLX-1, FLX-3, FLX-4, and FLX-5) solubilized inorganic phosphate, three (FLX-2, FLX-4, and FLX-5) produced IAA, and HCN (Figures 2 and 3). Phosphorus is a key element that restricts plant growth as well as nitrogen. PGPR assists plants absorb more phosphate by transforming it from insoluble to soluble forms. The Bacillus and Pseudomonas genera are the most influential bacteria that typically solubilize phosphate by producing organic acids (Qingwei et al., 2023). According to Rawat et al. (2021), the most prevalent phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. *Bacillus thuringiensis* FLX-1, *Pseudomonas boreopolis* FLX-3, *Bacillus mojavensis* FLX-4 and *Pseudomonas chlororaphis* FLX-5 dissolved inorganic phosphate in the current study. P solubilization index of bacterial isolates is defined to be between 3.56 to 5.47 on PKA agar medium. According to the Phosphate solubilization index of bacterial isolates in PKA agar medium, the maximum value was obtained from *Pseudomonas chlororaphis* FLX-5 with 5.47. This was followed by *Pseudomonas boreopolis* FLX-3 with 4.19 and *Bacillus mojavensis* FLX-4 with 3.85, respectively (Table 2).

Mukhtar et al. (2017) determined that *Bacillus safensis* PSB5 and *B. megaterium* PSB12 from wheat rhizosphere solubilized high phosphate levels. Prajakta et al. (2019) reported that *Bacillus mojavensis* PB-35 had a phosphate solubilization ability among 95 rhizobacterial strains isolated from soybean rhizosphere. Similarly, it was determined that *Bacillus mojavensis* FLX-4 had a higher phosphate solubilization ability (3.85) in the current study. Aliyat et al. (2022) reported that bacteria collected from the phosphate mining area were capable of dissolving various forms of phosphate (tricalcium phosphate, aluminum phosphate, and iron phosphate), with the maximum phosphate solubilization index (PSI) being 4.12 from *Pseudomonas brassicacearum* BT125. Similarly, it was determined that *Pseudomonas boreopolis* FLX-3 had a high (4.19) phosphate solubilization index in the current study. Amri et al. (2023) determined 28 phosphate-solubilizing bacteria from soil samples collected from different regions of Tunisia. They found that the solubilization index percentages of these bacteria ranged 2.14 to 3.51%, with the maximum PSI percentage belonging to *Pseudomonas fluorescens*. In the present study, it was determined that the phosphate solubilization index in the Pseudomonas genus was between 4.1 and 5.4. These results align with the findings of Roychowdhury et al. (2019), and Blanco-Vargas et al. (2020), which demonstrated that the solubilization index among various bacterial isolates, including *Pseudomonas* spp., ranged 2.56 to 4.50.

IAA controls and regulate plant growth, development, senescence, cell division, elongation, and fruiting. Approximately 80 % of PGPR are capable of producing IAA (Zahroya et al., 2020). In the present study, *Pseudomonas libanensis* FLX-2, *Bacillus mojavensis* FLX-4, and *Pseudomonas chlororaphis* FLX-5 produced varying amounts of IAA, as determined spectrophotometrically at 530 nm. The ability of Pseudomonas and Bacillus strains to produce IAA has been demonstrated in previous studies (Myresiotis et al., 2014; Rizvi & Khan 2017). Zainab et al. (2020) determined that *Bacillus gibsonii* (PM11) and *Bacillus xiamenensis* (PM14) strains inoculated on flax seeds produced high levels (79 µM ml^{-1} ve 91µM ml^{-1}) of IAA. Devanathan et al. (2021) reported that *Bacillus* sp. in the rhizosphere of *Capsicum annum* L. produced high levels of IAA. Khatami et al. (2023) reported that rhizospheric *Bacillus* sp. synthesized high amounts of IAA. Likewise, *Bacillus mojavensis* FLX-4 produced IAA in the present study (Table 2).

Table 2. IAA production and phosphate solubilization index (PSI) values of isolates

Bacterial isolate code	Isolates	IAA Production[1] (OD at 530nm) (X±Sx)	Phosphate Solubilization Index[2] (PSI) (X±Sx)
FLX-1	*Bacillus thuringiensis*	ND*	3.56±0.08[a]
FLX-2	*Pseudomonas libanensis*	12.45±0.60[a]	ND
FLX-3	*Pseudomonas boreopolis*	ND	4.19±0.22[b]
FLX-4	*Bacillus mojavensis*	10.39±0.65[a]	3.85±0.07[a]
FLX-5	*Pseudomonas chlororaphis*	16.61±0.18[b]	5.47±0.08[c]

*ND: Not Detected, Mean differences were statistically classified as significant ($p< 0.05$).

Nitrogen-fixing bacterial genera commonly found in the rhizosphere include *Rhizobium*, *Sinorhizobium*, *Bacillus*, *Pseudomonas*, *Azoarcus*, and *Burkholderia* (Shah et al., 2021). The current study demonstrated that *Bacillus thuringiensis* FLX-1, *Pseudomonas libanensis* FLX-2, *Pseudomonas boreopolis* FLX-3, and *Pseudomonas chlororaphis* FLX-5 have the ability to fix nitrogen. Reetha et al. (2014) determined that the *Pseudomonas fluorescens* strain isolated from the onion rhizosphere carried out nitrogen fixation ability. Afa et

al. (2020) reported that among 20 *Pseudomonas* isolates isolated from the rhizosphere of *Allium ascalonicum*, 6 of them (Twt04, Twb11, Tt01, Lw07, Lt01, and Lp03) fixed a high amount of nitrogen**.** Alnefai et al. (2020) determined that 8 isolates identified using MALDI-TOF MS showed strong nitrogen-fixing ability. Similarly, Singh et al. (2023) determined that *Pseudomonas koreensis* CY4 and *P. entomophila* CN11 isolated from sugarcane rhizosphere fixed nitrogen.

Some soil bacteria produce a volatile secondary metabolite called HCN. It inhibits electron transport pathways and energy sources in cells, resulting in pathogen death (Manasa et al., 2017). In the present study, *Pseudomonas libanensis* FLX-2, *Bacillus mojavensis* FLX-4 and *Pseudomonas chlororaphis* FLX-5 produced HCN. Ahmad et al. (2008) reported that 50% of Bacillus and 88% of Pseudomonas in the plant rhizosphere could produce HCN. Quesssaoui et al. (2017) determined that *P. fluorescens* Q110B and *P. fluorescens* Q036B, taken from the tomato rhizosphere, produced HCN. Similarly, Halimursyadah et al. (2023) determined that *P. fluorescens* produced HCN among 37 isolates from the patchouli rhizosphere. Singh et al. (2019) determined that *B. thuringiensis* SF 23, *P. aeruginosa* SF 44, *B. subtilis* SF 48, and *B. subtilis* SF 90 isolates produced HCN. The plant growth-promoting properties of the isolates are presented in Figures 2 and 3.

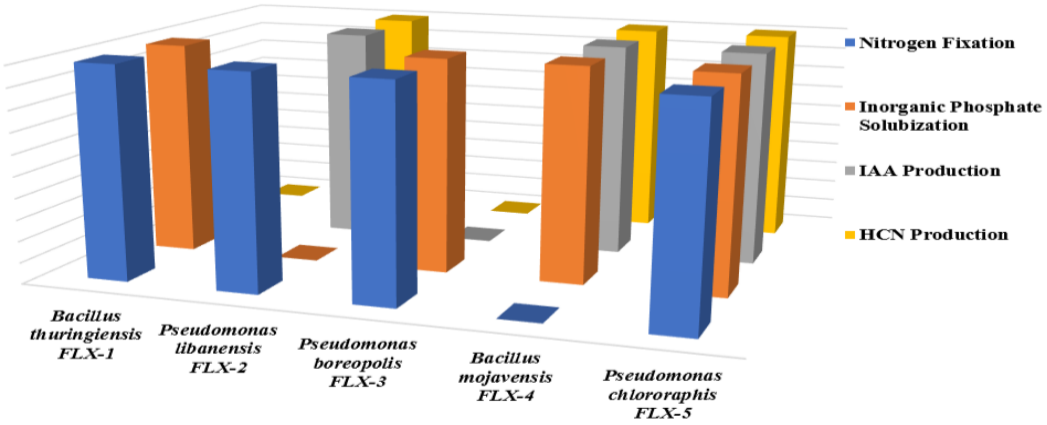


Figure 2. Distribution of plant growth promoting properties of isolates

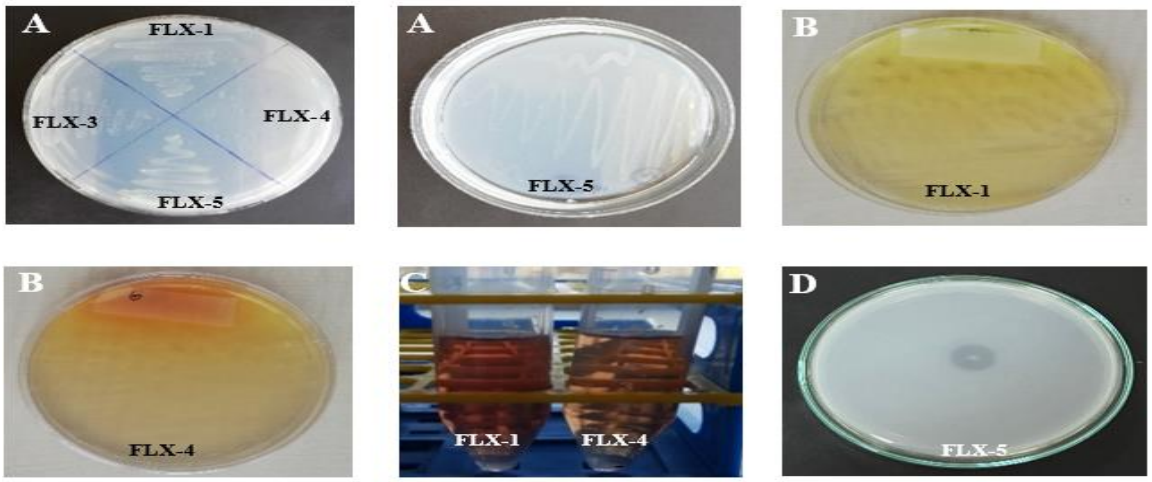


Figure 3. Plant growth promoting traits of the isolates. A: Nitrogen fixation B: HCN production C: IAA production D: Inorganic phosphate solubilization.

Germination tests

Germination Percentage (GP)

The study found that with each increase in copper concentration, the germination percentage decreased, and the flax seeds treated with bacteria germinated faster compared to the control group. This outcome can be attributed to the gradually increasing copper (Cu) stress, which exerts a toxic influence on seed germination and leads to a reduction in germination rate; however, bacterial treatment appears to be effective in alleviating this negative impact. In the evaluation made according to the rhizobacteria-Cu treatments, it was determined that the germination percentage ranged from 49.8% (control treatment) to 77.1% (FLX-5 rhizobacterium treatment) according to the average doses of Cu (Figure 4A, Table 3). Additionally, the maximum germination percentage for copper doses was 76.8% in the control group (0 mM), while the minimum germination percentage was 60 % after 20 mM treatment.

Mean Germination Time (MGT)

It was determined that the average germination time increased as the copper concentration increased in other bacterial treatments except for the FLX-2 rhizobacterium treatment. The evaluation based on the rhizobacteria-Cu treatments, it was determined that the average germination time ranged from 3.59 days (FLX-1 rhizobacterium treatment) to 2.89 days (FLX-2 rhizobacterium treatment) (Figure 4B). When considering the average copper doses, the maximum average germination time was 3.63 days in the control (0 mM) group, while the minimum germination time was detected on the 20 mM treatment after 2.8 days.

Germination Power Index (GPI)

The evaluation made according to the rhizobacteria-Cu treatments showed that the germination power index varied between 826.2 (FLX-5 rhizobacterium treatment) and 229.2 (control treatment) (Figure 4C). Additionally, considering the averages of copper doses, the maximum germination power index was 1116.5 in the control (0 mM) treatment, while the minimum germination power index was determined as 30.8 on 20 mM treatment.

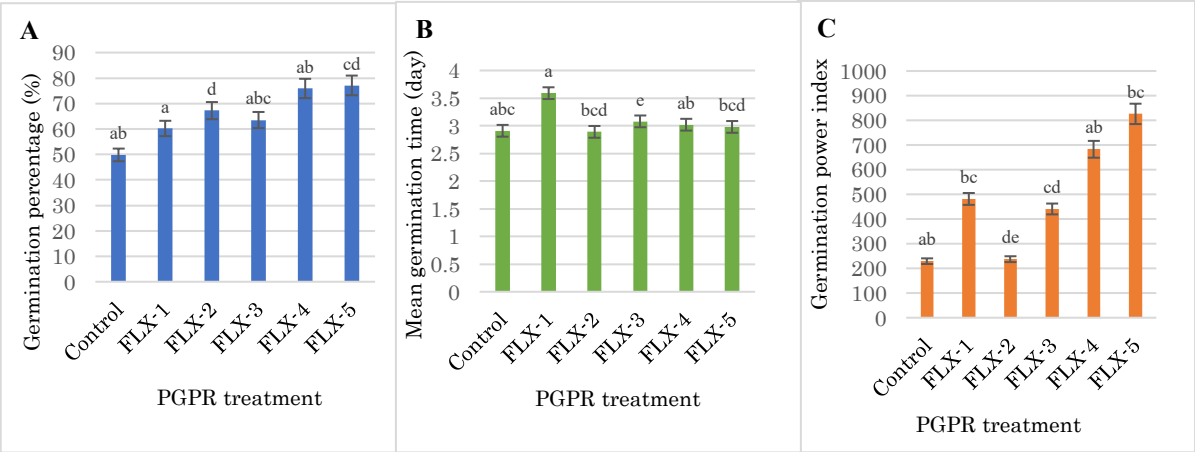


Figure 4. Effect of PGPR treatments on germination percentage (A), mean germination time (B) and germination power index (C) of flax seed

Many studies indicate that excessive copper accumulation in plants like corn, beans, cucumbers, and tomatoes negatively impacts plant growth (Mediouni et al., 2006; Feil et al., 2020). Copper stress in plants create biomass mobilization through glucose-fructose release, which prevents the breakdown of starch or sucrose in tissues and reduce seed germination. It has also been reported that copper toxicity inhibits seed germination by down-regulating the activity of α-amylase or enolase (Bajpai et al., 2021). Widawati and Suliasih (2018) reported applying PGPR (*Azospirillum* sp., *Azotobacter* sp., *Bacillus* sp.) to sorghum seeds promoted seed germination parameters as well as root and shoot growth in vitro. Similarly, Ghorbel et al. (2023) determined that inoculating barley seeds with the *Glutamicibacter* sp. MD36 strain, which has plant growth-promoting properties, increased barley germination under Cu heavy metal stress by 77 % at 50 mM, 63 % at 100 mM, and 38 % at 150 mM.

Germination percentage coefficient, germination percentage, germination power index, stem length and root length decreased due to the increase in Cu concentration; mean germination time was prolonged except for *Pseudomonas libanensis* FLX-2 treatment. It was determined that *Pseudomonas chlororaphis* FLX-5 bacteria treatment significantly increased the germination percentage, mean germination time, and germination power index compared to the control treatment. According to Zainab et al. (2020), IAA-producing rhizobacteria accelerate phytoremediation efficiency and increase the root and stem development of the plant. This improvement in germination parameters can probably be attributed to the ability of *Pseudomonas chlororaphis* FLX-5 to solubilize phosphate, produce IAA, and fix nitrogen. On the other hand, it was determined that *Pseudomonas*

boreopolis FLX-3 bacteria treatment increased the germination percentage, average germination time, and germination power index.

Evaluation of root and stem lengths

The maximum root length was determined as 5.14 cm after treatment with the FLX-5 bacteria, and the minimum root length was determined as 1.75 cm in the control treatment in this study. Moreover, considering the average copper doses, the maximum root length was determined as 7.33 cm in the control (0 mM) treatment; the minimum root length was determined as 0.35 cm in the 20 mM treatment (Table 3).

It was determined that *Pseudomonas chlororaphis* FLX-5 and *Bacillus mojavensis* FLX-4 strains provided longer roots compared to the control group in this study. In particular, it was determined that *Pseudomonas chlororaphis* FLX-5 strain sharp improvement in root length (5.14 cm) compared to control treatments. Whereas, the minimum root length (3.09 cm) was determined on plants treated with *Bacillus thuringiensis* FLX-1 (Figure 5A). In the evaluation made according to the bacteria-Cu treatments, the longest stem length of 4.91 cm was noted after treatment with *Pseudomonas chlororaphis* FLX-5 strain, and the shortest stem length of 2.10 cm was noted on the control treatment. Moreover, According to the average copper doses, the longest stems of 6.91 cm were noted on the control (0 mM) treatment. The minimum stem length of 0.27 cm was noted on the 20 mM treatment in the current study (Figure 5B).

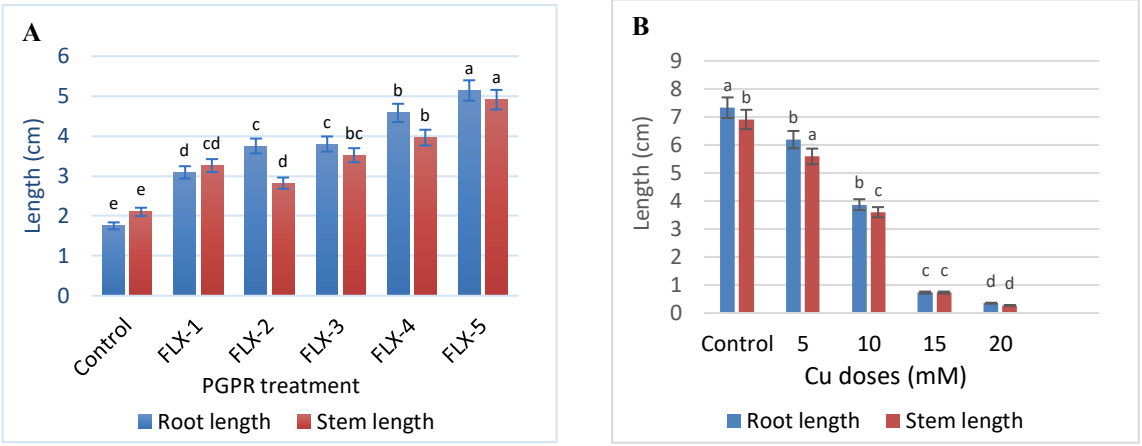


Figure 5. Effect of PGPR (A) and Cu (B) treatments on root and shoot development from flax seed

PGPR strains are beneficial microorganisms that influence the bioavailability of heavy metals (Gupta et al., 2024). Rizvi et al. (2020) investigated the Cr biosorption capacity of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Azotobacter chroococcum*. They determined that *B. subtilis* biosorbed Cr at a record rate of 96% at 25 g mL^{-1}. El-Saadony et al. (2024) determined that the treatment of *Pseudomonas azotoformans*, *Serratia rubidaea*, *Paenibacillus pabuli* and *Bacillus velezensis*, which were isolated from heavy metal contaminated soils and determined to have plant growth promoting properties, to pepper plants grown under heavy metal stress (Cd, Cu, Pb and Ni) increased photosynthetic pigment contents, relative water content and membrane stability index and germination parameters compared to plants without bacteria treatment.

Many researchers have reported that PGPR can tolerate heavy metals, break down or clean up soil pollutants, and convert them into less toxic forms (Nazli et al., 2020; Zhu et al., 2023). It has been noted that PGPR bacteria reduce the phytotoxicity of metal ions by sharing the metal load resulting from bioaccumulation in the presence of heavy metals (Zaidi et al., 2006). Pandey et al. (2013) reported that *Bacillus* species with plant growth promoting properties stimulated rice seed germination under copper stress and increased root and shoot lengths in rice plants compared to the control. According to Patel et al. (2016), the use of PGPR capable of adsorbing heavy metal ions is an eco-friendly alternative to chemical metal chelators. Hansda et al. (2017) determined that the plant growth-promoting *Kocuria* sp. CRB15 bacterial strain was resistant to various heavy metals like Cu, Ni, Cd, Zn, and Pb. Elsakhawy et al. (2019) reported that the halophilic bacteria *Chromohalobacter salexigens* KT989776 significantly improved all germination parameters of flax seeds under different heavy metal and salt concentrations. A study conducted by Abbaszadeh-Dahaji et al. (2021) reported that PGPR strains (*Pseudomonas cedrina* K4 and *Stenotrophomonas* sp. A22) were effective in reducing Cu toxicity in corn and sunflower grown in Cu-contaminated soil. It has been reported that the presence of toxic levels of copper in the soil prevents the growth and development of newly developing rootlets, resulting in decreases in root and shoot lengths (Sfaxi Bousbih et al., 2010; Pena et al., 2011). Soudek et al. (2010) determined that high levels of copper slowed down the root development of the flax plant when they studied the effects of different doses of various heavy metals (cadmium, cobalt, copper, zinc, nickel, lead, chromium, and arsenic) on its germination.

Minuț et al. (2022) reported that *Pseudomonas* sp. treatment increased the root and shoot lengths of flax by 178.38 % and 15.08 %, respectively, compared to the control treatment. In the current study, it was determined that *Pseudomonas libanensis* FLX-2, *Pseudomonas boreopolis* FLX-3 and *Pseudomonas chlororaphis* FLX-5 treatments increased root lengths by 114 %, 117 % and 193 %, respectively, compared to the control treatment. In addition, in the current study, it was determined that the maximum stem length was 4.91 cm after treatment with in the FLX-5 treatment, while the minimum stem length was 2.10 cm in the control group. This can be attributed to the ability of *Pseudomonas chlororaphis* FLX-5 to produce IAA and solubilize phosphorus. This result is consistent with Rizvi and Khan (2017), who investigated the effect of Cu stress on wheat germination by PGPR *Pseudomonas aeruginosa* strain CPSB1, which has the ability to produce IAA and solubilize phosphorus.

Evaluation of root and stem weights

In the evaluation of root fresh weight according to the bacteria-Cu treatments, the maximum root fresh weight was obtained from the FLX-5 bacteria treatment with 18.2 mg, while the minimum root fresh weight was obtained from the control treatment with 12.8 mg in the present study. According to the average values for copper doses, the maximum root dry weight was 18.9 mg in the control group (0 mM), followed in descending order by 5 mM (16.65 mg), 10 mM (15.05 mg), 15 mM (12.78 mg), and 20 mM (11.36 mg) concentrations (Table 3).

Many researchers have shown that PGPR treatments enhance the number of leaves, root and shoot length, fresh and dry weight, proline, and chlorophyll content. For example, in the study conducted by Yıldırım et al. (2015), it was reported that PGPR treatment in cucumber significantly increased stem diameter, seedling height, shoot and root weight compared to the control. Idder et al. (2019) stated that inoculating *Vicia faba* plant with *Pseudomonas* P1, P7 and P15 strains under copper stress significantly increased the root-stem fresh weight of the plants compared to uninoculated plants. Similarly, Madline et al. (2021) determined that the inoculation of growth-promoting rhizobacteria (*Mesorhizobium tamadayense*, *Enterobacter xiangfangensis*, *Pseudomonas azotifigens*, and *Streptomyces caelestis*) isolated from copper mining soils increases the root and shoot development of *Peganum harmala* and *Lactuca sativa* seeds.

There are many studies indicating that *Pseudomonas* sp. and *Bacillus* sp. bacteria, which are abundant in the soil, increase product yield by promoting plant growth. For example, In a similar study, Abbaszadeh-Dahaji et al. (2021) reported that inoculating corn seeds with *Pseudomonas fluorescens* P22 and *Pseudomonas* sp. Z6, isolated from copper-contaminated soils, increased shoot dry weight by 12 % and 16 %, respectively, and shoot length by 20 % and 22 %, compared to the control. According to Omer et al. (2022), they reported that the treatment of *Pseudomonas geniculata*, which supports plant growth, to flax seeds increased root and shoot lengths and fresh and dry weights in flax compared to the control group. In the current study, it was determined that *Pseudomonas libanensis* FLX-2 strain treatment provided an 11 % increase in root fresh weight compared to the control treatment, but had no effect on stem fresh weight (Figure 6A). On the other hand, it was determined that *Pseudomonas chlororaphis* FLX-5 strain treatment increased the root and shoot fresh weights by 42 % and 23 %, respectively, compared to the control. Figure 6 shows the fresh weight of the roots and stems of flaxseed under different PGPR and copper treatments.

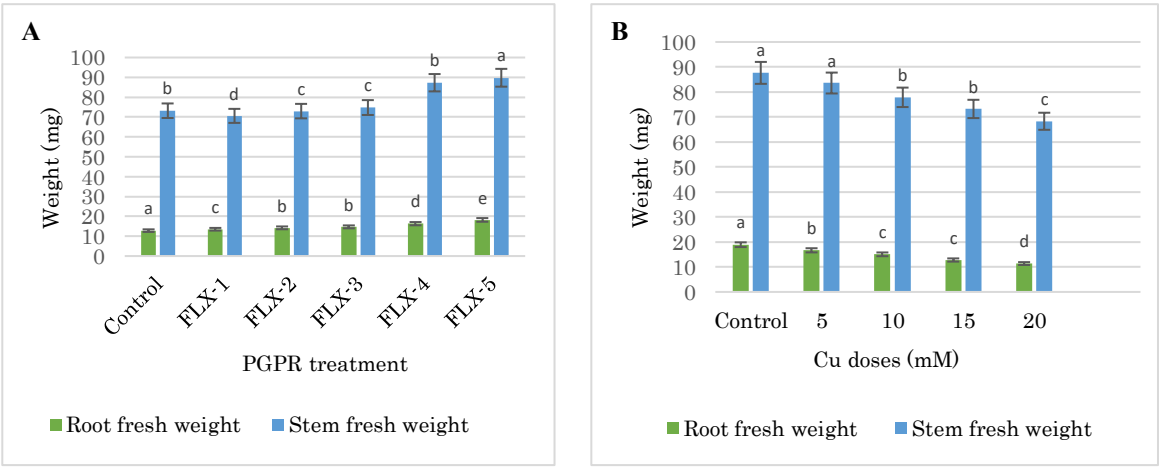


Figure 6. Effect of PGPR (A) and Cu (B) treatments on root and shoot fresh weight of flax seedlings

In the evaluation of root dry weight according to the bacteria-Cu treatments, the maximum root dry weight was determined in FLX-5 bacteria with 1.24 mg, and the minimum root dry weight was determined in FLX-1 bacteria with 1.05 mg in the current study. According to the average copper doses, the maximum root dry weight was determined as 1.50 mg in the control (0 mM) treatment, followed by 5 mM (1.42 mg), 10 mM (1.26 mg), 15 mM (1.0 mg) and 20 mM (0.77 mg) concentrations in decreasing order. In the evaluation of stem dry weight according to bacterial treatments, the maximum stem dry weight was determined as 10.4 mg in FLX-5 bacteria, and the minimum body dry weight was determined as 10.08 mg in FLX-2 bacteria. In addition, according to copper concentrations, the maximum stem dry weight was determined as 12.19 mg in the control (0 mM) treatment, followed by 5 mM (11.72 mg), 10 mM (10.93 mg), 15 mM (9.12 mg) and 20 mM (6.90 mg) concentrations in decreasing order (Table 3). According to our results, the isolated *Pseudomonas chlororaphis* FLX-5 not only promotes plant growth but also exhibits resistance to different copper concentrations, thereby exerting a positive effect on flax seed germination. Furthermore, *Pseudomonas chlororaphis* FLX-5 may possess significant potential for the remediation of areas contaminated with copper heavy metal.

Numerous studies have shown that inoculating plants with PGPR causes significant changes in various growth parameters, such as plant height, root and stem dry weight (Mazhar et al., 2020; Liu et al., 2022). Moreover, It has been reported that strains belonging to the Bacillus genus, which are abundant in the plant root, increase root development by producing nitrogen fixation and IAA, and thus play a role in plant development (Myresiotis et al., 2014). In a study conducted by Zainab et al. (2020), it was reported that the treatment of *Bacillus gibsonii* (PM11) and *Bacillus xiamenensis* (PM14), which have the ability to produce IAA and ACC-deaminase, to flaxseed significantly increased the root and shoot length, fresh and dry weight, proline and chlorophyll content of the plant. Similarly, Ke et al. (2021) reported that inoculating the grass plant with *Bacillus thuringiensis* and *Bacillus cereus* isolates obtained from Cu and Cd contaminated mines increased the stem and root biomass by 11.49 % and 44.50 %, respectively. Our current study revealed that treatment of *Bacillus thuringiensis* FLX-1 and *Bacillus mojavensis* FLX-4 strains increased stem length by 55 % and 88 %, respectively, compared to the control group. When evaluating fresh root and fresh stem weights, it was determined that the *Bacillus thuringiensis* FLX-1 strain treatment resulted in a 15 % increase in fresh root weight compared to the control group, while it had no positive effect on fresh stem weight (Figure 7A). On the other hand, the *Bacillus mojavensis* FLX-4 strain treatment was observed to provide increases of 27 % and 19 % in fresh root and fresh stem weights, respectively, compared to the control group. Figure 7 presents data on root and stem dry weight of flaxseed in different PGPR and Cu treatments. Table 3 shows the effects of PGPR and Cu treatments on parameters such as root-shoot length, root fresh-dry weight and shoot fresh-dry weight.

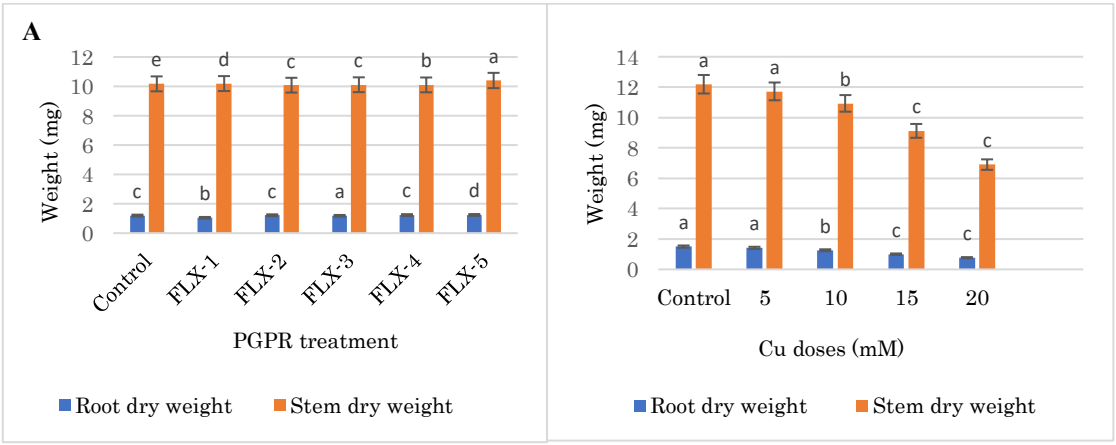


Figure 7. Effect of PGPR (A) and Cu (B) treatments on root and stem dry weight of flax seedlings

Table 3. Effects of PGPR and Cu treatments on root-shoot length, root fresh-dry weight and shoot fresh-dry weight of flax seedlings.

Bacterial isolate code	**Cu (mM)**	**Root length** ** **(cm)** (X±Sx)	**Stem length (cm)** (X±Sx)	**Fresh Weight (mg)** (X±Sx)		**Dry Weight (mg)** (X±Sx)	
				Root	**Stem**	**Root**	**Stem**
Control	**0**	3.20±0.11^{a}	2.90±0.11^{b}	18.5±0.45^{a}	86.3±2.55^{a}	1.74±0.05^{a}	12.64±0.02^{a}
	5	2.44±0.10^{b}	3.70±0.12^{a}	16.0±0.35^{b}	84.8±0.95^{a}	1.59±0.03^{a}	12.21±0.01^{a}
	10	2.08±0.10^{b}	1.78±0.07^{c}	13.5±0.80^{c}	69.2±0.75^{b}	1.32±0.02^{b}	9.8±0.32^{b}
	15	0.76±0.09^{c}	1.96±0.09^{c}	8.5±0.25^{c}	68.5±1.40^{b}	0.76±0.04^{c}	8.06±0.03^{c}
	20	0.29±0.07^{d}	0.2±0.12^{d}	7.5±0.20^{d}	57.2±0.92^{c}	0.67±0.02^{c}	8.14±0.01^{c}
	Mean	**1.75^{e}**	**2.10^{e}**	**12.8^{a}**	**73.2^{b}**	**1.20^{c}**	**10.17^{e}**
FLX-1*	**0**	5.43±0.24^{a}	6.02±0.08^{a}	19.5±0.56^{a}	88.6±0.70^{a}	1.36±0.02^{a}	12.67±0.04^{a}
	5	4.66±0.20^{a}	4.23±0.07^{b}	16.4±0.15^{b}	80.1±0.62^{b}	1.28±0.03^{ab}	11.29±0.03^{b}
	10	3.78±0.17^{b}	5.13±0.12^{c}	13.7±0.30^{c}	74.7±0.72^{c}	1.16±0.02^{b}	10.55±0.02^{c}
	15	1.12±0.16^{c}	0.61±0.12^{d}	9.5±0.35^{d}	57.3±1.48^{d}	0.77±0.02^{c}	9.81±0.04^{d}
	20	0.46±0.09^{c}	0.32±0.02^{d}	8.4±0.15^{d}	52.3±1.26^{e}	0.72±0.07^{c}	6.67±0.03^{e}
	Mean	**3.09^{d}**	**3.26^{cd}**	**13.5^{c}**	**70.6^{d}**	**1.05^{b}**	**10.19^{d}**
FLX-2	**0**	8.21±0.12^{a}	5.54 ±0.14^{a}	17.3±0.15^{a}	85.5±1.65^{a}	1.60±0.04^{a}	11.98±0.06^{a}
	5	6.34±0.09^{b}	4.87±0.38^{a}	15.2±0.30^{b}	79.3±1.47^{ab}	1.45±0.01^{b}	12.23±0.02^{b}
	10	3.0±0.41^{c}	3.14±0.14^{b}	14.6±0.20^{b}	71.5±2.63^{bc}	1.39±0.03^{b}	10.58±0.05^{c}
	15	0.75±0.09^{d}	0.32±0.10^{c}	14.3±0.20^{b}	68.6±0.72^{c}	1.14±0.03^{c}	8.73±0.01^{d}
	20	0.47±0.06^{d}	0.27±0.04^{c}	9.6±0.20^{c}	60.1±0.56^{d}	0.56±0.02^{d}	6.88±0.01^{e}
	Mean	**3.75^{c}**	**2.82^{d}**	**14.2^{b}**	**73.0^{c}**	**1.22^{c}**	**10.08^{c}**
FLX-3	**0**	6.31±0.08^{a}	7.45±0.67^{a}	18.7±0.36^{a}	82.4±0.4^{a}	1.47±0.02^{a}	12.06±0.29^{a}
	5	7.54±1.58^{a}	5.88±0.12^{b}	16.4±0.25^{b}	76.5±0.68^{b}	1.31±0.04^{ab}	11.67±0.09^{a}
	10	4.40±0.28^{a}	3.43±0.06^{c}	13.7 ±0.30^{c}	74.4±0.41^{bc}	1.19±0.03^{bc}	11.45±0.02^{a}
	15	0.61±0.07^{b}	0.56±0.02^{d}	12.8±0.15^{cd}	71.5±1.08^{cd}	1.10±0.04^{c}	9.48±0.03^{b}
	20	0.37±0.07^{b}	0.30±0.11^{d}	11.9±0.26^{d}	69.2± 0.46^{d}	0.83±0.02^{d}	5.85±0.04^{c}
	Mean	**3.80^{c}**	**3.52^{bc}**	**14.7^{b}**	**74.8^{c}**	**1.18^{a}**	**10.11^{c}**
FLX-4	**0**	9.43±0.19^{a}	8.9±0.55^{a}	19.6±0.45^{a}	89.5±0.92^{a}	1.33±0.02^{a}	11.39±0.02^{a}
	5	7.56±0.07^{b}	5.8±0.34^{b}	17.3±0.55^{b}	88.7±0.72^{ab}	1.54±0.02^{b}	11.27±0.03^{b}
	10	4.67±0.08^{c}	4.19±0.11^{c}	16.9±0.26^{b}	87.0±0.25^{abc}	1.28±0.03^{b}	12.41±0.03^{b}
	15	0.9±0.30^{d}	0.62±0.18^{d}	14.1±0.35^{c}	86.1±0.58^{bc}	1.03±0.02^{c}	9.02±0.03^{c}
	20	0.38±0.03^{d}	0.33±0.10^{d}	13.6±0.26^{c}	85.2±0.41^{c}	0.97±0.06^{c}	6.45±0.04^{d}
	Mean	**4.58^{b}**	**3.96^{b}**	**16.3^{d}**	**87.3^{b}**	**1.23^{c}**	**10.10^{b}**
FLX-5	**0**	11.4±0.28^{a}	10.7±0.25^{a}	19.8±0.55^{a}	93.5± 0.37^{a}	1.55±0.01^{a}	12.45±0.01^{a}
	5	8.6±0.92^{b}	9.1±1.05^{a}	18.6±0.30^{ab}	92.1± 0.25^{a}	1.36±0.02^{b}	11.68±0.05^{b}
	10	5.3±0.26^{c}	4.2±0.20^{b}	17.9±0.35^{b}	90.4± 0.52^{b}	1.22±0.02^{c}	10.82±0.04^{c}
	15	0.28±0.08^{d}	0.35±0.06^{c}	17.5±0.35^{b}	87.3± 0.20^{c}	1.20±0.01^{c}	9.65±0.01^{d}
	20	0.14±0.02^{d}	0.21±0.03^{c}	17.2±0.20^{b}	85.7± 0.32^{c}	0.88±0.03^{d}	7.43±0.01^{e}
	Mean	**5.14^{a}**	**4.91^{a}**	**18.2^{e}**	**89.8^{a}**	**1.24^{d}**	**10.4^{a}**

*FLX-1: *Bacillus thuringiensis*, FLX-2: *Pseudomonas libanensis*, FLX-3: *Pseudomonas boreopolis*, FLX-4: *Bacillus mojavensis*, FLX-5: *Pseudomonas chlororaphis*. Each value represents the mean of at three replications **Values sharing the same letter within a column are not statistically different at the 0.05 level of confidence.

CONCLUSION

Heavy metal accumulation in soil due to industrial and agricultural waste is increasing, affecting soil fertility and food safety. Excess copper in soil harms plant growth. Plant growth-promoting rhizobacteria (PGPR) are used to reduce heavy metal toxicity by altering metal pathways and converting them into harmless forms. This study found that PGPR strains positively affect flax seed germination and physiological traits, and *Pseudomonas chlororaphis* FLX-5 treatment effectively reduced copper's negative effects.

Compliance with Ethical Standards
Peer-review
The authors declare that there is no ethical issue
Declaration of Interests
The authors declare that there is no conflict of interest
Author contribution
The authors declare that they have contributed equally to the article

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Prediction model of albumen index and height in Japanese quail eggs via external quality characteristics

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Abstract

This study used multiple regression analysis to estimate the relationships between egg albumen index and height and external quality traits of eggs in Japanese quails. Egg albumen index and height were selected as dependent variables, while egg weight, width, length, shape index and Haugh unit were determined as independent variables. In the multiple regression analysis, although the overall fit of the model was high, it was determined that there were multicollinearity problem among the independent variables. To solve this problem, the Principal Components Regression (PCR) method, which is widely used in the literature, was applied. With this method, the following regression equations were obtained, respectively, by using egg weight (X_1, Z_1), width (X_2, Z_2), length (X_3, Z_3), shape index (X_4, Z_4) and Haugh unit (X_5, Z_5) variables in estimating albumen index and height:

$$\hat{Y} = -\widehat{19.95} - \widehat{0.02}X_1 - \widehat{0.22}X_2 - \widehat{0.03}X_3 + \widehat{0.01}X_4 + \widehat{0.43}X_5 + \hat{e}$$

$$\hat{T} = -\widehat{13.21} + \widehat{0.09}Z_1 + \widehat{0.03}Z_2 - \widehat{0.004}Z_3 - \widehat{0.005}Z_4 + \widehat{0.18}Z_5 + \hat{e}$$

The regression equations obtained were found to be statistically significant ($P<0.05$) and the goodness of fit of the models were determined as Detaermination coefficient (R^2) = 0.86 and 0.99, respectively. Principal Components Regression (PCR) method reduced the errors caused by multicollinearity problem by decreasing the standard errors of the parameters and increased the accuracy of the model. The results show that Principal Components Regression (PCR) method effectively solved the multicollinearity problem and ensured the reliability of the model by increasing the prediction accuracy. These findings reveal that Principal Components Regression (PCR) method can be used effectively in poultry breeding and selection studies.

Keywords: Multicollinearity problem, Least Squares Method (LSM), Quail egg characteristics, Principal Components Regression(PCR) method

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INTRODUCTION

Quail eggs have become a food product that has attracted egg is used as a food from the hunting-gathering age, especially with their high nutritional value and health benefits. These eggs are widely used in both fresh consumption and processed food products. The external quality characteristics of quail eggs are an important part of quality control in production processes. The physical and chemical properties of egg white play an important role in determining the external quality criteria of quail eggs (Pahm et al., 2012). In this context, objective evaluation of the quality characteristics of quail eggs is of great importance for producers and consumers.

The main factors affecting egg quality are physical parameters such as egg weight, shape index, width, length, egg albumen height and Haugh unit (Haug et al., 2010). However, the relationships between these characteristics can be complex and correct modeling of these relationships is important in order to make accurate quality estimates. Multiple regression analysis allows modeling the relationship between a dependent variable and more than one independent variable, and this method is frequently used in cases where multiple factors are effective, such as quail eggs (Kutlu and Erdem, 2013).

In studies conducted on quail eggs, multiple regression analysis has been used to model the relationships between egg external quality characteristics and egg albumen. However, in cases where more than one independent variable is effective at the same time, the presence of high correlations between independent variables may negatively affect the accuracy of the model. This situation causes a problem known as multiple collinearity (Oktay and Yıldız, 2017). Multicollinearity problem reduces the reliability of the regression coefficients and makes it difficult to interpret the results of the model.

Principal Components Regression (PCR) method is an effective method in solving such problems. Principal Components Regression (PCR) method eliminates the problem of multiple collinearity by using new components consisting of linear combinations of highly correlated in dependent variables (Jolliffe, 2002). This method allows the development of reliable and robust models for determining quail egg quality (Wang et al., 2015). Principal Components Regression (PCR) method is often preferred to increase the generalizability of the model, especially in high-dimensional data sets (Bai, 2017).

This study aims to model the relationships between quail egg external quality traits and dependent variables such as egg albumen and height using multiple regression analysis and Principal Components Regression (PCR) method the findings of the study may help quail egg producers obtain more reliable results in quality control processes.

MATERIALS AND METHODS

The data set in this study was obtained from egg analysis performed to determine internal and external quality criteria on 90 eggs obtained from a 13-week-old Japanese quail (*Coturnix Coturnix Japonica*) flock in the Quail Breeding Unit of the Research and Application Farm of the Faculty of Agriculture of Çukurova University. Egg quality analyses were performed in the Poultry Laboratory of the Department of Animal Science of the Faculty of Agriculture of Çukurova University. The width and length of the eggs were measured with a digital caliper (± 0.01 mm). Egg weight was determined using an electronic scale with a precision of 0.01 g. The eggs were broken one by one on a glass breaking bench and the dimensions of the egg yolk and egg white (height, width and length) were recorded using a digital caliper (± 0.01 mm). The obtained data were used in the following formulas to calculate shape index, white index, yellow index and haugh unit values (Yannakopoulos and Tserveni-Gousi, 1986; Kaya and Aktan, 2011; Olawumi and Chiristiana, 2017).

$$\text{Shape Index} = \frac{\text{Egg Width (mm)}}{\text{Egg Length (mm)}} \text{x} 100 \quad (1)$$

$$\text{Albumen Index} = \frac{\text{Albumen heigth (mm)}}{\text{Average of Albumen Width and Length (mm)}} \text{x} 100 \quad (2)$$

$$\text{Haugh Unit} = 100 \log (\text{Albumen Height} + 7.57 - 1.7 \text{ x Egg Weight}^{0.37}) \quad (3)$$

$$\text{Yolk Index} = \frac{\text{Yolk heigth (mm)}}{\text{Yolk Width (mm)}} \text{x} 100 \quad (4)$$

In the study, two prediction models were established between two dependent variables (albumen index and height) and five independent variables (egg weight, width, length, shape index and Haugh unit). In order to evaluate how compatible these models are, multiple regression analysis was performed using the SPSS 26 statistical package program, which is frequently preferred in the literature.

Multiple regression analysis is a statistical technique used to model the relationship between a dependent variable and more than one independent variable. The general model used in this analysis can be expressed as follows:

$$Y = \beta_0 + \beta_1 X_1 + \cdots \beta_n X_n + \varepsilon \quad (5)$$

Here, Y is the dependent variable, β_0 is the regression scalar, β_1, β_2, …, βn are the regression coefficients, X_1, X_2, …, Xn are the independent variables and ε is the error term (Üçkardeş et al., 2012).

In multiple regression analysis, it is important to examine whether there is any relationship between the independent variables in order to evaluate the accuracy and reliability of the model. Because, any relationship between the independent variables causes the problem of multicollinearity (Maxwell, 2000; Montgomery et al., 2001). Some of the common methods used to detect this problem are as follows:

Simple Correlation Coefficient: If the correlation between the independent variables ($r \geq 0.75$) is high, the problem of multicollinearity should be suspected (O'Brien, 2007).

Variance Increasing Factor (VIF): This method measures how strong the relationship between each independent variable is with the other independent variables. When the Variance Increasing Factor (VIF) value is 10 and above, there is a multicollinearity problem. It can also be calculated with the help of the formula below (Albayrak, 2005).

$$\text{VIF}: C_{ij}: \frac{1}{1-R_{ij}} \quad (6)$$

Tolerance Value (TV): It is calculated by subtracting the coefficient of determination (R^2) from 1 (TV = 1 - R^2). A small Tolerance Value (TV) is associated with a high Variance Increasing Factor (VIF) value, which may indicate a multicollinearity problem (Albayrak, 2005).

Each of these methods should be used from different perspectives, because each of them may not give accurate results on its own. Therefore, it is important to consider all these methods when determining the presence of a multicollinearity problem.

There are various suggestions in the literature to eliminate the multicollinearity problem removing the variable from the model, combining related variables, adding a variable to the model, Principal Component Regression (PCR) and Ridge Regression methods, etc.). However, the most widely and reliably used Principal Component Regression (PCR) method is recommended in the literature (Albayrak, 2005).

Principal Component Regression (PCR) method, developed by Pearson (1901) and Hotelling (1933), is a more powerful alternative developed for situations where the classical Least Squares Method (LSM) is ineffective when faced with a multicollinearity problem (Jolliffe, 2004). Although it is based on the basic principles of the Least Squares Method (LSM), it is a method of expressing the data set consisting of the original p variables with fewer new variables that are linear components of these variables. In other words, the method of expressing the structure explained by p number of variables with correlation between them with k number of variables that are linear components of the original variables that are not correlated and are fewer than the number of original variables ($k<p$) is called principal components analysis (Johnson and Wichern, 2002). In Principal Component Regression (PCR) method, the variance lag length must first be determined in order to determine the appropriate model. This process begins by determining at which point the regression becomes stationary. Augmented Dickey-Fuller (ADF) unit root test is often used for stationarity testing. After the data becomes stationary, Vector Auto Regression (VAR) model is used to determine the variance lag length of the appropriate model. Thus, by determining the correct lag length, the reliability of the model is increased and more accurate estimates can be made.

Augmented Dickey-Fuller (ADF) unit root test: A commonly used test to determine whether time series data is stationary is the Augmented Dickey-Fuller (ADF) unit root test. A stationary time series is a series whose statistical properties (such as mean and variance) do not change over time and remain constant. The Augmented Dickey-Fuller (ADF) unit root test whether the series contains a unit root. If there is a unit root in the series, the time series is non-stationary, meaning that there is a trend or tendency in the series. If there is no unit root, the time series is stationary. The mathematical model of the Augmented Dickey-Fuller (ADF) unit root test is usually expressed as follows:

$$\Delta Y_t = \alpha + \beta_t + \gamma Y_t - 1 + \sum_{i=1}^{p} \delta_i \Delta Y_{t-i} + \varepsilon_t \quad (7)$$

Here,

Yt= Time series data,

ΔY_t = Time series change,

α = Constant term,

β = Trend coefficient,

γ = Unit root coefficient,

δ _i= Lagged values coefficients,

p: Lag length,

ε _t= Error term,

(Dickey Fuller, 1979).

Vector Auto Regression (VAR) model: In econometric analyses, when the relationships between independent variables are complex and reciprocal, the use of simultaneous equation systems becomes important. In such systems, taking into account the interactions of each variable with other variables provides more accurate and reliable results. One of the methods widely used in solving simultaneous equations is the Vector Auto Regression (VAR) model. The Vector Auto Regression (VAR) model makes it possible to model using the past values of each endogenous variable together with all other endogenous variables in time series analyses. This method is a powerful tool in examining dynamic relationships and interactions. The mathematical formula of the Vector Auto Regression (VAR) model can be expressed as follows with an equation with a lag length of p:

$$y_t = A_1 y_{t-1} + \cdots + A_p y_{t-1} + B_{x_t} + \varepsilon_t \quad (8)$$

Here, $y_t p \times 1$ dimensional value vector, $x_t d \times 1$ dimensional deterministic variables, $A_p p \times p$ dimensional parameter matrices and the error terms vector (Johansen, 1995).

In the research, it was determined that there was a multicollinearity problem in the estimated models established with the help of simple correlation coefficient, Variance Increasing Factor (VIF) and Tolerance Value (TV). It was aimed to eliminate this problem with the help of the Principal Components Regression (PCR) method, which is widely used in the literature.

RESULTS AND DISCUSSION

In the data set obtained as a result of internal and external quality analyses of quail eggs, firstly, egg albumen-yolk indexes and heights were tested in determining dependent variables, the most appropriate Detaermination coefficient ($R^2 \geq 0.75$) albumen index and height were found appropriate, and the hypotheses and estimated models were determined as follows, respectively.

H_0: There is no relationship between egg albumen index and external egg quality characteristics.

H_1: There is a relationship between egg albumen index and external egg quality characteristics.

H_0: There is no relationship between egg albumen height and external egg quality characteristics.

H_1: There is a relationship between egg albumen height and external egg quality characteristics.

$$\hat{Y} = \widehat{\beta_0} + \widehat{\beta_1}X_1 + \widehat{\beta_2}X_2 + \widehat{\beta_3}X_3 + \widehat{\beta_4}X_4 + \widehat{\beta_5}X_5 + \hat{e} \quad (9)$$

$$\hat{T} = \widehat{\beta_0} + \widehat{\beta_1}Z_1 + \widehat{\beta_2}Z_2 + \widehat{\beta_3}Z_3 + \widehat{\beta_4}Z_4 + \widehat{\beta_5}Z_5 + \hat{e} \quad (10)$$

$\hat{Y}$ = Egg albumen index
X_1 = Egg weight
X_2 = Egg width
X_3 = Egg length
X_4 = Shape index
X_5 = Haugh unit

$\hat{T}$ = Egg albumen height
Z_1 = Egg weight
Z_2 = Egg width
Z_3 = Egg length
Z_4 = Shape index
Z_5 = Haugh unit

Afterwards, the multiple regression equation obtained by the Least Squares Method (LSM) determined that the linear relationship between egg albumen index and height and external quality characteristics was 94 % and 99 % strong, respectively. This finding reveals that the changes in albumen index and height are largely explained by egg external quality characteristics. However, this strong relationship also raises the issue of multicollinearity problem, and reveals that the Ho hypothesis is rejected and the regression relationships are statistically significant ($P<0.05$). This indicates that the model is significant and the obtained results are unlikely to be random. In addition, parameter estimates and the significance levels of these parameters are given in Tables 1 and 2, while Variance Increasing Factor (VIF) and Tolarance Value (TV) are given in Table 3 to determine the relationship between independent variables and the risks of multiple linear connections, and the distribution graphs of each independent variable are given in Figures 1, 2, 3, 4 and 5.

Table 1. Parameter estimate and significance level of the multiple regression equation obtained by the the Least Squares Method (LSM; where the albumen index is the dependent variable).

	Regression Coefficient	Std. Error of the Estimate	t	p
$\widehat{\beta_0}$	-28.329	4.824	-5.873	0.000
$\widehat{\beta_1}$	0.362	0.172	2.099	0.039
$\widehat{\beta_2}$	-1.092	0.324	-3.371	0.001
$\widehat{\beta_3}$	0.181	0.076	2.378	0.020
$\widehat{\beta_4}$	0.226	0.078	2.914	0.005
$\widehat{\beta_5}$	0.449	0.018	24.908	0.000

Table 2. Parameter estimate and significance level of the multiple regression equation obtained by the the Least Squares Method (LSM; where the albumen height is the dependent variable).

	Regression Coefficient	Std. Error of the Estimate	t	p
$\widehat{\beta_0}$	-13.561	0.383	-35.391	0.000
$\widehat{\beta_1}$	0.114	0.014	8.345	0.000
$\widehat{\beta_2}$	-0.002	0.026	-0.070	0.944
$\widehat{\beta_3}$	0.005	0.006	0.758	0.451
$\widehat{\beta_4}$	0.003	0.006	0.532	0.596
$\widehat{\beta_5}$	0.184	0.001	128.185	0.000

Table 3. The relationship between the independent variables of the multiple regression equation obtained by the Least Squares Method (LSM), Variance Increasing Factor (VIF) and Tolerance Value (TV).

Correlation Matrix							
Variables	Egg Weight	Egg Width	Egg Length	Shape Index	Haugh Unit	TV	VIF
Egg Weight	1					0.099	11.210
Egg Width	0.757**	1				0.095	11.548
Egg Length	0.232*	0.098	1			0.039	25.84
Shape Index	-0.122	0.200	-0.918**	1		0.033	30.30
Haugh Unit	-0.087	-0.085	-0.168	0.096	1	0.926	1.08

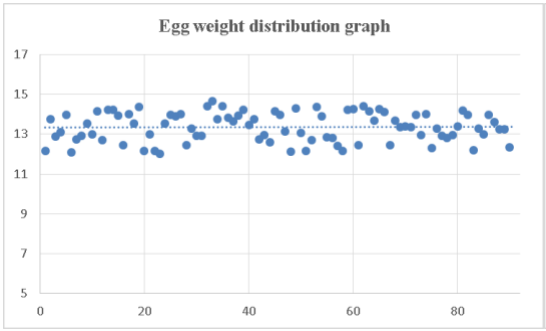


Figure 1. Egg weight distribution graph.

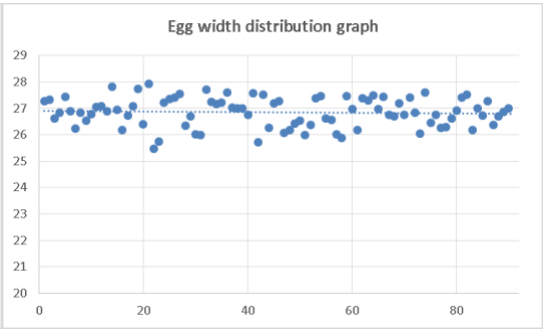


Figure 2. Egg width distribution graph.

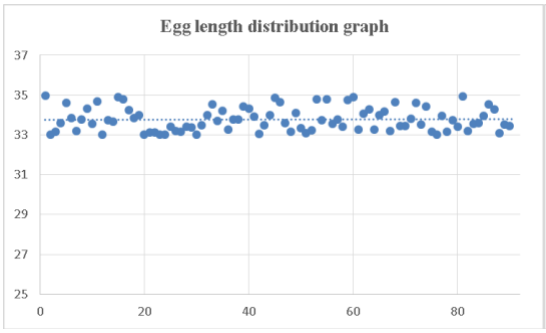


Figure 3. Egg length distribution graph.

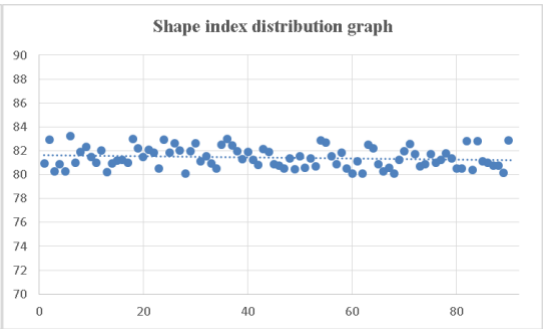


Figure 4. Shape index distribution graph.

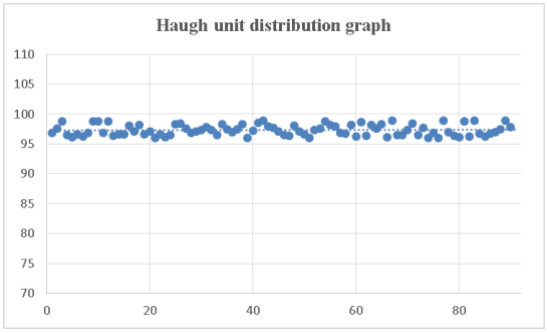


Figure 5. Haugh unit distribution graph.

When Figures 1, 2, 3, 4 and 5 were examined, it was determined that the data were linear because the independent variables in the obtained data set (egg weight, width, length, shape index and Haugh unit) moved around a certain line.

When Table 1 is examined, it is determined that all coefficients are significant in the model where the albumen index is the dependent variable, while in Table 2 only the coefficients $\widehat{\beta_0}$, $\widehat{\beta_1} ve\ \widehat{\beta_5}$ were found significant ($p<0.05$). In Table 3, the relationship between the independent variables is also examined and it is observed that the Variance Increasing Factor (VIF) value are high. While the Variance Increasing Factor (VIF) value of the shape index, egg length and width values are high, it is found that the Tolerance Value (TV) of these variables are low. The high Variance Increasing Factor (VIF) value (VIF≥10) indicate the presence of a multicollinearity problem in the established estimated model. In order to determine this problem precisely;

VIF: $C_{ij}: \frac{1}{1-R_{ij}}$ (11)

with the help of the formula,

The albumen index is the dependent variable VIF: $C_{ij} = \frac{1}{1-0.94} = \frac{1}{0.06} = 16.66$ (12)

The albumen height is the dependent variable VIF: $C_{ij} = \frac{1}{1-0.99} = \frac{1}{0.01} = 100$ (13)

calculated.

In the estimated models where the albumen index and height are dependent variables, the presence of a multicollinearity problem has been determined since the condition (C_{ij}≥10) is met. In order to determine the

appropriate lag length to eliminate this problem, the data set was first tested for stationarity with the help of Augmented Dickey-Fuller (ADF) unit root test using the Eviews package program. Then, the appropriate lag number was determined with the Vector Auto Regression (VAR) model in the estimated models. The results obtained in this process are given in Tables 4, 5 and 6 respectively.

Table 4. Augmented Dickey-Fuller (ADF) unit root test results.

	Critical Values			Level	
ADF- t statistics	% 1	% 5	% 10	t- statistics	Probability (p)
Egg Albumen Index	-3.505	-2.894	-2.584	-8.981	0.000
Egg Albumen Height	-3.505	-2.894	-2.584	-9.188	0.000
Egg Weight	-3.505	-2.894	-2.584	-8.997	0.000
Egg Width	-3.505	-2.894	-2.584	-10.359	0.000
Egg Length	-3.505	-2.894	-2.584	-9.539	0.000
Shape Index	-3.505	-2.894	-2.584	-9.702	0.000
Haugh Unit	-3.505	-2.894	-2.584	-8.992	0.000

Table 5. Vector Auto Regression (VAR) model lag length (where the albumen index is the dependent variable) result.

Lag	LogL	LR	FPE	AIC	SC	HQ
0	-846.21	NA	94.43	21.57	21.75	21.64
1	-828.01	33.19	148.57	22.02	23.28	22.53
2	-809.66	30.65	235.88	22.47	24.81	23.40
3	-794.76	22.62	418.68	23.00	26.42	24.37
4	-779.44	20.94	763.59	23.53	28.02	25.33
5	-758.37	25.60	1271.27	23.90	29.48	26.14
6	-734.99	24.86	2155.26	24.22	30.88	26.89
7	-683.85	46.60	2015.49	23.84	31.58	26.94
8	-628.85	41.77	1994.45	23.36	32.18	26.89
9	-546.72	49.90	1252.13	22.19	32.09	26.16
10	-451.32	43.47	817.64	20.69	31.66	25.08
11	-270.15	55.04	122.03	17.01	29.07	21.84

LR: sequential modified LR test statistic (each test at 5% level) FPE: Final prediction error,
AIC: Akaike information criterion, SC: Schwarz information criterion, HQ: Hannan-Quinn information criterion.

Table 6. Vector Auto Regression (VAR) model lag length (where the albumen height is the dependent variable) result.

Lag	LogL	LR	FPE	AIC	SC	HQ
0	-645.83	NA	0.59	16.50	16.68	16.57
1	-630.14	28.60	0.99	17.01	18.27	17.52
2	-617.99	20.29	1.84	17.62	19.95	18.55
3	-605.02	19.70	3.43	18.20	21.62	19.57
4	-588.05	23.19	6.00	18.68	23.18	20.48
5	-555.87	39.10	7.54	18.78	24.36	21.01
6	-527.64	30.01	11.31	18.97	25.63	21.64
7	-496.58	28.30	17.59	19.10	26.84	22.20
8	-419.63	58.44	9.98	18.06	26.88	21.59
9	-332.49	52.95	5.52	16.77	26.66	20.73
10	-265.27	30.63	7.36	15.98	26.95	20.37
11	-140.36	37.94	4.56	13.73	25.78	18.56

LR: sequential modified LR test statistic (each test at 5% level) FPE: Final prediction error,
AIC: Akaike information criterion, SC: Schwarz information criterion, HQ: Hannan-Quinn information criterion

When Tables 4, 5 and 6 are examined, it is determined that there is a maximum of 11 lag lengths suitable for the data set and the series are stationary at a high level. This situation shows that the effect of egg external quality characteristics on albumen index and height is zero starting from the 11th lag length in the established estimated model. In addition, since the minimum values are taken as the zeroth lag length in terms of all comparison criteria, the appropriate lag length is taken as zero in the Variance Increasing Factor (VIF) value calculation and the Principal Components Regression (PCR) method in the literature is used in order to eliminate the multicollinearity problem that emerged in the model. In the multiple regression equation obtained with the Principal Components Regression (PCR) method, the linear relationship between egg albumen index and height and external quality characteristics was re-examined and determined as 86 % and 88 %, respectively. It also revealed that the Ho

hypothesis was rejected and the regression relationships were statistically significant (P<0.05), and parameter estimates, significance levels, Variance Increasing Factor (VIF) and Tolerance Value(TV) are given in Tables 7 and 8.

Table 7. Parameter estimates, Variance Increasing Factor (VIF) and Tolerance Values (TV) of the multiple regression equation obtained by the Principal Components Regression (PCR) method (where the albumen index is the dependent variable).

	Regression Coefficient	Std. Error of the Estimate	VIF	TV
$\widehat{\beta_0}$	-19.95	-	-	-
$\widehat{\beta_1}$	-0.02	0.11	2.12	0.18
$\widehat{\beta_2}$	-0.22	0.13	2.58	0.09
$\widehat{\beta_3}$	-0.03	0.01	1.46	0.038
$\widehat{\beta_4}$	0.01	0.009	0.77	0.033
$\widehat{\beta_5}$	0.43	0.01	1.44	0.92

Table 8. Parameter estimates, Variance Increasing Factor (VIF) and Tolerance Values (TV) of the multiple regression equation obtained by the Principal Components Regression (PCR) method (where the albumen height is the dependent variable).

	Regression Coefficient	Std. Error of the Estimate	VIF	TV
$\widehat{\beta_0}$	-13.21	-	-	-
$\widehat{\beta_1}$	0.09	0.008	2	0.19
$\widehat{\beta_2}$	0.03	0.01	2	0.22
$\widehat{\beta_3}$	-0.004	0.001	1.03	0.08
$\widehat{\beta_4}$	-0.005	0.0007	0.03	0.02
$\widehat{\beta_5}$	0.18	0.001	1.01	0.99

When Tables 7 and 8 are examined, it is observed that there are significant differences between the parameters obtained from the Principal Components Regression (PCR) method and the Least Squares Method (LSM). Principal Components Regression (PCR) method significantly reduces the standard errors by limiting the size of the parameters thanks to the penalty term added to the parameters. This increases the estimation accuracy of the model and provides more reliable results. In the Least Squares Method (LSM), problems such as multicollinearity and overfitting can lead to an increase in the standard errors of the parameters. However, Principal Components Regression (PCR) method effectively eliminates such problems and provides a more robust and reliable model. As a result, Principal Components Regression (PCR) method has reduced the standard errors of the parameters and provided more accurate and reliable regression models. The estimated models are given below, respectively.

$$\hat{Y} = -\widehat{19.95} - \widehat{0.02}X_1 - \widehat{0.22}X_2 - \widehat{0.03}X_3 + \widehat{0.01}X_4 + \widehat{0.43}X_5 + \hat{e} \quad (14)$$

$$\hat{T} = -\widehat{13.21} + \widehat{0.09}Z_1 + \widehat{0.03}Z_2 - \widehat{0.004}Z_3 - \widehat{0.005}Z_4 + \widehat{0.18}Z_5 + \hat{e} \quad (15)$$

In Table 1, in the regression analysis results obtained with the Least Squares Method (LSM) (where the albumen index is the dependent variable), the regression parameters of the entire estimation equation were found to be significant, while in Tables and 2 (where the albumen height is the dependent variable), the regression parameters (except for $\widehat{\beta_0}, \widehat{\beta_1} ve\ \widehat{\beta_5}$) were insignificant (P<0.05). In addition, the standard error of the $\widehat{\beta_0}$ parameter was found to be higher than the others, it is in accordance with the studies of Aktan (2004) and Akçay and Sarıözkan (2015).

When the relationship between independent variables is examined in Table 3, it was determined that there was a relationship between egg weight and egg width (75 %) and between egg length and shape index (91 %). These results are similar to the reports of Kul and Şeker (2004), Alkan et al., (2010), Gök ve ark., (2023).

In order to solve the multicollinearity problem detected in the estimation models in Tables 4, 5 and 6, the Principal Components Regression (PCR) method, which is frequently used in the literature, was preferred. In the Principal Components Regression (PCR) method, the lag length was determined as zero in the Variance Increasing Factor (VIF) calculations in accordance with the literature recommendations. This application is compatible with the findings obtained in the studies conducted by Gök et al., (2022), Gök and Şahin (2023), Gök and Şahin (2023), Gök and Şahin (2024) Gök and Şahin (2024).

In the Principal Components Regression (PCR) method performed to solve the multicollinearity problem in Tables 7 and 8, lower parameter, Variance Increasing Factor (VIF; 7.14, 8.33) and Tolerance Values (TV) were obtained compared to the Least Squares Method (LSM). These findings are; Rathert et al. (2011), Üçkardeş et al.

(2012), Yalçınöz and Şahin (2020), Demir et al., (2021), Tolun et al. (2023), Yavuz et al. (2023), Çetenak et al. (2024) and Tolun et al. (2024) are equivalent to the results in the studies.

CONCLUSION

In poultry farming, accurate prediction of egg yield and quality is of critical importance for the success of the research. In particular, the presence of multicollinearity problem in the prediction of variables such as albumen index and height, which are among the internal egg quality parameters, may limit the effectiveness of traditional Least Squares Method (LSM). In this case, the use of alternative methods such as Principal Components Regression (PCR) method stands out as an important approach that increases the accuracy of the model. Principal Components Regression (PCR) method minimizes the prediction errors that occur due to high correlation, providing more consistent and accurate results. This allows for a more reliable assessment of egg quality. Therefore, this study shows that Principal Components Regression (PCR) method can be a more effective tool in poultry breeding and selection studies and highlights the critical points to be considered in model selection. Such innovative models not only increase productivity, but also help analyze quality more objectively and accurately.

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Sustainable agricultural practices: investigating the impact of microalgae use on green plant (Lettuce, Basil, Mint, Green Tatsoi and Chervil) development in soilless agriculture

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Abstract

The increasing global population and environmental degradation make agricultural sustainability essential. Soilless farming and microalgae usage are innovative and environmentally friendly approaches in this field. Soilless farming grows plants using water and nutrient solutions, reducing water consumption while enhancing yield and quality. Microalgae serve as biological fertilizers, decreasing the need for chemical fertilizers and minimizing environmental impact. This study evaluates the potential of organic fertilizers in soilless farming by examining the effects of different microalgae concentrations on plant size and leaf development. Today, combating climate change and promoting sustainable agricultural practices are becoming increasingly important. In this context, zero-waste approaches and the use of organic materials as plant nutrients contribute to environmental sustainability and offer an effective strategy in the fight against climate change. The research findings indicate a significant increase in growth (45% and 30%) and leaf development (35% and 30%) in lettuce *(Lactuca sativa)* and basil *(Ocimum basilicum)* seedlings. However, the effects were more limited in mint *(Mentha spicata)* and green tatsoi *(Brassica rapa* subsp. *Narinosa)* seedlings. A slight growth increase (22%) was observed in chervil *(Anthriscus cerefolium)* seedlings. These results highlight the varying responses of different plant species to microalgae applications and emphasize the importance of determining the optimal concentration for plant species to maximize benefits.

Keywords: Soilles farming, Microalgae, *Chlorella vulgaris*, Sustainability, Biological fertilize

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INTRODUCTION

The rapid increase in the world population, especially in the 21st century, has brought significant challenges in agricultural production and food security. It is projected that the global population will reach 9.1 billion by 2050, which increases the pressure on existing agricultural systems. Traditional farming methods threaten environmental sustainability due to the intensive use of chemical fertilizers and pesticides, leading to long-term soil degradation. Meeting the growing demand for food while adopting sustainable agricultural practices has become one of the greatest challenges of our time. In this context, alternative methods such as soilless agriculture provide a significant opportunity to reduce environmental impacts and enhance agricultural productivity (Alexandratos & Bruinsma, 2012).

The rapid growth of the global population and the impact of economic development are expected to increase food demand by 70% to 100% by 2050 (Fukase & Martin, 2020). This raises serious concerns about whether existing resources will be sufficient to meet future food needs. The scarcity of natural resources, particularly land and water, is one of the main factors threatening the sustainability of agricultural production. In this context, water scarcity has a direct impact on agricultural production and poses a significant threat to food security (Yuan et al., 2024).

Therefore, it is essential to quickly implement policies and actions that mitigate the effects of climate change. This includes the development of crop varieties resistant to sudden changes in temperature and rainfall, as well as the adoption of biological and sustainable agricultural methods. The use of biofertilizers and biopesticides can reduce dependence on chemical fertilizers and pesticides, lowering greenhouse gas emissions and reducing environmental pollution. Chemical fertilizers play a vital role in sustaining soil fertility and boosting crop yields in modern intensive farming However, their large-scale production and continuous application have led to several significant issues (Shahid et al., 2024). Long-term reliance on chemical fertilizers not only degrades soil quality but also triggers various environmental problems, such as nutrient runoff, pollution of surface and groundwater, eutrophication, greenhouse gas emissions, and the decline of aquatic biodiversity (Chandini et al., 2019; Zhang et al., 2024). Consequently, adopting organic and biofertilizers has become essential to mitigate the environmental challenges linked to chemical fertilizer use in agriculture.Additionally, the resilience of crops can be enhanced by employing different planting techniques and crop types (Furtak & Wolińska, 2023).

Soilless agriculture is defined as an innovative production technique that meets the nutritional needs of plants without relying on soil, utilizing water and nutrient solutions. This system directly supplies nutrients and water to the plant root zone, ensuring the optimal uptake of all necessary elements. One of the most significant advantages of soilless agriculture is its ability to reduce the risk of pest and disease spread while improving the efficiency of water and nutrient use. Additionally, these systems save both land and water, creating a more sustainable agricultural model (Savvas et al., 2013).

Soilless agriculture offers numerous benefits not only for environmental sustainability but also in terms of economic advantages and product quality (Ayele et al., 2025; Mir et al., 2022). Particularly in regions with limited water resources, this method reduces production costs by conserving water. Additionally, soilless farming systems enable year-round production, minimizing the adverse effects of climate change (Yavuz et al., 2023). The controlled environmental conditions provided by these systems—such as optimal temperature, humidity, and light levels—allow for higher yield and quality (Fussy & Papenbrock, 2022).

The most common applications include hydroponic, aquaponic, and aeroponic systems (Shrouf, 2017). The advantages of soilless farming include reduced water and fertilizer usage, lower disease risk, increased productivity, and prevention of soil fatigue. However, these systems also have disadvantages, such as high initial costs, the need for skilled labor, and the requirement for continuous monitoring. Soilless farming offers an important alternative for sustainable food production, particularly in regions facing water and soil scarcity, and can play a key role in shaping agricultural production in the future.

The use of microalgae in soilless farming systems presents a sustainable approach. Utilizing microalgae as organic fertilizers can reduce the reliance on chemical fertilizers, thereby minimizing environmental impact (Renganathan et al., 2024). Microalgae support plant growth by producing plant growth-promoting hormones and biostimulants, enhancing both development and productivity (Parmar et al., 2023). Furthermore, they optimize nutrient cycling, ensuring more efficient uptake of essential nutrients by plants. Certain microalgae species enhance soil organic carbon levels by capturing atmospheric carbon dioxide (CO_2) and utilizing it through photosynthesis to produce nutrient-rich biomass. This process helps mitigate the greenhouse effect, global warming, and climate change. Additionally, microalgal biomass can sequester other greenhouse gases, such as nitrous oxide, further contributing to climate change mitigation (Zhang et al., 2024).

In recent years, regulatory frameworks have increasingly emphasized sustainability in agricultural systems, including soilless cultivation methods. The European Union, through its Green Deal and Farm to Fork Strategy, encourages innovative practices that reduce environmental impacts and enhance resource efficiency. Similarly, in the United States, the USDA and FDA have introduced guidelines addressing sustainable practices in controlled-environment agriculture. Although regulatory approaches vary across regions, they commonly aim to ensure food safety, environmental protection, and economic viability. Considering legal aspects, even at a basic level, can support the alignment of research and practice with broader policy objectives (Boros et al., 2025; Steines et al., 2024). Interest in sustainable agricultural practices has been increasing in Turkey, with soilless cultivation gaining particular attention. The Ministry of Agriculture and Forestry promotes sustainability through regulations such as the "Good Agricultural Practices" and "Organic Agriculture" directives, which aim to reduce environmental impact and ensure efficient resource use. Although there is currently no comprehensive and up-to-date legislation specifically focused on soilless agriculture, related frameworks—such as controlled environment agriculture, plant nutrition regulations, and production permits—form the basis of the legal landscape in this field. In line with Turkey's 2023 Agricultural Vision and its commitment to sustainable development goals, strengthening the legal support for soilless farming offers significant environmental and economic opportunities. Therefore, incorporating legal perspectives into future research may facilitate better alignment with policy development and implementation.

This study aims to examine the effects of using *Chlorella vulgaris* microalgae as organic fertilizers in soilless farming systems on plant growth. Experiments conducted on various green plant species such as lettuce, mint, and basil will evaluate the impact of these materials on plant development. The study seeks to contribute to the advancement of sustainable agricultural practices and the widespread adoption of soilless farming systems. By

offering sustainable and efficient solutions for agricultural production in the future, the research aims to enhance food security. The use of microalgae as organic fertilizers can enable more efficient utilization of natural resources, providing a long-term solution for agricultural production.

MATERIAL ve METHODS

In this study, pure cultures of *Chlorella vulgaris* was used. The algal cultures were sourced from the Hacettepe University laboratory. To cultivate the algae, BBM (Bold's Basal Medium) culture medium was prepared using laboratory-grade chemicals. BBM contains precise concentrations of the following compounds: $NaNO_3$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, K_2HPO_4, NaCl, Na_2EDTA, KOH, $FeSO_4 \cdot 7H_2O$, H_3BO_3, $ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, MoO_3, $CuSO_4 \cdot 5H_2O$, and $Ca(NO_3)_2 \cdot 6H_2O$. During inoculation, an algae-to-medium ratio of 1:10 was maintained. The cultivation of algal cultures was carried out in the ESCO AC2-4E8 Biological Safety Cabinet within the laboratory. Freshly cultivated cultures were then transferred to an orbital shaker inside an air-conditioned cabinet, where they were exposed to LED panel modules emitting red and blue light optimized for algal growth. For optimal growth, a Photosynthetic Photon Flux Density (PPFD) value nearly 700 µmol/m²/s has been utilized, providing the ideal light intensity to support photosynthetic activity and rapid growth in fast-growing algae species. The cultivation conditions were set at 120 rpm, a temperature of 28°C and 45% humidity (Poleko KK 240 INOX climate chamber and Infors/HT orbital shaker system).

In the study, two different growing media, both suitable for soilless agriculture and easily accessible, were selected: perlite, an inorganic material, and cocopeat, an organic material. Perlite is notable for its lightweight structure and excellent drainage properties, while coco peat stands out for its high water retention capacity. The solid growing medium used in the study consisted of a mixture of 80% cocopeat and 20% perlite. Coco peat is a natural material derived from the processing of coconut husks and has a remarkably high water retention capacity, capable of absorbing 8–9 times its weight in water. Due to this property, it is widely used in soilless agriculture. Its natural pH level allows plants to easily absorb essential nutrients, positively influencing plant growth. Perlite, on the other hand, offers excellent drainage and aeration properties, enhancing oxygen uptake by the roots while ensuring the efficient utilization of water and nutrients (Fazlil Ilahi & Ahmad, 2017; Mohammadi Ghehsareh et al., 2011).

The growth of lettuce *(Lactuca sativa)*, basil *(Ocimum basilicum)*, mint *(Mentha spicata)*, chervil *(Anthriscus cerefolium)* and green tatsoi *(Brassica rapa* subsp. *Narinosa)* plants in different growing media using soilless agriculture methods was examined. The seedlings were transferred to hydroponic medium from beginning November to ending of December 2024, nearly 60 days. An experimental design with 2 replicates and 5 plants per replicate was used in each solid aggregat medium. 15 ml of irrigation water was provided per day. As a nutrient supplement, microalgae-based biofertilizer feeding was applied to the seedlings once a week at different doses (0.2, 0.3, 0.5 and 0.75 mg mL^{-1}).

(1) Control: Fresh media + Plant
(2) 0.2 mg mL^{-1} microalgae + Media + Plant
(3) 0.5 mg mL^{-1} microalgae + Media + Plant
(4) 0.75 mg mL^{-1} microalgae + Media + Plant

One plant from each replicate was reserved as a control, and it was only watered (Figure 1). In this way, the effects of different media on plant development were compared, and the potential benefits of microalgae supplementation were examined.



Figure 1. Soilless farming system

RESULTS AND DISCUSSION

The effect of microalgae supplementation varies depending on the plant species and the applied dose. The minimum, maximum, and average measured seedling heights are shown in the Figure 2. In lettuce seedlings, a 0.3 mg mL^{-1} microalgae resulted in the highest growth, allowing the seedlings to reach a height of 11 cm. In contrast, seedlings receiving 0,75 mg mL^{-1} ml of microalgae remained shorter at 10.5 cm, while those in the control group measured 7.8 cm. These data indicate that the optimal microalgae dose for lettuce seedlings is 0,3 mg mL^{-1}.

For basil seedlings, the most effective result was observed with a 0.5 mg mL^{-1} microalgae supplement, leading to a height of 17 cm. Seedlings given 5 ml remained the shortest at 13 cm, while those in the control group measured 11.9 cm. These findings suggest that the optimal microalgae dose for basil seedlings is 0.3 mg mL^{-1}.

In mint seedlings, the effect of microalgae supplementation was more limited. Seedlings receiving 0.2mgmL^{-1} of microalgae reached the highest height at 19 cm, while those in the control group measured 17 cm. These results suggest that microalgae application has a minimal effect on mint seedlings.

For green tatsoi seedlings, microalgae supplementation was found to be ineffective. The applied microalgae concentrations did not produce a significant growth difference compared to the control group. This indicates that microalgae application does not result in significant growth for green tatsoi seedlings.

In chervil seedlings, a 0.5 mg mL^{-1} microalgae supplement resulted in the highest growth, with seedlings reaching 4.9 cm. Seedlings receiving 0.2 mg mL^{-1} remained the shortest at 4.2 cm, while those in the control group measured 4.4 cm. These findings suggest that the optimal microalgae dose for chervil seedlings is 0.5 mg mL^{-1}.

While significant growth increases were observed in lettuce and basil seedlings, the effects were more limited in mint and green tatsoi seedlings. In chervil seedlings, a slight growth increase was observed. These findings indicate that the effect of microalgae supplementation on plant growth depends on the plant species and the determination of the appropriate dose.

Furthermore, the results suggest that while microalgae can act as an effective biofertilizer for certain plant species, its impact is not universal. The varying responses among different seedlings highlight the importance of species-specific optimization when using microalgae as a growth enhancer. Additionally, excessive microalgae supplementation may not always lead to greater growth, as seen in the lettuce seedlings where higher doses resulted in reduced height. This emphasizes the need for careful dose selection to maximize the benefits of microalgae application.

Overall, these findings contribute to a better understanding of how microalgae supplements can be integrated into plant cultivation strategies. Further studies exploring the long-term effects, nutrient uptake mechanisms, and interactions with soil microbiota could provide deeper insights into the potential of microalgae in sustainable agriculture.

The species- and dose-dependent effects of microalgae supplementation on plant growth can be attributed to several physiological and biochemical factors. Microalgae such as *Chlorella vulgaris* and *Scenedesmus sp.* are known to be rich in proteins, amino acids, phytohormones (e.g., indole-3-acetic acid [IAA]), vitamins, and micronutrients, all of which can promote plant growth (Renuka et al., 2013; Win et al., 2018). However, the degree to which plants can absorb and utilize these compounds varies among species, depending on their specific nutrient uptake mechanisms, root architecture, and growth stage (Ronga et al., 2019). In lettuce and basil seedlings, where significant growth improvements were observed, the applied doses of microalgae may have matched the optimal nutritional needs of these plants, particularly in terms of phytohormonal stimulation and nitrogen supplementation (Kumar et al., 2022). On the other hand, in mint and green tatsoi seedlings, the limited or negligible response could indicate either a lower sensitivity to the bioactive compounds in microalgae or possible nutrient saturation, where additional inputs do not translate into further growth (Sharma et al., 2014). Furthermore, the observation that higher doses (e.g., 0.75 mg mL^{-1} in lettuce) resulted in reduced growth suggests that excessive microalgae supplementation may lead to nutrient imbalances or osmotic stress, which can inhibit seedling development (Fiorentino et al., 2025). This aligns with findings from previous studies that emphasized the importance of dose optimization when using microalgae-based biofertilizers. The slight increase observed in chervil also underscores that not all species respond equally to biofertilizer input, and that certain species may require a more tailored approach. These species-specific responses highlight the importance of optimizing both the type and concentration of microalgae supplements to maximize their benefits.

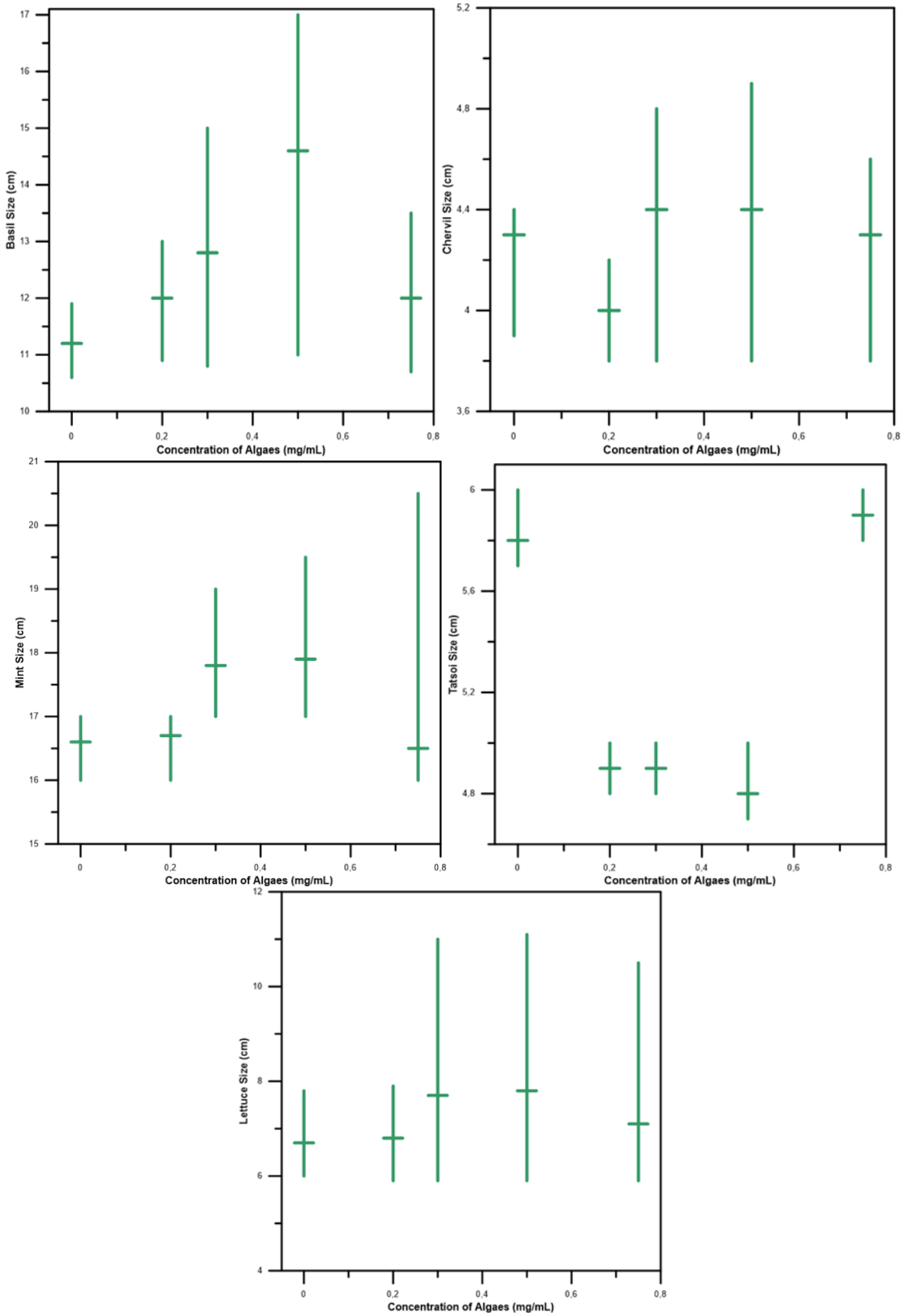


Figure 2. Effect of different concentrations of algal density on green plants height (Basil, chervil, mint, lettuce, tatsoi)

The effect of microalgae application on the number of leaves also varies depending on the plant species and the applied concentration (Figure 3). In experiments conducted on lettuce plants, a 0.3 mg mL^{-1} microalgae application resulted in the highest leaf increase, leading to the formation of 14 leaves per seedling. In contrast, seedlings treated with 0.75 mg mL^{-1} of microalgae had 10 leaves, while those in the control group had 9 leaves. These findings indicate that a 0.3 mg mL^{-1} microalgae application is the most effective dose for lettuce, whereas 0.75 mg mL^{-1} does not provide a significant difference compared to the control group.

In studies on basil plants, a 0.5 mg mL^{-1} microalgae application led to the highest increase, with seedlings developing 39 leaves. Seedlings treated with 0.3 mg mL^{-1} had 37 leaves, while the lowest leaf count was recorded at 0.2 and 0.75 mg mL^{-1} concentrations. The control group had 23 leaves. These results suggest that 0.5 mg mL^{-1} is the optimal microalgae dose for basil plants.

For mint plants, seedlings treated with 0.2 mg mL^{-1} of microalgae developed 10 leaves, while those in the control group had 8 leaves. These findings indicate that while microalgae application does not cause a significant increase in leaf production for mint plants, it has a slight positive effect.

Experiments on green tatsoi plants showed that microalgae concentrations did not lead to a significant leaf increase compared to the control group. This suggests that microalgae application does not have a meaningful impact on green tatsoi plants therefore, it has not been provided in the graphs.

In chervil plants, a 0.3 mg mL^{-1} microalgae application resulted in the highest leaf increase, while the lowest leaf count was observed in seedlings treated with 0.2 mg mL^{-1} of microalgae. These findings suggest that while microalgae supplementation provides a slight benefit for chervil plants, the most effective concentration is 0.3 mg mL^{-1}.

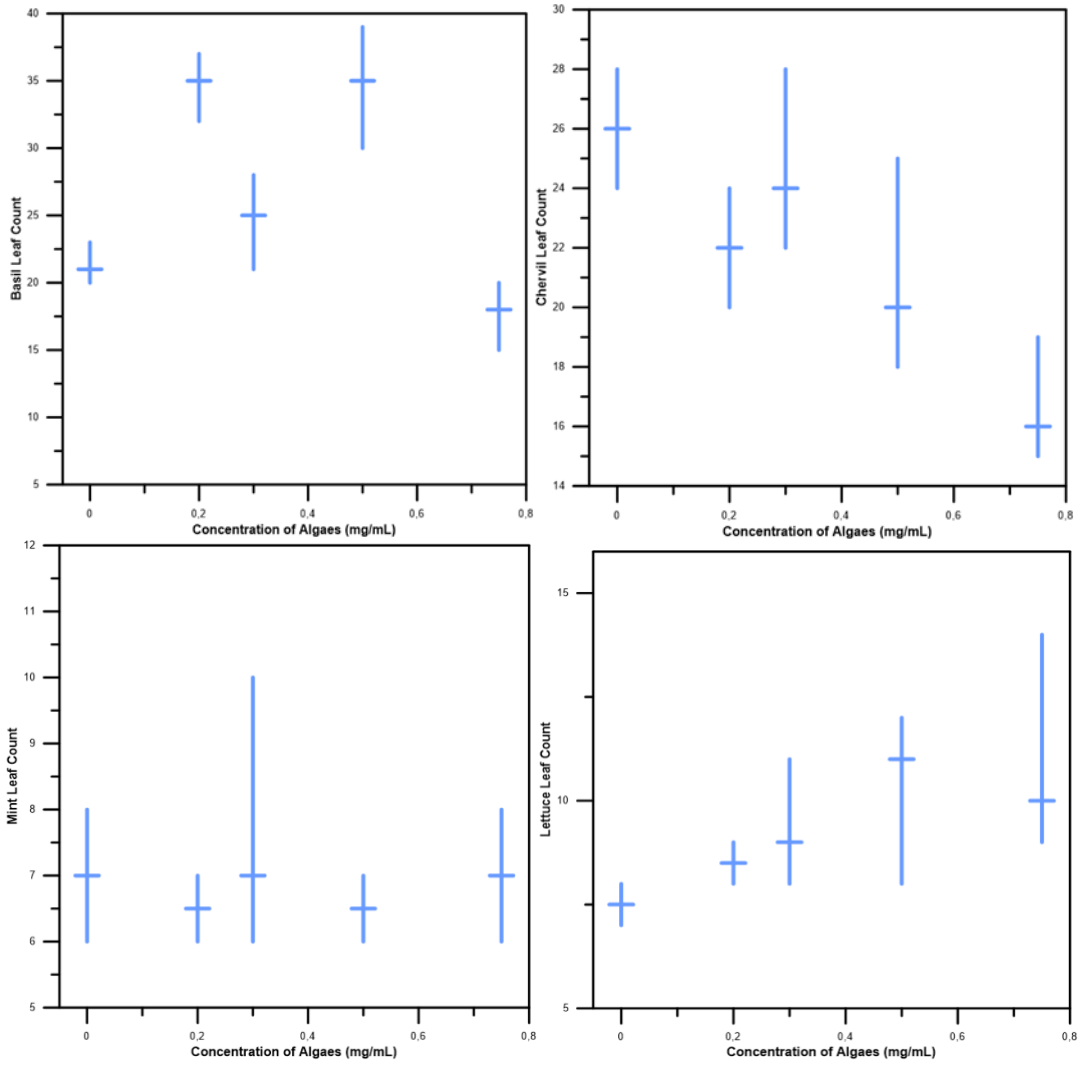


Figure 3. Effect of different concentrations of algal density on green plants leafs number (Basil, chervil, mint, lettuce)

The differences observed in leaf production in response to microalgae application could be due to several factors (Gonçalves et al., 2023). For example, the varying effects of microalgae on different plant species might be influenced by species-specific nutrient requirements, growth habits, or photosynthetic efficiency (Salah et al., 2023). Lettuce and basil, which showed significant increases in leaf count, may be more responsive to the added nutrients from the microalgae or have a higher photosynthetic capacity, allowing them to utilize the applied microalgae more effectively. On the other hand, mint and green tatsoi, which showed minimal response, may have different nutrient uptake mechanisms, or their growth may be more influenced by other environmental factors such as light intensity or soil composition, which were not controlled in the experiment. These differences highlight the need for a more thorough investigation into the physiological and environmental factors that govern plant responses to microalgae applications (Melo et al., 2022).

CONCLUSION

This research evaluates the potential of organic fertilizers in soilless agriculture by examining the effects of different microalgae concentrations on plant size and leaf development. Today, combating climate change and promoting sustainable agricultural practices are becoming increasingly important. In this context, zero-waste approaches and the use of organic matter as plant nutrients contribute to environmental sustainability and provide an effective strategy in the fight against climate change. Soilless agriculture, which includes techniques such as hydroponics and aeroponics, is gaining significant attention as a viable solution to conventional farming methods. This is due to its potential to conserve water, reduce land use, and promote more efficient nutrient uptake by plants. Today, combating climate change and promoting sustainable agricultural practices are becoming increasingly important as the global population continues to rise, placing additional pressure on agricultural systems. In this context, zero-waste approaches, such as recycling organic waste into microalgae biomass, and the use of organic matter as plant nutrients, contribute significantly to environmental sustainability. By utilizing microalgae as biofertilizers, we can reduce reliance on synthetic fertilizers, which often have detrimental environmental impacts (Nur et al., 2025). Moreover, these organic fertilizers provide essential nutrients to plants, improving their growth potential while minimizing soil degradation and water pollution, thus providing an effective strategy in the fight against climate change.

The findings of this study reveal that microalgae supplementation positively influenced lettuce and basil seedlings, resulting in notable improvements in plant height and leaf development. These results are consistent with prior studies that report enhanced root and shoot biomass, chlorophyll content, and nutrient uptake in leafy greens treated with microalgae-based fertilizers (Spolaore et al., 2006). In contrast, mint and green tatsoi seedlings demonstrated a more limited response, which may be attributed to species-specific physiological traits, root morphology, or differing nutrient demands (González et al., 2022). A slight increase in growth was also observed in parsley seedlings, suggesting a moderate responsiveness to microalgae supplementation.

These varied responses underscore the importance of optimizing both the dosage and the formulation of microalgae biofertilizers according to plant species. Over-application can lead to nutrient imbalances or osmotic stress, while under-application may result in suboptimal growth effects (Pekkoh et al., 2024). Therefore, species-specific trials are essential to develop effective guidelines for microalgae use in soilless systems.

In summary, this research highlights the dual environmental and agronomic benefits of integrating microalgae into sustainable soilless agriculture. It emphasizes the need for targeted application strategies based on plant-specific responses, ultimately contributing to resource-efficient and climate-smart farming systems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

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

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Effect of organic and inorganic nutrient sources on yield attributes of African marigold

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Abstract

Marigold holds significant cultural and religious importance, making them widely cultivated flowers in Nepal. However, its yield remains comparatively low due to the loss of nutrients through leaching and volatilization, which diminishes nutrient availability and soil fertility. This study aimed to identify an appropriate nutrient source for higher flower yield in marigolds while ensuring sustainable soil health by providing essential nutrients to the soil. For this, a field experiment was conducted in Biratnagar, Morang, Nepal in a Randomized Complete Block Design (RCBD) with four replications and five treatments. The treatments include Recommended Dose of Fertilizers (T1), 75% Recommended Dose of Fertilizers (T2), 125% Recommended Dose of Fertilizers (T3), Poultry Manure (T4) and Vermicompost (T5). Vermicompost (T5) demonstrated superior performance, with the tallest plants (19.25 cm at 24 DAT, and 58.48 cm at 69 DAT), the highest fresh flower weight (7.4 g), and maximum flower yield (12.54 t ha^{-1}). Similarly, T2 (75% RDF) resulted in significantly taller plants (52.88 cm) at 54 DAT. T3 (125% RDF) promoted highest number of branches (17.53) at 54 DAT. T1 had the thinnest stem diameter at 24 DAT, whereas other treatments were on par with each other. No significant differences were observed at days to first flowering. Vermicompost was observed as the most effective nutrient source, which significantly improved the growth and yield of marigold when compared to poultry manure and chemical fertilizers. Hence, the farmers in Biratnagar Metropolitan City are recommended to use vermicompost for higher and sustainable yield, along with sustainable soil health

Keywords: Marigold, Fertilizers, Organic nutrients, Growth, Yield

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INTRODUCTION

Marigold is a plant that has been grown for a long time. It is loved not just for its bright flower color, but also for its diverse uses in different areas. It is mainly used for decorative purposes and is popular in gardens and landscapes. Marigold plants are not only important for their ornamental value, but also for their applications in medicine, industry, and agriculture. They are used in traditional medicine for their anti-inflammatory, antimicrobial, and wound-healing properties (Singh et al., 2004). The African marigold is a key flower in Nepal, and it is loved for its crucial role in culture and traditions. It comes in many colors, shapes, and sizes. Furthermore, its fragrance is long-lasting, and it is the reason that most people prefer it (Bhosale & Gobade, 2025). Growing marigold is easy because of their rapid growth in nature and short gestation period. They are mostly used for cut purpose all over the world (Singh et al., 2015). In South Asia, people use marigolds for making garlands, decorating places, and improving garden's view. They also use them in many religious and social events (Dixit et al., 2013). Marigold also acts as repellent for bugs, insects and mosquitoes. It is also used in chicken feed, as it helps egg yolks become yellow. People also plant them for the management of pests such as tomato bugs. In addition, oils extracted from marigold is used in perfume industries (Singh et al., 2015). Nepal's need for flowers is big and

growing, but it cannot grow enough on its own, and hence, is imported from countries such as India and China. The peak demand for marigold in Nepal is during festive seasons in Autumn, on the occasions such as Dashain and Tihar. However, due to improper timing of the plantation of this crop, most people cannot harvest flowers at the right time (i.e. during the festive season), and hence, the flower has to be imported (Dhakal & Bhattarai, 2017). Nepal imports around 150,000 marigold garlands during Tihar, spending NRs. 8,100,000 on marigold flowers (FAN, 2013). Tihar, a major festival in Nepal (widely known as Diwali in India), involves worshipping different animals over its first four days. On the fifth and final day, sisters perform rituals for their brothers to protect and bless them.

Among several genera of marigold, African marigold (*Tagetes erecta*) and French Marigold (*Tagetes patula*) are common worldwide among people (Kumar et al., 2015). The French Marigold is one of the sub-species of the African Marigold. Beyond its use in landscaping, African Marigold is also cultivated extensively for its loose flowers, which are commonly used in religious and cultural ceremonies and garland-making (Kumar et al., 2019, Patel et al., 2019). As one of the most important herbaceous annuals, French Marigold is commonly used in flower beds (Cicevan et al., 2016). The African Marigold grows upright, producing large flower heads measuring 7-12 cm, and blooms 80-90 days after planting. In contrast, the French Marigold forms compact clumps with smaller flowers, about 3-6 cm in diameter, and flowers earlier, within 30-55 days after sowing (Zhang et al., 2022).

In Nepal, traditional farming practices dominate, and the use of fertilizers is often based on inherited knowledge rather than scientific research. This leads to inconsistent yields and suboptimal plant health, which directly impacts the economic returns for farmers. There is an abundance of organic manures available in Nepal, which are cheap and effective. These not only improve soil health but also improve plant growth (Acharya et al., 2020). However, the nutrient content and release rates of organic manures can be highly variable, leading to inconsistent results. On the other hand, inorganic fertilizers help provide exact nutrients immediately to plants such as potassium, zinc, nitrogen, phosphorus, and many more nutrients for quick growth and bloom in marigold plants. However, they are expensive and unsustainable because of the number of chemical residues left behind in the land (Marschner, 2012). Fertilizers are crucial for the rapid growth and flowering of marigold plants but can be costly and may have adverse environmental impacts if not used judiciously. When organic and inorganic sources of nutrients are mixed, marigold's production increases, along with positive results in soil health (Chaupoo & Kumar, 2021). However, research related to organic manures and inorganic fertilizers is done very rarely in Nepal. This is because marigold cultivation is primarily confined to the summer and autumn seasons in Nepal. Furthermore, successful production during winter and spring has not yet been achieved due to challenges such as low temperatures, and insufficient light intensity (Dhakal et al., 2021). In addition, most of the researchers from universities, research centers, NGOs and INGOs prioritize other cereal and vegetable crops because of their importance in food security. Hence, due to these reasons, there are only a limited number of researches in the floriculture sector.

Understanding the comparative effects of organic and inorganic fertilizers on the morphological growth and yield of marigolds is crucial for farmers, agricultural advisors, and policymakers. Such knowledge can aid in developing sustainable agricultural practices that optimize crop yield and quality while preserving soil health and environmental integrity (Yadav et al., 2024). Moreover, the export potential of marigolds could be improved drastically because of their cultural and religious importance in the neighboring country, India, when proper research on marigolds is prioritized in Nepal. The rationale for this study is to provide a comprehensive comparison of the effects of organic and inorganic fertilizers on the morphological growth and yield of marigolds in Nepal. By evaluating various growth parameters and yield outcomes, this research aims to identify the most effective fertilization strategy for marigold cultivation that balances immediate productivity with long-term soil health and sustainability.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out in the farmer's field located in Rajbanshi Chowk, Biratnagar, Nepal from March to June 2024 to find out the effect of organic and inorganic nutrient sources on morphological and yield attributes of *Tagetes erecta* L. Geographically, it is located at 26°27'15" N and 87°16'47" E, where the climate is tropical with an elevation of 80 meters above sea level.

Experimental Design

Randomized Complete Block Design was used for the field experiment where five different treatments were replicated four times. The treatment details are given below in (Table 1). Calcuttia Orange variety of African marigold was used for the field trial. The individual plot was 2 m × 1.8 m. The spacing was kept at 40 cm × 30 cm with a total number of 30 plants per plot (Nain et al., 2017). 5 random plants per plot were selected and tagged for recording data. The nutrient sources for T1, T2 and T3 were chemical fertilizers with a dose of 100% RDF, 75% RDF and 125% RDF respectively. Similarly, the nutrient sources for T4 and T5 were organic and received poultry manure and vermicompost respectively. A full dose of P and K and a half dose of N were applied in T1, T2 and T3 during field preparation. The remaining half dose of N was applied as top dressing 30 days after transplanting. Soil sampling and analysis were performed, and the results are given below in (Table 2).

Table 1. Treatment details

SN	Treatments	
T_1	Recommended dose of NPK (45: 90: 75 N: P_2O_5:K_2O kg/ha)	(Tnau, 2009)
T_2	75% of the recommended dose of NPK (33.75:67.5:56.25 N: P_2O_5:K_2O kg/ha)	(Divya et al., 2017)
T_3	125% of the recommended dose of NPK (56.25:112.5:93.75 N: P_2O_5:K_2O kg/ha)	(Divya et al., 2017)
T_4	Poultry manure (3.16t/ha)	(Dikr and Belete,2017)
T_5	Vermicompost (5t/ha)	(Dikr and Belete,2017)

Table 2. Soil chemical and physical properties

Parameters	Units	Analysis result
pH		5.7
Total nitrogen	%	0.010
Available P205	ppm	165.000
Available K205	ppm	263.000
Soil organic matter	%	0.150
Textural class		Silty loam

Climatic conditions

The recorded highest temperature was 40°C in April and May, whereas the minimum temperature was recorded 15°C in March. The highest amount of rainfall was recorded during monsoon season, i.e. June and July, with records of 27 cm and 15 cm respectively. The climatic information is shown in the (Figure 1). There was no research during July, which is represented below, and hence, the climatic data for July can be neglected.

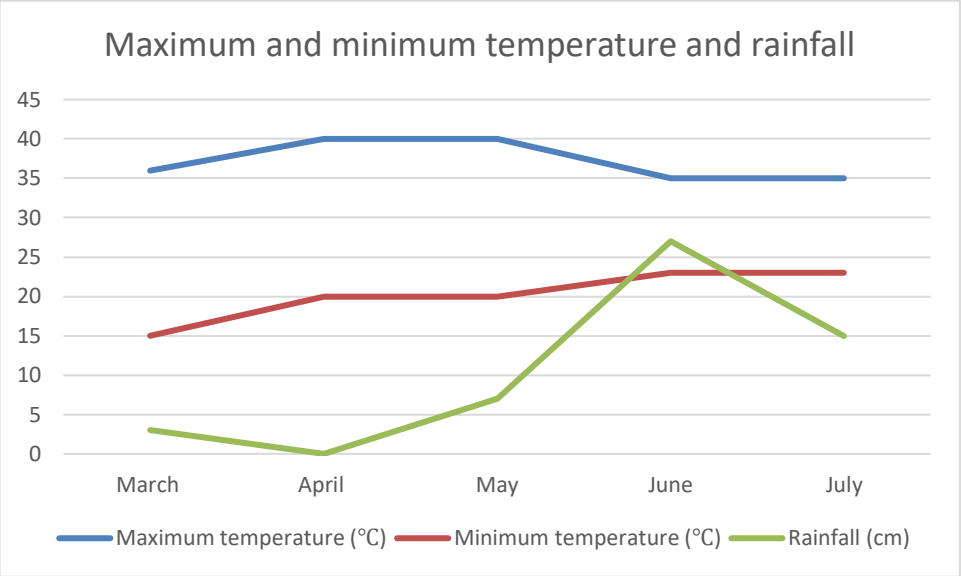


Figure 1. Maximum and minimum temperature and rainfall of the experimental site

Field preparation and irrigation

A deep plow of the land with a 4-wheel drive tractor-driven cultivator was executed after the previous crop was harvested. 2-3 harrows were done after deep ploughing. The clods were crushed and made fine tilth with a spade. The whole land was leveled properly. Stubbles, weeds and pebbles were removed clearly from the experimental plot. The plots were slightly raised to avoid water logging during monsoon. A furrow was dug around the trial plot to provide proper drainage during rainfall. Marigold plants require adequate moisture throughout their growth period. The first irrigation was performed immediately after transplanting. When the plants are small, frequent water is necessary in the root zone, and hence, irrigation was done daily based upon the weather conditions.

Statistical analysis

Data entry and processing were carried out using Microsoft Excel. Analysis of Variance (ANOVA) and mean estimation were performed using R-studio version 4.1.1. The treatment means were compared by Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

RESULTS AND DISCUSSIONS

Plant height (cm)

Plant height is a key indicator of vegetative growth and overall plant health. Significant differences were observed in plant height at 5%, 0.1% and 1% levels of significance at 24, 54 and 69 DAT. The maximum plant height at 24 days after transplanting was recorded from vermicompost application (19.25 cm) as shown in (Table 3). Similarly, at 39 DAT, plant height is non-significant. At 54 DAT the maximum plant height was seen in the plot with 75% of the recommended dose of NPK (52.88 cm). At 69 DAT, though there were not any significant differences between vermicompost and 75% of the recommended dose of NPK, pots fertilized with vermicompost had taller plants (58.48 cm).

The results of Dikr and Belete (2017) are in conformity with this research. Vermicompost made plants grow tall from the start to the end days. This is because, it helps take more nutrients such as nitrogen from the sub-soil, which lets chlorophyll accumulate in the leaves for photosynthesis. Additional studies by different researchers support this observation, emphasizing that organic amendments improve soil structure, water retention, and microbial activity, which collectively enhance plant growth (Arancon et al., 2003). Vermicompost facilitates plant growth by offering increased nutrient availability (Dikr and Belete, 2017).

Table 3. Effect of organic and inorganic nutrient sources on plant height of African marigold

Treatment	Plant height (cm)			
	24 DAT	39 DAT	54 DAT	69 DAT
Recommended dose of NPK	16.9^{c}	26.95	38.45^{b}	46.95^{b}
75% of the recommended dose of NPK	17.5^{bc}	28.45	52.88^{a}	54.25^{a}
125% of the recommended dose of NPK	18.2^{ab}	30.1	40.45^{b}	47.45^{b}
Poultry manure	18.55^{bc}	27.9	37.25^{b}	45.65^{b}
Vermicompost	19.25^{a}	30.95	40.33^{b}	58.48^{a}
F-test	*	ns	***	**
SE(m)	0.18	0.56	0.77	00.96
LSD	1.23	5.68	5.33	6.64
Grand mean	18.08	28.87	41.87	50.55

Note: ns: non-significant ***Significant at 0.1% level; **Significant at 1% level; * significant at 5% level; SE(m): Standard error of mean; Value with same letter(s) in a column are not significantly different at 5% level by DMRT.

Stem diameter

Stem diameter is crucial to plants because it helps plants withstand extreme external forces such as storms and animals. There was a significant difference in plant stem diameter at 24 DAT, with a significance level of 5%. The highest stem diameter at 24 days after transplanting (DAT) was recorded from 125% of the recommended dose of NPK as shown in (Table 4). The stem diameter was non-significant at 39 DAT, 54 DAT and 69 DAT, but most of the treatments at 24 DAT were at par. Even though the treatments were insignificant at 39 DAT, the one with a 125% NPK dose had the thickest stem. This is because of the higher nutrient availability at the initial stages of plant growth.

Dikr and Belete (2017) & Singh et al. (2015) found the same result regarding the effect on stem girth by NPK fertilizer. They recorded that the right amount of NPK made the stems wider. Furthermore, nitrogen helped the plants grow more, phosphorus helped the roots, and potassium made the plants stronger and steadier. Similarly, a proper dose of nitrogen, phosphorous and potassium to plants improve their vigor and growth, along with structural integrity (Brady & Weil, 2008).

Table 4. Effect of organic and inorganic nutrient sources on stem diameter of African marigold

Treatment	Stem diameter (mm)			
	24 DAT	39 DAT	54 DAT	69 DAT
Recommended dose of NPK	2.1^{ab}	7.25	10.24	12.74
75% of the recommended dose of NPK	4.35^{a}	7.30	10.18	12.25
125% of the recommended dose of NPK	5.24^{a}	7.95	10.16	11.75
Poultry manure	4.98^{a}	7.40	9.94	11.25
Vermicompost	4.32^{a}	7.71	9.89	12.45
F-test	*	ns	ns	ns
SE(m)	0.31	0.18	0.22	0.26
LSD	2.10	1.26	1.53	1.79
Grand mean	4.20	7.52	10.08	12.09

Note: ns: non-significant ***Significant at 0.1% level; **Significant at 1% level; * significant at 5% level; SE(m): Standard error of mean; Value with same letter(s) in a column are not significantly different at 5% level by DMRT.

Number of branches

The number of branches in a plant is very important because of its proportionality to the yield. It was non-significant at 39 DAT and 69 DAT. However, at 54 DAT, a significant difference with a significance level of 5% was observed. The plot with 125% of the recommended dose of NPK (17.53) recorded the highest number of branches. Except for this treatment, the other treatments were at par, meaning that there were not any significant differences between those treatments (Table 5).

Regarding the number of branches each plant had, the plots with 125% of NPK had more branches. Sinha & Tandon (2020) indicated that nitrogen is important for branch development, phosphorus fosters the health of roots, and potassium enhances overall plant stability. Similarly, Marschner (2012) stated the role of macronutrients in the regulations of hormones, which is essential for branch development. Hence, a higher supply of nutrients fosters the development of branches in the plants.

Table 5. Effect of organic and inorganic nutrient sources on number of branches of African marigold

Treatment	Number of branches		
	39 DAT	54 DAT	69 DAT
Recommended dose of NPK	11.50	14.05[b]	13.63
75% of the recommended dose of NPK	12.15	13.53[b]	12.87
125% of the recommended dose of NPK	13.70	17.53[a]	15.65
Poultry manure	12	13.65[b]	14.15
Vermicompost	11.80	14.40[b]	14.06
F-test	ns	*	ns
SE(m)	0.28	0.35	0.60
LSD	1.91	2.42	4.13
Grand mean	12.23	14.63	14.07

Note: ns: non-significant ***Significant at 0.1% level; **Significant at 1% level; * significant at 5% level; SE(m): Standard error of mean; Value with same letter(s) in a column are not significantly different at 5% level by DMRT.

Phenological and yield traits

There was no significant difference among treatments in the case of days to first flowering. This was also seen in the results by Singh et al. (2015) and Dikr and Belete (2017) (Table 6).

However, a significant difference at a 0.1% significance level was observed in the case of fresh-weight of flowers. The plots treated with vermicompost were seen superior in the case of both the fresh weight of flowers (7.4 grams) and the total yield (12.54 t/ha) during the cropping season. In the case of the individual weight of flowers, all other plots except for the one treated with vermicompost were on par. This result is similar to that of Singh et al. (2015). They found that plots with vermicompost had higher flower weight. Vermicompost has many key things plants need, such as nitrogen, phosphorus, potassium, and other micronutrients. It becomes efficient and convenient for plants to consume nutrients from such sources (Saranraj & Stella, 2012). Moreover, Arancon et al. (2006) demonstrated that vermicompost improves soil microorganism's population, which helps in the proper utilization of nutrients and improves plant productivity.

Similarly, there was a significant difference in flower yield at a significance level of 1% among treatments. The plots with RDF had the lowest yield, but it was also on par with the plots fertilized with poultry manure and 75% of RDF (Table 6). The highest yield was obtained from the plots treated with vermicompost. The plots with vermicompost gave out the highest yield than the ones with 125% of the advised NPK. This finding was also noted by Singh et al. (2015) and Dikr with Belete (2017). Gupta et al. (2014) indicated that vermicompost releases nutrients slow and steadily, which promotes vegetative and reproductive (floral) development. Moreover, Edwards et al. (2011) reported that vermicompost improves soil aeration and root development, which are directly linked with higher yield.

The recommended dose of NPK did not perform satisfactorily, due to leaching and volatilization. Nitrogen loss is a significant problem, which not only impacts plant's performance but also affects the environment negatively (Roosta & Schjoerring, 2007). Furthermore, though poultry manure is organic, a lower yield was observed because of nutrient imbalances and saline conditions. Though poultry manure fosters the growth of plants, its performance is limited in comparison to organic and inorganic fertilizers. In addition, the high amount of ammonia in poultry manure damages the root, which limits nutrient uptake (Pan et al., 2016).

Table 6. Effect of fertilizer on phenological characters at different days in Biratnagar, Morang, 2024

Treatment	Phenological characters		
	Days to first flowering	Fresh weight of flower (gm)	Yield(t/ha)
Recommended dose of NPK	52.25	3.20[b]	3.97[c]
75% of the recommended dose of NPK	55.75	3.13[b]	5.63[bc]
125% of the recommended dose of NPK	53.50	2.93[b]	9.12[ab]
Poultry manure	55.50	2.30[b]	6.90[bc]
Vermicompost	55	7.4[a]	12.54[a]
F-test	ns	***	**
SE(m)	0.45	0.15	0.52
LSD	3.09	1.07	3.61
Grand mean	54.40	3.79	7.62

Note: ns: non-significant ***Significant at 0.1% level; **Significant at 1% level; * significant at 5% level; SE(m): Standard error of mean; Value with same letter(s) in a column are not significantly different at 5% level by DMRT.

CONCLUSION

Based on the results from above, vermicompost performed satisfactorily. It resulted in superior plant height, stem diameter, fresh weight of flowers and total yield of flowers. Furthermore, it supplied nutrients to plants slow and steadily, lowering the leaching and volatilization effect. Such leaching and volatilization problems are common in the case of inorganic fertilizers. In addition, vermicompost does not contain ammonia in excess amount, like that of poultry manure. The excess amount of ammonia is the reason for the low production of flowers by poultry manure in this research. Hence, given its outstanding performance, vermicompost is recommended for local farmers and residents across the district. Its consistent nutrient release and environmentally friendly nature make it a sustainable choice for improving marigold production.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there is no conflict of interest.

Author contribution

Melina Rai: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Resources, Data curation, Writing- Original Draft, Writing- Review & Editing, Visualization, Project administration. **Krishna Dahal:** Conceptualization, Methodology, Validation, Visualization, Supervision, Project administration. **Khem Raj Ghimire:** Methodology, Investigation, Resources, Writing- Original Draft, Writing- Review & Editing, Visualization. **Supriya Niroula & Uma Devi Bhandari:** Methodology, Investigation, Resources, Project administration. **Soniya Koirala, Chandani Sunuwar & Lilam Karki:** Methodology, Investigation, Resources.

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Enhancing soil health and nitrogen efficiency in cotton (*Gossypium hirsutum* L.) plants through organic and mineral fertilizations in saline and non-saline soils

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Abstract

Soil salinity significantly influences nitrogen dynamics within the soil matrix. Therefore, monitoring the nitrogen use efficiency (NUE) by plants in saline conditions is imperative. This investigation assesses the impact of mineral and organic fertilizers on nitrogen assimilation and various soil chemical characteristics under both saline and non-saline conditions in cotton (*Gossypium hirsutum* L.) cultivation. The study employed the Candia cotton cultivar along with diammonium phosphate (DAP, 18-46-0), cattle manure, and vermicompost as fertilization strategies. Vermicompost (1.58 dS m^{-1}) and cattle manure (1.49 dS m^{-1}) applications markedly alleviated the adverse effects of salinity by reducing electrical conductivity (EC) in saline soils ($P<0.05$). Organic fertilizers were observed to significantly increase the soil organic carbon (SOC) levels in saline soils compared to mineral fertilizers ($P<0.05$). The maximum plant height in saline conditions was recorded with vermicompost treatment (26.00 cm), while in non-saline environments, the greatest height was observed with cattle manure application (32.33 cm). In saline scenarios, DAP fertilization resulted in elevated nitrogen concentrations in the root (0.94%) and stem (2.03%) tissues over leaf tissues. Organic fertilizers markedly improved the nitrogen content in cotton across all tissue types, under both saline conditions and with both fertilizer categories ($P<0.05$). Additionally, organic amendments decreased the EC levels in saline soils and enhanced soil organic matter content. Contrary to mineral fertilizers, the use of organic amendments positively influenced nitrogen acquisition by cotton in saline conditions.

Keywords: Saline soil, Cotton, Nitrogen dynamics, Organic fertilizers

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.) plays a significant role as a fiber crop, contributing fundamentally to the economies of many countries worldwide (Ahmad et al., 2018; Abbas and Ahmad, 2018; Koukouli and Georgiou, 2018; Rahman et al., 2018). It is also an important oilseed crop (Semizer-cuming et al., 2015) Grown in various geographical regions, cotton production spans approximately 80 countries today (FAO, 2018). Leading producers include China, India, the USA, Pakistan, and Turkey (Jabran et al., 2019). According to the International Cotton Advisory Committee's data for the 2021/2022 production season, the global cotton planting area reached 33.4 million hectares, with production totaling 26.4 million tons. Global cotton consumption was reported at 26.2 million tons (ICAC, 2022).

Like other plants, cotton requires a variety of nutrients for its growth and development, with nitrogen fertilizers being among the most commonly preferred in cotton production (Weir et al., 1996). A deficiency in nitrogen prevents the plant from achieving sufficient vegetative and generative growth (Sutton et al., 2011). Soil salinity is

one factor that affects nitrogen uptake in plants (Zhang et al., 2018). The accumulation of salts, especially in the plant root zone, reduces the soil solution's osmotic potential, hindering water uptake by plants (Setia et al., 2013). An increase in soil salinity not only hampers plant growth but also adversely affects the size and activity of soil microbial biomass, as well as the biochemical processes essential for soil organic matter decomposition (Mavi et al., 2012). Soil salinity leads to nitrogen losses in the soil (Reddy and Crohn, 2014; Li et al., 2020). Elevated soil salt concentrations and high humidity conditions result in reduced nitrogen mineralization, with high salinity conditions potentially increasing nitrogen losses in the form of ammonia. Salinity impacts microbial activity in nitrification, the process of converting ammonium to nitrate, thereby reducing the efficiency of this conversion (Zhang et al., 2018).

To ensure sustainability in agricultural production, preserving soil health—the foundation of field agriculture is essential. Soil health encompasses chemical, physical, and biological properties that enable a wide range of functions. The dynamic roles of microorganisms in enhancing soil fertility and productivity hinge on enzyme activity, organic matter decomposition, and nutrient cycling (Antonious et al., 2020). Environmentally friendly applications of animal and plant-based fertilizers notably contribute to soil fertility, thereby increasing sustainable agricultural production (Kılbacak et al., 2021). Among these practices, the application of vermicompost is significant, enriching the soil with organic matter and nutrients, and stands out as a key organic fertilizer (Huang et al., 2013; Emperor and Kumar, 2015). Vermicompost, produced through the digestion of organic matter by earthworms, has been shown to have a greater positive impact on plant growth, soil reclamation, plant health, and the environment compared to traditional compost (Fritz et al., 2012; Bellitürk et al., 2013; Bellitürk et al., 2015).

This study aimed to evaluate the effects of organic and mineral fertilization strategies on nitrogen uptake and key soil chemical properties in cotton cultivation under saline and non-saline soil conditions.

MATERIAL AND METHOD

Material

The Candia cotton (*Gossypium hirsutum* L.) variety cleaned seeds served as the plant material. Organic fertilizers used in the study were cattle manure (Biofarm Fertilizer) and vermicompost (Ekosolfarm), while the chemical fertilizer employed was diammonium phosphate (DAP 18-46). The fertilizer treatments comprised four different approaches in both saline and non-saline soil types: 2000 kg ha^{-1} of cattle manure, 1500 kg ha^{-1} of vermicompost, 300 kg ha^{-1} of DAP fertilizer, and a control group without fertilizer application. The saline soils for the experiment were sourced from the Bozyazı area in the Harran plain, a region affected by salinity. The chemical properties of the soil types utilized in the experiment are detailed in Table 1.

Table 1. Characteristics of soil used in the experiment.

Soil Type	EC [ds m^{-1}]	pH	Lime [%]	Organic matter [%]
Saline soil	3.40	7.20	26.62	3.74
Non-saline soil	0.88	7.82	10.96	3.03

Method

The study was carried out in May 2019 at the Plant Laboratory of Harran University Şanlıurfa Technical Sciences Vocational School. The Candia cotton variety was cultivated in both non-saline and saline soils. Germination proceeded under laboratory conditions within a temperature-controlled chamber, adhering to the International Seed Testing Association (ISTA) guidelines. A test system was established for both non-saline and saline soils, utilizing seeds as control with three types of fertilizers and a control group. To prevent contamination, surface sterilization of the cotton seeds was performed by initially rinsing them with deionized water, followed by immersion in a 2% sodium hypochlorite solution for 3-5 minutes. Subsequently, the seeds were thoroughly rinsed 2-4 times with sterile distilled water. Additionally, for surface sterilization, the seeds underwent a 30-second soak in 70% ethanol, then a 10-minute immersion in a 10% sodium hypochlorite (NaOCl) solution. Soils were sifted through a 2 mm sieve and filled into 5 kg plastic pots. Surface-sterilized seeds were sown in pots prepared with fertilizers, applied according to manufacturers recommendations. Post-sowing, pots were watered to facilitate germination. Irrigation was adjusted based on plant water requirements, generally occurring twice weekly, using deionized water. The average temperature for plant growth was maintained daily at 27±1°C (30 °C day/26 °C night), with plant illumination set to 14 hours of light and 10 hours of darkness. Both sunlight and fluorescent light served as light sources. Harvesting occurred 12 weeks post-germination.

Soil reaction (pH) and electrical conductivity (EC) were assessed using saturation extract mud (Jackson, 1958). The soil calcium carbonate ($CaCO_3$) content was determined via the Scheibler calcimeter method (Allison and Moodie, 1965). Organic carbon was quantified through the wet oxidation method as developed by Nelson and Sommer (1982). Total soil nitrogen content (Bremner and Mulvaney, 1983), as well as nitrate (NO_3-N), were extracted using a 2M potassium chloride solution and quantified at a wavelength of 410 nm with a spectrophotometer (Cataldo et al., 1975). The nitrogen content in different parts of the cotton plant (root, stem,

and leaf) was measured employing the Kjeldahl method (Bremner and Mulvaney, 1983). Plant height was determined by measuring from the soil surface to the apex of the tallest part of the plant.

Statistical analysis

The experiment was laid out according to the split plots divided in random plots. The descriptive statistics of the data were carried out and the mean values were obtained. Variance analyzes were performed using the JMP 13.2 statistical software. The mean values for the treatments were grouped by the Tukey-HSD test.

RESULTS AND DISCUSSION

Result

This study investigated the potential effects of soil salinity on the development of cotton plants and certain soil chemical properties, applying organic and mineral fertilizer sources across two contrasting soil types. Specifically, organic fertilizers (cattle manure and vermicompost) were found to positively contribute to both plant nitrogen uptake and the improvement of some soil chemical properties. The application of organic fertilizers led to a greater reduction in soil electrical conductivity (EC) compared to mineral fertilizers in both soil types, as detailed in Table 2. Particularly in the saline soil type, the application of cattle manure and vermicompost resulted in a more than twofold decrease in EC values relative to the control group. The effects of organic and mineral fertilizers on saline soils were also statistically significant in terms of soil EC values ($P<0.05$). In the non-saline soil type, due to the low EC value of the soil used in the experiment and the absence of salinity issues (as indicated in Table 1), there was no statistically significant variation in soil EC values between organic and mineral fertilizers applied in the non-saline soil type ($P<0.05$), as shown in Figure 1.

Table 2. Effect of treatments on some soil chemical properties

Soil Type	Treatments	EC [dS m^{-1}]	pH	CaCO3 [%]	Organic matter [%]
Saline soil	Control	3.72	7.80	28.95	2.55
	DAP	3.40	7.20	22.73	2.95
	Cattle manure	1.49	8.10	23.97	4.63
	Vermicompost	1.58	8.10	26.31	4.23
Non-saline soil	Control	1.01	7.80	12.30	3.49
	DAP	1.00	7.50	12.14	2.55
	Cattle manure	0.88	7.70	11.52	3.56
	Vermicompost	0.77	7.70	9.34	2.55

The application of both mineral and organic fertilizers in the contrasting soil types did not lead to any alterations in the pH values of the soils, as demonstrated in Table 2 and Figure 1. In the saline soil type, compared to the control group, the use of both mineral and organic fertilizers was associated with a reduction in the soil $CaCO_3$ content, as detailed in Table 2. This reduction was statistically significant ($P<0.05$), as illustrated in Figure 1. While no statistically significant difference was observed in the $CaCO_3$ content within the non-saline soil type, the application of vermicompost was notably associated with a partial decrease in the $CaCO_3$ content of the soils, as indicated in Table 2.

In the saline soil type, organic fertilizer applications significantly increased the soil organic carbon content (Table 2), and the changes induced by the applications in soil organic carbon content were also statistically significant ($P<0.05$). However, in the non-saline soil type, it was observed that the organic fertilizer sources applied to the soil had values closer to the control group. Organic fertilizers, containing high levels of carbon in their structure, are seen to be particularly effective in improving the properties of problematic soils such as saline soil. In the experiment terminated with the harvest of the cotton plant, the remaining total nitrogen and nitrate concentrations in the soil were determined.

In the conducted experiment, soils treated with both organic and mineral fertilizers exhibited lower nitrogen content compared to the control group soils across both soil types, as detailed in Table 3. Within the saline soil type, the application of cattle manure resulted in the lowest nitrogen content, with diammonium phosphate (DAP) and vermicompost applications following in ascending order of nitrogen content, respectively, as shown in Table 3.

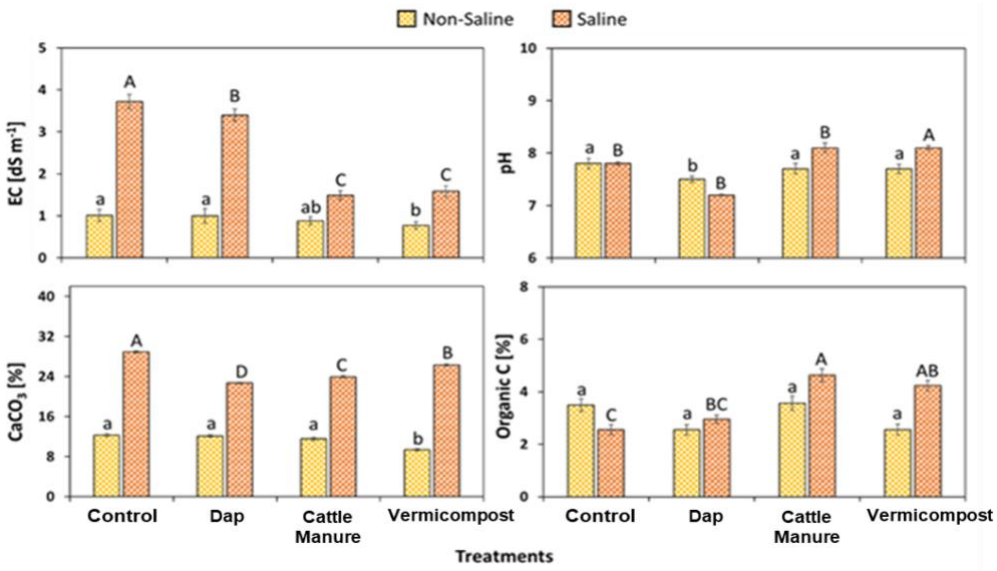


Figure 1. The effect of organic and mineral fertilizer application on certain soil chemical properties

Table 3. Soil total nitrogen and nitrate concentration.

Soil Type	treatments	Soil Nitrogen Concentration [mg N kg]	Soil Nitrate Concentration [mg N kg]
Saline soil	Control	1806	127.58
	DAP	1132	4.04
	Cattle manure	698	0.72
	Vermicompost	1181	11.17
Non-saline soil	Control	2015	2.61
	DAP	610	18.96
	Cattle manure	781	7.18
	Vermicompost	699	11.55

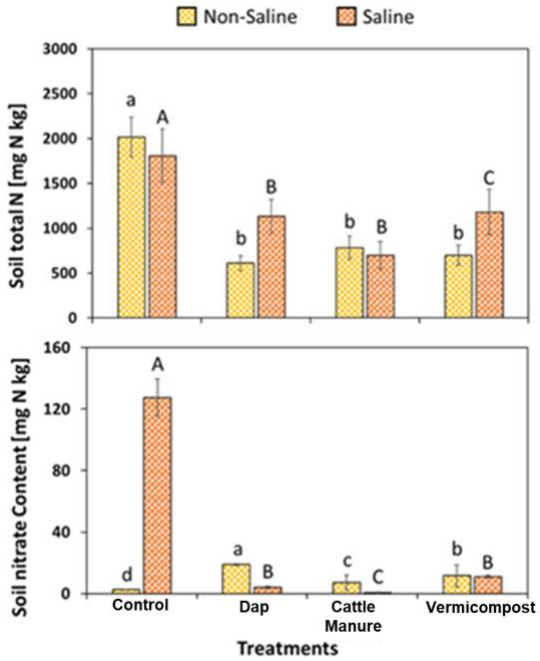


Figure 2. The effect of organic and mineral fertilizer application on soil nitrogen and nitrate content.

In the saline soil, the application of organic and mineral fertilizers did not result in any statistically significant differences ($P < 0.05$) in the soil nitrogen content remaining after harvest, as illustrated in Figure 2. Conversely, in the non-saline soil, the nitrogen content post-harvest was significantly lower for both organic and mineral fertilizer treatments compared to the control group, as detailed in Table 2. Additionally, no statistically significant difference was observed between the organic and mineral fertilizer treatments in the non-saline soil, as shown in Figure 2. Intriguingly, the control group in the saline soil, which received no fertilizer application, exhibited higher residual nitrate content after harvest compared to treated soils, as indicated in Table 3. For both mineral and organic fertilizer applications, soil nitrate levels were markedly lower than those in the control group, with this difference being statistically significant, as depicted in Figure 2. Despite the reduced soil nitrate contents post-harvest in the non-saline soil, the differences between treatments were statistically significant ($P < 0.05$), as shown in Figure 2.

Plant height, a critical agronomic parameter, demonstrated varied responses across the different soil types and fertilizer treatments. In the non-saline soil, the highest plant height (32.33 cm) was observed in the cattle manure treatment, whereas the lowest plant height (24.33 cm) occurred in the DAP-treated soil. In the saline soil, the highest plant height was noted in the vermicompost treatment (26.00 cm), with the lowest height recorded in the control group. Statistically significant differences in plant height were observed across both mineral and organic fertilizer applications in both soil types, as evidenced in Figure 3.

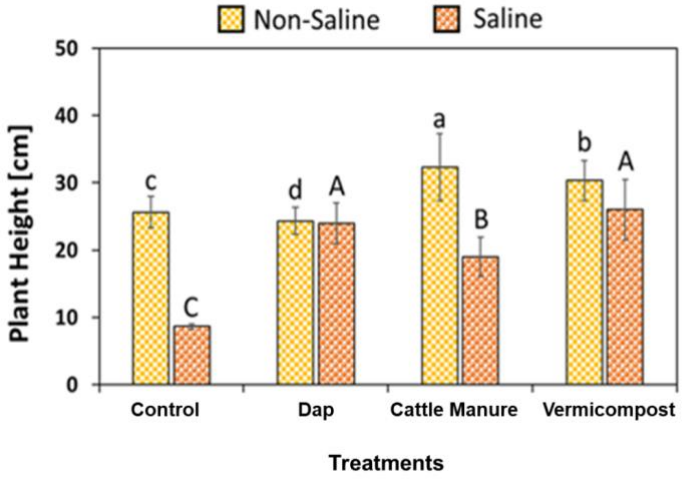


Figure 3. The effect of treatments on plant height.

Total nitrogen analysis was performed on various parts of the cotton plants (roots, stems, and leaves) to assess the nitrogen absorption from the soil during the trial period. In the saline soil, the nitrogen content in the roots and the stem and leaf tissues of cotton plants grown in the control soil exhibited very low levels. This suggests that the presence of salt in the environment may have significantly impeded physiological development, as evidenced by the notably low levels of nitrogen in the cotton plants from the control group (Table 4), coupled with their reduced height relative to other treatments. Notably, in saline soils, the application of diammonium phosphate (DAP) resulted in lower nitrogen content across the roots, stems, and leaves of the plant compared to the applications of the two organic fertilizers. Overall, in the saline soil type, organic fertilizers had a more pronounced effect on the plant nitrogen uptake than mineral fertilizer applications, as demonstrated in Figure 4.

Table 4. Effects of organic and mineral fertilizers on nitrogen uptake in cotton plants

Soil Type	Fertilizer treatment	Root [%]	Stem [%]	Leaf [%]
Saline soil	Control	0.10	0.10	0.10
	DAP	0.94	2.03	0.05
	Cattle manure	1.43	2.78	1.32
	Vermicompost	1.81	3.36	1.49
Non-saline soil	Control	3.05	3.73	1.47
	DAP	3.62	4.48	0.20
	Cattle manure	2.65	0.28	0.09
	Vermicompost	1.84	1.97	1.63

The analysis of nitrogen content in the roots, stems, and leaves of cotton plants grown in control group soil revealed uniform nitrogen levels across all three plant parts. This uniformity, coupled with the observed reduced

plant height compared to other treatments, suggests that the presence of salt in the environment may have hindered the plant ability to thrive. In treatments with organic fertilizers, the nitrogen content was predominantly higher in the stem part of the plant, indicating a differential uptake and allocation pattern. Conversely, in the DAP (diammonium phosphate) treatment, nitrogen content was lower in the leaves but higher in the stems, pointing to a specific influence of mineral fertilizer on nitrogen distribution within the plant.

In the context of non-saline soil, DAP application was associated with significantly higher nitrogen content in both the root and stem parts of the plant, while the nitrogen content in the leaves was comparatively lower. The vermicompost treatment stood out, showing a more balanced nitrogen distribution across all three parts of the plant, as indicated in Table 4. The challenging conditions created by soil salinity significantly impair the plant nitrogen uptake from the soil. However, this study found that the adverse effects of salinity on nitrogen absorption could be substantially mitigated through the application of organic fertilizers. Organic fertilizers have been observed to enhance plant nitrogen content more effectively than mineral fertilizers, underscoring their potential to improve nutrient uptake in saline conditions.

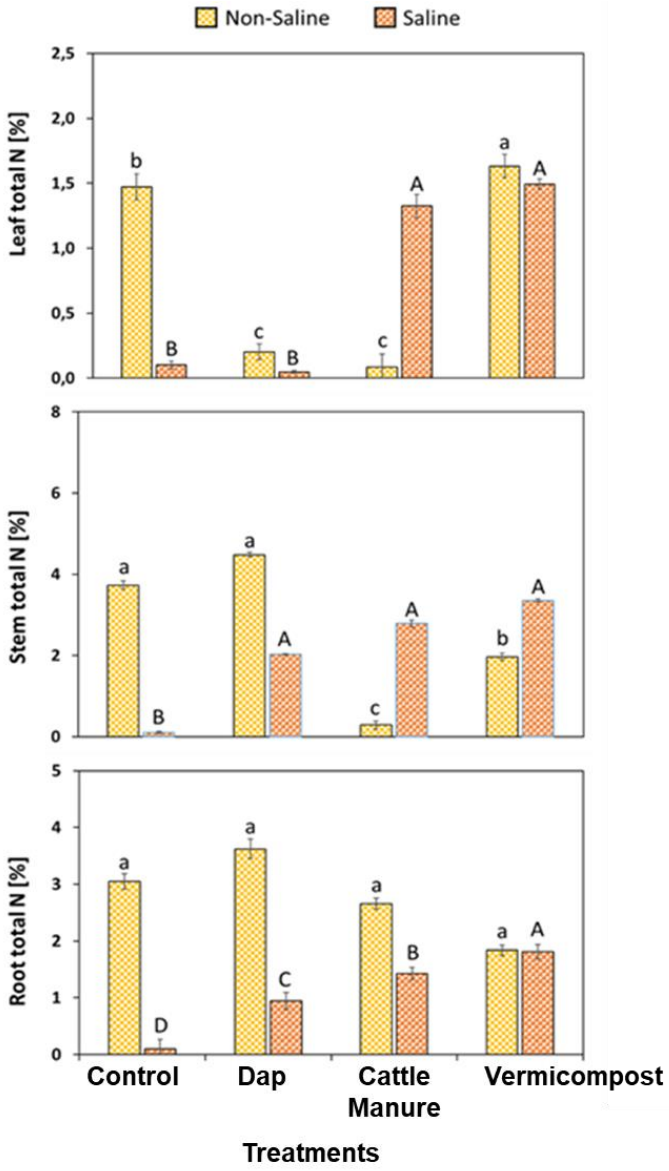


Figure 4. Nitrogen content in cotton plant root, stem, and leaf tissues

The examination of the impact of various treatments on the nitrogen uptake in cotton plants, as depicted in Figure 4, reveals that in non-saline soil conditions, the nitrogen absorption by the plant roots, stems, and leaves was comparable between the control group and the DAP (diammonium phosphate) fertilizer treatment. This observation underscores the primary aim of the study, which was to explore the effects of salinity on plant development. The conclusion of the experiment upon achieving the requisite plant measurement parameters in saline soils may have obscured the potential distinctions in treatment effects within the non-saline soil category.

The utilization of pots as the medium for conducting this experiment was a limiting factor for extending its duration. A longer experimental period might have highlighted more pronounced differences, with expectations pointing towards the control group exhibiting the lowest nitrogen content.

In non-saline soil, the DAP application was observed to result in the highest nitrogen content across the entire plant. Among the organic fertilizers, vermicompost was identified as more efficacious than cattle manure in enhancing nitrogen uptake. In conditions of saline soil, applications of organic fertilizers markedly improved the nitrogen uptake in cotton plants, with vermicompost standing out as the most advantageous for nitrogen absorption in such environments.

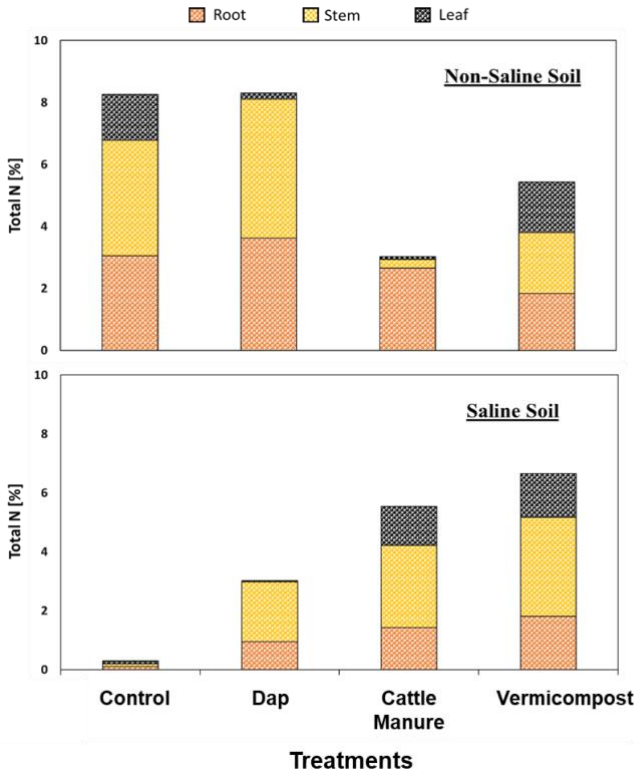


Figure 5. Nitrogen distribution in various parts of the cotton plant

Cattle manure and materials such as vermicompost, owing to their high carbon content, significantly enhance the levels of soil organic matter and biological activity in the soils to which they are applied (Deng et al., 2017). An increase in soil organic matter levels is pivotal for the rapid amelioration of saline soils, particularly those characterized by poor aeration and microbial activity (Makkar et al., 2017). Soil salinity profoundly influences nitrogen dynamics in the soil, affecting processes such as mineralization, nitrification, and denitrification (Zeng et al., 2013). This influence directly impedes nitrogen uptake, a critical nutrient for plants, thereby substantially reducing crop yields (Han et al., 2015). In saline soil types, the elevated soil nitrate content post-harvest can be attributed to the intensified competition between chloride ions (Cl^-) and nitrate ions (NO_3^-) due to the increased salt concentration, which obstructs the plants absorption of nitrate (Mengel and Kirkby, 2001). Nitrogen plays a crucial role in metabolic processes that enhance plant photosynthetic efficiency through the synthesis of chlorophyll and certain enzymes (Wen et al., 2019). The presence of salt disrupts the protein bond between chlorophyll and chloroplasts, leading to a rapid decline in the photosynthetic activity of plants (Jia et al., 2020).

Several factors contribute to the diminished development of plants in saline soil compared to those in non-saline soil types. These include the impediment to the plants access to water and nutrients caused by soil salinity, the formation of small capillary roots during early developmental stages, which in turn restricts growth and hinders the synthesis of enzymes involved in photosynthesis. The growth of cotton seeds in saline conditions may significantly slow or even result in plant mortality (Gouia et al., 1994). The lower nitrogen content observed in the roots of plants grown in saline soils, as opposed to other plant parts, may be due to the dynamic distribution of salt which interferes with irrigation and root water uptake (Malash et al., 2008). It has been documented that cotton plants accumulate nitrogen absorbed from the soil in their roots and stems, while the leaves exhibit lower nitrogen content. These observations align with the findings of our study (Chen et al., 2010). Ma et al. (2021) observed that salt application during early stages reduced nutrient and water uptake through the roots of sunflower plants. However, the roots were not significantly affected by salt applied during periods of increased plant growth, which was attributed to the absence of a rapid decrease in photosynthetic activity due to growing and expanding leaf area.

The dissolution of organic fertilizers in the soil releases humic substances, which enhance the availability of certain nutrients that plants are deficient in under saline conditions (Lakhdar et al., 2009). Notably, the application of vermicompost significantly improves nitrogen uptake in plants. Akhzari et al. (2016) reported that vermicompost application substantially increases plant nitrogen content, positively affecting plant growth parameters. The use of mineral fertilizers in saline conditions may further increase soil salinity. This is because mineral fertilizers inherently contain salts, and their excessive use in saline soils can exacerbate plant damage (Liu et al., 2019).

CONCLUSION

The application of both mineral and organic fertilizers to saline and non-saline soil types significantly influences soil chemical properties and the nitrogen uptake in cotton plants. Specifically, the application of organic fertilizers in saline soil types has been shown to reduce soil electrical conductivity (EC) values, thereby mitigating the adverse effects of salinity on plant growth. This reduction in EC values in saline soils contributes to enhanced plant development and more efficient utilization of nitrogen from the soil. It is commonly expected that plants grown under saline conditions tend to accumulate absorbed nitrogen predominantly in their roots and stems. A pivotal finding of our study is that the application of organic fertilizers to cotton plants cultivated in saline soils prevents the excessive accumulation of nitrogen in both the root and stem parts. Furthermore, the analysis of nitrogen content in leaf tissues reveals that organic fertilizer applications bolster nitrogen uptake by plants in saline conditions. In the specific soil conditions examined in this study, the utilization of 200 kg ha^{-1} of cattle manure and 150 kg ha^{-1} of vermicompost significantly improves various chemical properties of saline soils, as well as the nitrogen uptake and overall development of cotton plants.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

The responsible author evaluated the analysis and results of the experiment and wrote the article. Other researchers collaborated on studies such as setting up the trial, supplying materials, statistical evaluation, and editing the article.

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Soil moisture deficit drives assimilate remobilization and grain yield variability in bread wheat genotypes

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Abstract

Soil moisture deficit and drought, exacerbated by climate change, frequently affect crop yields. This study aimed to evaluate the physiological and agronomic responses of bread wheat (*Triticum aestivum* L.) genotypes under soil moisture deficit conditions. Field experiments were conducted using a randomized complete block design during the 2015–2016 growing season at the Agricultural and Natural Resources Research Station in southwestern West Azerbaijan Province, Iran. The study treatments were full irrigation and irrigation cutoff at the flowering stage with seven bread wheat genotypes and the Orum, Zare, Zarin, and Mihan cultivars. The bread wheat grain yield and its components, and some physiological traits like the contribution of photosynthesis, assimilate remobilization, and harvest index (HI) were determined in this study. A significant reduction was observed in yield components (spikelets/spike, grains/spike, grain weight/spike, and spike weight) under drought stress. The Mihan cultivar exhibited the highest spikelets/spike (15), grains/spike (38), grain weight/spike (1.62 g), and spike weight (2.17 g). The intensification of drought stress increased the contribution of remobilization of stored assimilates by 25%. The Mihan cultivar had the highest remobilization rate at 52%, while line C-91-7 and line C-91-5 had the lowest at 27 %. The HI decreased by 20% under soil water deficient stress. The Mihan cultivar, with a HI of 50%, and C-91-8, with 41%, had the highest and lowest HI, respectively. The mean comparison of the contribution of the current photosynthesis trait to wheat grain yield from full irrigation to severe water shortage dropped to 41.2%. Under full irrigation and irrigation cutoff conditions, line C-91-4 had the highest grain yield, with 8747 kg/ha and 5039 kg/ha, respectively. Furthermore, the highest grain yield under full irrigation and irrigation cutoff treatment was related to the Mihan cultivar, with 7569 kg/ha and 4856 kg/ha. Based on these findings, the Mihan cultivar and C-91-4 line are recommended for cultivation in semi-arid regions facing water limitations. Understanding physiological traits and yield components is critical for selecting drought-tolerant wheat varieties in the context of climate change.

Keywords: Soil water deficit, Bread wheat, Assimilate remobilization, Photosynthesis, Yield components

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INTRODUCTION

Soil moisture deficit and drought stress are among the most critical and widespread environmental factors negatively impacting agricultural production worldwide (Rahdari & Hoseini, 2012). Wheat, one of the major cereal crops, is cultivated globally and serves as a staple food for approximately one-third of the world's population (Rahman et al., 2008; Abdelsalam et al., 2019). Among the various abiotic stresses, water deficit is particularly detrimental, adversely affecting chlorophyll content, qualitative traits, and the individual components of wheat grain yield, ultimately leading to substantial yield losses (Raza et al., 2012; Tesfaye et al., 2013; Raza et al., 2014;

Wasaya et al., 2021; Ahmad et al., 2022). Drought stress severely impacts photosynthesis, leading to stomatal closure and reduced CO_2 supply, making it a highly sensitive process (Li et al., 2021; Naseer et al., 2022).

The selection of drought-tolerant genotypes can reduce damage to crops, and testing these genotypes can lead to the development of new cultivars and potential genetic improvement (Zafar-ul-Hye et al., 2007; Chowdhury et al., 2021). Wheat genotypes and varieties impact genetic engineering success, and genotype screening aids in selecting parents and breeding processes by assessing transformation, regeneration, tissue culture, and callus induction efficiency (Vendruscolo et al., 2008; Abid et al., 2014). Various indices have been proposed for selecting genotypes based on grain yield. Among these, a higher harvest index (HI) under drought stress conditions is one of the important criteria for selecting genotypes (Giunta et al., 1995; Rezadoost & Roshdi, 2006; Sanjarei Pirvatlou & Yazdansepas, 2009). The increase in grain yield potential of new wheat varieties has primarily been achieved by enhancing the harvest index (Araus et al., 2002). Remobilizing photosynthetic materials can be different under soil moisture scarcity between wheat genotypes (Meng et al., 2017). Consistent with this, Elyassi et al. (2010) reported that the Shahryar wheat cultivar had the highest remobilization of photosynthetic materials (80 g/m^2) among other studied varieties under pre-anthesis drought stress. They also reported that the maximum remobilization efficiency under pre-anthesis stress treatment belonged to the Shahriar cultivar (53%), while the highest efficiency under post-anthesis stress was observed in the tall Toos cultivar (34%). Aligned with this, Gent (1994), over a three-year experiment, concluded that the carbohydrates stored in the wheat stem are important for grain filling but stated that the amount of current carbohydrates for grain filling is limited under drought stress conditions.

Additionally, wheat genotypes can exhibit different ranges of yield components including spike weight, number of spikelets per spike (spikelets/spike), grain weight/spike, number of grains per spike (grains/spike), and 1000-grain weight under drought stress (Ahmad et al., 2022; Javed et al., 2022). Furthermore, Subhani and Chowdhry (2000), by correlation and causation analysis of bread wheat under drought and irrigation conditions, also found that grain yield had a direct positive correlation with the traits of flag leaf area, plant height, spike length, grains/spike, 1000-grain weight, plant biomass, and HI under drought stress, but negative correlation with the trait of days to spike emergence. Emam et al. (2007) reported that the reduction in the grains/spike and the 1000-grain weight were the main causes of decreased grain yield under drought stress conditions under post-anthesis, while drought stress had no significant effect on the spikelets/spike and spikes/m^2.

Therefore, selecting genotypes with high tolerance to limited soil moisture according to yield components and physiological traits (Mutanda et al., 2024) is significant in gaining higher grain yield, especially in arid ecosystems. This research investigates the impact of soil water deficit stress on photosynthesis and remobilization in wheat genotypes and the relationship between yield and yield components under drought conditions. In the context of these facts, this study screened wheat genotypes in semi-arid regions of Northwest Iran (West Azerbaijan Province) for moisture deficit resistance. We hypothesized that changes in physiological traits and yield components under soil water deficiency, which affect the grain yield of different wheat genotypes, can help us introduce a wheat genotype or cultivar with a higher yield than other studied genotypes for cultivation in these cold and arid regions.

MATERIALS AND METHODS

Study area

Field research was conducted during 2015-2016 at the Agricultural and Natural Resources Research Station in the southeastern part of West Azerbaijan Province, Iran (46°05' E longitude and 36°00' N latitude). The elevation of the study area was 1142 m above sea level. The meteorological data for the experimental site during the 2015–2016 growing season are presented in Table 1. Long-term meteorological data showed the area's mean annual rainfall was 280 mm. The area's minimum and maximum mean temperatures were about -5.7°C and 26.2 °C, respectively.

The soil at the experimental site is a silty loam. Composite soils were collected from a depth of 0-30 cm, processed and analyzed for particle size analysis, pH, calcium carbonate ($CaCO_3$) concentration, and electrical conductivity (ECe) using the standard methodologies (Bauyoucos, 1954; McLean, 1965; Allison & Moodie, 1965; and Black et al. 1983). The modified Walkley-Black method was used to determine soil organic carbon (SOC) content (Jackson, 1958). The Kjeldahl digestion and distillation method was used for measuring total nitrogen (Bremner, 1982), while Olsen (1954), the ammonium acetate method (Carson, 1980), and DTPA extraction (Lindsay & Norvell, 1978) were used to determine available phosphorus (P), potassium (K), and micronutrients (iron and zinc). The soil had an ECe of 0.81 dS/m and a pH of 8 (Table 2).

Experiment design

The experiment followed a randomized complete block design with three replicated plots of 4.8 m^2 for each treatment. Treatments were full irrigation and irrigation cutoff at the flowering stage with seven bread wheat genotypes and the Urum, Zare, Zarin, and Mihan cultivars, with the cultivar as the control. According to the soil analysis results, 73.6 kg N ha^{-1} was applied in the form of 160 kg/ha urea fertilizer (46% nitrogen) in three stages: 20 kg/ha during soil preparation (before planting), 70 kg/ha at the tillering stage, and 70 kg/ha at the stem elongation stage of wheat. Additionally, 38.25 kg K/ha was applied as 85 kg/ha potassium sulfate (45% potassium),

and 23 kg P /ha was applied as 100 kg/ha diammonium phosphate (23% phosphorus) concurrently with planting. Zinc was applied at a rate of 3.38 kg Zn/ha in the form of 15 kg/ha zinc sulfate, applied in a band at a depth of 3–4 cm beneath the plants.

Table 1. Meteorological Data of the Experimental Site in the 2015–2016 Growing Season

Months	Temperature (°C)			Relative humidity (%)			Total Evaporation (mm)	Total Precipitation (mm)
	Average	Min	Max	Average	Min	Max		
October	16	7.1	25	53	28	77	145.1	0.8
November	11	2.9	19	55	31	79	61.4	5.6
December	3.7	-1.8	9.1	64	41	87	0	17.8
January	-5.7	-11.4	-1.0	65	43	88	0	1.3
February	-2.6	-7.1	2	72	54	90	0	42.3
March	6.2	0.1	12.5	61	36	86	0	38.2
April	8	-4	20	57	30	84	72.9	37.6
May	15.8	1.6	31.2	50	2	77	182.2	36.1
June	19.4	6.4	32.4	46	21	71	225.7	9.6
July	25.1	11.4	38.8	47	26	69	290	8.8
August	26.2	12	40.4	45	24	66	308	0
September	23.5	9.4	37.6	53	28	78	207	23.3

Table 2. Selected soil physical and chemical properties

Soil properties	Unit	Values
Electrical conductivity (ECe)	(dS/m)	0.81
pH		8
Saturation moisture	(%)	33
Calcium Carbonate	(%)	11.4
Sand	(%)	16
Silt	(%)	58
Clay	(%)	26
Soil organic carbon	(%)	0.83
Total nitrogen	(%)	0.12
Exchangeable potassium	(mg/kg)	250
Available phosphorus	(mg/kg)	11.2
Available iron	(mg/kg)	6.24
Available zinc	(mg/kg)	0.74

Wheat planting was performed in October with clean, disinfected seeds, with a planting density of 450 seeds/m^2. Following planting, one irrigation in the fall was applied to facilitate the germination and establishment of the seedlings. Weed management was executed utilizing the pesticide 2,4-D (2,4-Dichlorophenoxyacetic acid).

To assess dry matter remobilization to the grains at two growth stages—pollen shedding and physiological maturity—20 randomly selected plants from each experimental plot were harvested at ground level and oven-dried at 72°C for 48 hours. Upon crop maturation, the total plant weight, spike weight, grain weight/spike, and number of grains per spike (grains/spike) were measured from the harvested samples. The amount, efficiency, and ultimately the contribution of the remobilization of dry matter from vegetative tissues to the grain were calculated using the methods of Cox et al. (1986) and Papakosta and Gagianas (1991) as follows:

Assimilate Remobilization contribution (%) = (Transferred dry matter / Grain yield) × 100

Assimilate Remobilization contribution - 100 = Current photosynthesis contribution

The grain yield was harvested by removing 50 cm from the beginning and end of each plot using a Wintersteiger cereal combine and then converted to kg/ha.

Statistical analyses

Statistical analyses and graph plotting were performed using SPSS and Excel software. To identify significant variations, analysis of variance and the mean data comparison were performed using Duncan's multiple range test at a $p \leq 0.05$ unless otherwise mentioned.

RESULTS AND DISCUSSION

Grain yield components

The effects of soil moisture deficit (drought stress) and genotypes on the wheat yield components were significant. The highest values of wheat yield components were observed in the full irrigation treatment (Table 3). In contrast, one of the effects of water deficit stress in the early growth stages of wheat stem elongation and spike formation is the reduction in the number of spikelets per spike. Kilic and Yağbasanlar (2010) indicated that the spikelets/spike were positively correlated with wheat yield under soil moisture deficit. The spikelets/spike exhibited a 14.3% reduction under soil moisture deficit compared to full irrigation conditions. The difference between the highest and lowest spikelets/spike among the wheat genotypes was 30.7% (Table 3).

The highest number of spikelets per spike (15) was observed in Mihan, Zare, and C-91-9, while the lowest (11) was recorded in C-91-5. Water scarcity stress led to the formation of fewer grains in the spike, such that the highest grains/spike was determined in full irrigation with 29 grains, and the lowest was in the water scarcity stress treatment with 25 grains/spike (Table 3). Our results correlated with the results of Praba et al. (2009), who reported that water stress significantly reduces plant height, grains/spike, spike weight, and grain weight/spike. Likewise, Başer et al. (2024) reported a significant reduction in the grains/spike under limited soil moisture conditions.

Wheat genotypes showed significant differences in grains/spike (53.3%). The Mahin variety had the highest grains/spike (38), while C-91-5 had the lowest (22) grains/spike. Water stress in all genotypes caused a reduction (21.7%) of grain weight/spike. Pour-Aboughadareh et al. (2020) reported a significant reduction in the grains/spike of the wheat genotypes under drought stress compared to the control. Similarly, Akbari Mogaddam et al. (2002) stated that water stress during the spike emergence stage reduces grain yield, with the most significant impact on grain weight among the yield components. The highest (1.62 g) and lowest (0.98 g) grain weight/spike were observed in genotypes Mihan and C-91-8, respectively. Furthermore, under soil water deficit, spike weight exhibited a 23.4 % reduction compared to full irrigation. Denčić et al. (2000) concluded that kernel weight/spike positively impacted wheat grain yield under drought stress.

The Mihan cultivar had the highest spike weight (2.17 g), while line number 5 (C-91-5) had the lowest (1.32 g). The grain yield exhibited significantly different values under irrigation treatments. Under soil water deficit stress, the grain yield was reduced by 41.7% (Table 3). Considering that increasing soil water deficiency led to a reduction in grain yield components such as spikelets/spike, grains /spike, and grain weight/spike, the decrease in grain yield with increasing water stress was not unexpected. Javed et al. (2022) found a positive correlation between wheat grain yield and yield components under soil water deficit. Shahryari and Mollasadeghi (2011) reported that water stress decreased grain yield and its components across all studied genotypes.

Several studies (Siddique et al., 2000; Guttieri et al., 2001) have reported reductions in wheat grain yield under water deficit stress. According to Sanjari and Shiri (2000), this reduction is primarily associated with a decrease in grains per spike and the number of spikes per unit area. Among the cultivars studied, the highest grain yield (6894 kg/ha) was recorded for Mihan, while the lowest (5025 kg/ha) was observed in Line C-91-5 (Table 3). Tahmasebi Sarvestani et al. (2001) found that the Sabalan variety had the highest average yield (2214 kg/ha) under different irrigation treatments, whereas the Sardari variety had the lowest (1502 kg/ha). They noted that applying a single irrigation at the milk stage in Sabalan led to a 54% increase in grain yield compared to the control.

Table 3. Irrigation and genotype effects on yield components of wheat.

Treatments	Spikelets /spike	Grains /spike	Grain weight /spike (g)	Spike weight (g)	Harvest index (HI)	Grain yield (kg/ha)
Irrigation						
Full irrigation	14x$^{\phi}$	29x	1.29x	1.75x	50x	7410x
Irrigation cutoff	12y	25y	1.01y	1.34y	40y	4317y
Genotypes						
Orum	13bc$^{\neq}$	26cde	1.16bc	1.59bc	46bc	5951bc
Zare	15a	25cde	1.05bcd	1.38ef	46bc	5465bc
Mihan	15a	38a	1.62a	2.17a	50a	6213ab
Zarrin	14ab	26cde	1.09bcd	1.41cdef	45bcd	5951abc
C-91-4	14ab	30b	1.20b	1.58bcd	43cd	6894a
C-91-5	11d	22e	1.02cd	1.32f	43cd	5025c
C-91-6	12c	24de	1.12bcd	1.49cdef	44cd	5475bc
C-91-7	13bc	27bcd	1.15bc	1.54bcde	44cd	5724bc
C-91-8	13bc	23e	0.98d	1.46cdef	41d	5893bc
C-91-9	15bc	24de	1.05bcd	1.40def	44cd	5642bc
C-91-10	13bc	28bc	1.20b	1.68b	48ab	6265ab

In each column, the means that have the same lower-case letter (x vs. y$^{\phi}$) or (a, b, c, d, e, f$^{\neq}$) are not significantly different between irrigation treatments or wheat genotypes, based on DMRT at the 5% probability level

The HI was determined at 50% and 40% in the full irrigation and irrigation cutoff treatments, respectively. The difference between the two irrigation treatment levels in HI was 20% in this study. Previous studies (Bayoumi et al., 2008; Khakwani et al., 2012) reported that wheat genotypes' physiological and agronomic traits and HI significantly decreased under drought conditions. Ahmadi et al. (2006) observed a significant interaction between drought stress and the timing of nitrogen fertilizer application on grain yield and related physiological traits in wheat. They highlighted that the soil moisture regime notably influences grain yield and HI. These traits significantly decreased under drought-stress conditions. Likewise, Tavakoli (2003) concluded that supplementary irrigation of rainfed wheat fields directly and positively increased the HI and grain yield. Sharifi and Rahmanian (2001) also reported that applying soil moisture stress in wheat fields reduces the ripening period, the HI, and the grain yield. The average of the HI of the studied genotypes in the experiment varied, with a difference of 18% between the lowest and highest harvest indexes among the genotypes. The Mihan cultivar, with a harvest index of 50%, and line C-91-8, with 41%, had the highest and lowest HI, respectively (Table 3).

Both irrigation wheat genotypes significantly affected wheat grain yield (Figure 1). Among genotypes, Line C-91-4, with 8747 kg/ha under full irrigation treatment, had the highest grain yield, while C-91-5, with 6066 kg/ha under soil water stress treatment, had the lowest grain yield. Considering that line C-91-4 had a higher grains/spike and grain weight under full irrigation treatment, the high grain yield of this genotype was predictable. With increased irrigation intervals and intensified water stress in all genotypes, grain yield was decreased. In cultivars such as Zarin and Urum, the reduction in grain yield under drought conditions compared to full irrigation was more than 50%, whereas in the Mihan cultivar, this difference was about 33% (Figure 1). The highest grain yield in the irrigation cutoff treatment was related to the Mihan cultivar, with 4856 kg/ha. Although the Zarin and Urum cultivars had the highest grain yield under full irrigation conditions, their grain yield significantly decreased under water stress conditions. One of the main reasons for the severe reduction in their grain yield was the wrinkling of the grains and a reduction (40%) in grain weight. In contrast, the Mihan cultivar showed a 22% reduction in grain weight under water stress conditions.

Considering that the trend of changes in the traits of the spikelets/spike and the grains/spike of the genotypes at different irrigation levels was almost the same, and water stress uniformly reduced these traits in all genotypes, the high grain weight (or thousand-grain weight) should be considered as one of the most important components of grain yield under water stress conditions as a criterion for selecting drought-tolerant genotypes (Akbari Mogaddam et al., 2002; Rezadoust & Roshdi, 2006; Sanjari Pireivatlou & Yazdansepas, 2008). Under drought stress conditions, the genotype C-91-4 and Mihan cultivar, probably due to higher grain weight, produced high grain yields of 5039 and 4856 kg/ha, respectively (Figure 1)

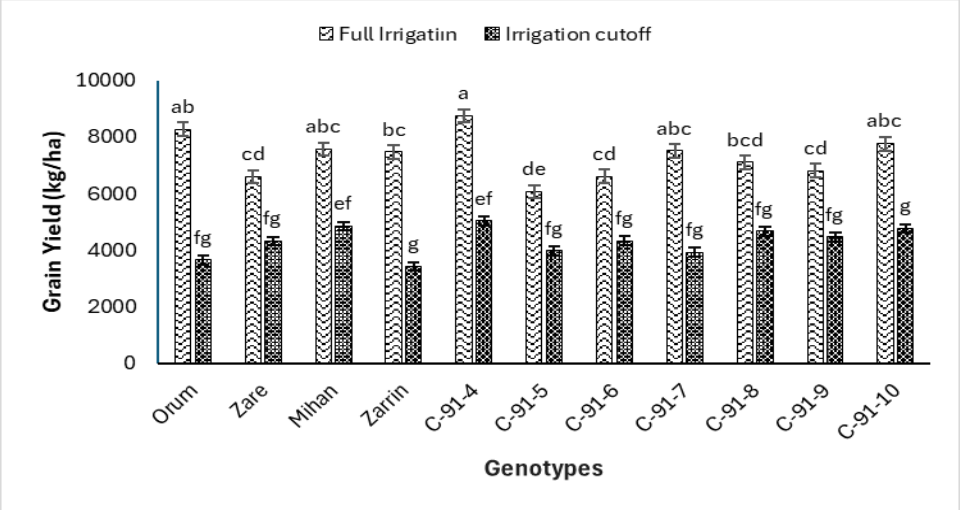


Figure 1. The mean of grain yield of wheat genotypes under different levels of full irrigation and irrigation cutoff (water stress) treatments (Different letters indicate significant differences at the 5% and 1% probability level)

Photosynthesis and assimilate remobilization

Results indicated that varying irrigation levels significantly influenced the contribution of remobilized photosynthetic materials (assimilate remobilization) to grain yield. Under non-stress conditions, remobilized assimilates were determined to be 32%, which increased to 40% as drought stress intensified (Figure 2). Soil water stress significantly enhanced stem reserve mobilization, ranging from 16% to 68%, compared to non-stress conditions (Gurumurthy et al., 2023).

Gent (1994), in a three-year study, concluded that carbohydrates stored in wheat stems are crucial for grain filling, particularly under drought conditions where current photosynthesis is limited. Similarly, Tiryakioğlu (2024) reported that, under water-limited regimes, wheat dry matter remobilization was primarily derived from pre-anthesis reserves, especially under irrigation until anthesis and until physiological maturity.

Vosoghi Rad et al. (2022) found that the peduncle internode significantly affected stem height across all durum wheat genotypes, with lower internodes showing the highest levels of dry matter remobilization under both drought and normal conditions. In the present study, significant genotypic differences in assimilate remobilization were observed, with rates ranging up to 63.3%. The Mihan cultivar showed the highest remobilization rate (52%), while lines C-91-7 and C-91-5 exhibited the lowest (27%) (Table 4).

Plaut et al. (2004) also reported considerable variation in dry matter remobilization from vegetative organs to grains among wheat cultivars under drought conditions, with the Sonka variety outperforming the Batavia variety in this regard. These findings align with Ehdaie et al. (2006), who, in evaluating 11 wheat genotypes, observed significant genetic diversity in remobilization capacity. The remobilized dry matter stored in the peduncle, lower internode, and lower stem nodes ranged from 43 to 171 mg, 81 to 272 mg, and 198 to 474 mg, respectively, under water deficit conditions.

Both irrigation regime and genotype significantly influenced the contribution of current photosynthesis to grain yield (Figure 2 and Table 4). On average, current photosynthesis was determined to be 68% under non-stress conditions, but this dropped to 40% under severe water stress (Figure 2). This underscores the critical role of current photosynthesis as a selective mechanism in grain filling. Since remobilization processes—both in accumulation and transfer—require energy, their activity diminishes when current photosynthetic assimilates are sufficient for grain development (Naderi et al., 2000).

The studied genotypes also differed significantly in their reliance on current photosynthesis, with a 34% variation. Lines C-91-5 and C-91-7 showed the highest contributions from current photosynthesis (73%), while the Mihan cultivar had the lowest (48%) (Table 4). However, despite their higher current photosynthesis rates, C-91-5 and C-91-7 exhibited reduced grain yields due to lower yield component performance. In contrast, the Urum variety, with a high current photosynthesis rate (67%) and favorable yield components, achieved the highest grain yield (8274 kg/ha) under full irrigation (Figure 1). This highlighted the importance of current photosynthesis for grain filling under optimal conditions.

Therefore, maintaining an active and healthy green leaf area is essential to sustain photosynthetic capacity, particularly under non-stress environments. In plant communities, solar energy capture through photosynthesis is fundamental. Research in crop physiology has consistently shown that the final yield of agricultural crops depends largely on both the size and efficiency of their photosynthetic systems.

Table 4. Irrigation and Genotypes effects on Assimilate remobilization contribution and Current photosynthesis contribution

Treatments	Assimilate remobilization contribution (%)	Current photosynthesis contribution (%)
Irrigation		
Full irrigation	32y$^{\phi}$	68x
Irrigation cutoff	40x	40y
Genotypes		
Orum	33cd$^{\neq}$	67b
Zare	39bc	61cd
Mihan	52a	57d
Zarrin	34cd	66b
C-91-4	41b	59cd
C-91-5	27d	73a
C-91-6	28d	72a
C-91-7	27d	73a
C-91-8	32cd	68b
C-91-9	38bc	62c
C-91-10	43b	48e

In each column, the means that have the same lower-case letter (x vs. y$^{\phi}$) or (a, b, c, d, e, f$^{\neq}$) are not significantly different between irrigation treatments or wheat genotypes, based on DMRT at the 5% probability level

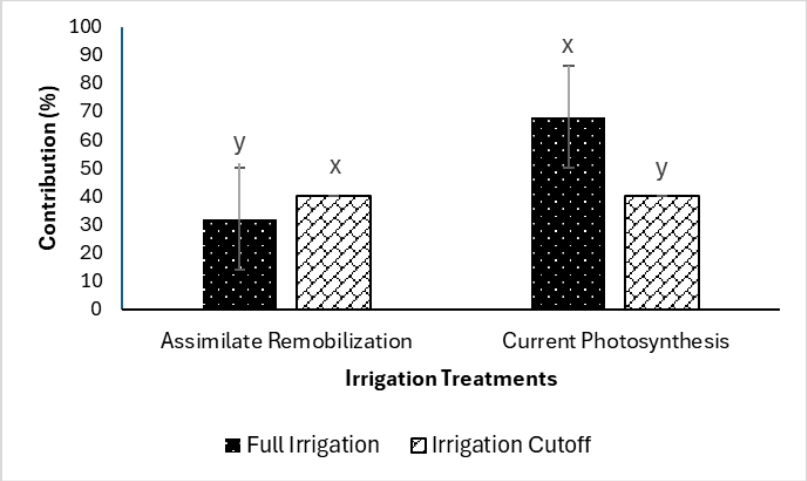


Figure 2. The mean comparison of assimilate remobilization and current photosynthesis contributions under full irrigation and irrigation cutoff (drought stress) treatments

CONCLUSION

This study highlights the significant impact of soil moisture deficit on wheat grain yield and its key components. Drought stress notably reduced spikelets/spike, grain weight/spike, spike weight, and grains/spike, especially during critical growth stages such as spike development and grain filling. Physiological responses, including a reduced harvest index and diminished contribution from current photosynthesis, further contributed to yield losses, shifting reliance toward assimilate remobilization for grain filling.

Among the genotypes tested, drought-tolerant lines like Mihran and C-91-4 maintained comparatively higher yields under stress conditions, underscoring the importance of selecting and breeding for traits associated with stress resilience. The Mihran cultivar, with a high harvest index (50%), remobilization rate (52%), and moderate current photosynthesis contribution (57%), demonstrated strong adaptability and yield stability in semi-arid environments.

The findings underscore the necessity of incorporating traits such as enhanced assimilate remobilization, sustained photosynthetic activity, and optimized grain weight into breeding programs. Developing wheat varieties with these adaptive traits will be crucial for maintaining productivity under increasing water constraints. This research offers valuable insights for wheat breeders and agronomists working to improve drought resilience and ensure food security in water-limited regions.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

The author confirms sole responsibility for the study's conception, design, analysis, interpretation, and manuscript preparation

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Bread wheat responses to post-generative drought and heat stress: physiological, yield and quality implications

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Abstract

Post-generative drought and heat stress significantly impact wheat physiology, yield, and grain quality. This study investigated the effects of four different soil moisture levels (100%, 75%, 50%, and 25% of (WHC) water holding capacity) combined with heat stress (≥30ºC) conditions on four bread wheat cultivars from stem elongation to harvest in semi-controlled green house conditions. This study examined the physiological responses of the plants as assessed by flag leaf area, SPAD chlorophyll measurements and changes in dry matter, from anthesis to harvest. Yield formation parameters, including the number of grains per ear and ear yield, were analyzed alongside grain quality traits such as crude protein, fiber, starch content, and flour color parameters (L*, a*, b*). Additionally, total phenolic content and antioxidant activity were evaluated to determine the impact of water stress on wheat's bioactive compounds.The results revealed that severe water deficit (50% and 25% WHC) led to a significant decline in flag leaf area, SPAD chlorophyll values and dry matter accumulation in anthesis and post anthesis stages, and also grain yield. The cultivars responded to increasing water stress conditions as accelerated senescence as a results of a decrease in chlorophyll and biomass production values. However, water deficiency (50 and 25% WHC) influenced quality traits differently, with causing increases in protein content, total phenolic content and antioxidant activity. It is clear that increasing temperature and drought conditions will cause wheat yield reductions supported by physiological responses. The findings provide significant insights into wheat yield and quality response under water-limited conditions, simulating spring rainfall deficiency and increasing temperatures in post-generative growth stages.

Keywords: *Triticum aestivum* L., Drought, Phenology, SPAD, Yield, Quality, Antioxidant

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INTRODUCTION

Wheat is grown in many regions of the world because of its adaptability to different environments. It has long been a staple food, providing daily starch and energy. It is also one of the most important cereals in terms of health and nutrition, as it contains phytochemicals, vitamins and minerals that contribute to good health (Shewry and Hey, 2015). However, this plant will face significant challenges in production levels, which is of critical importance with regard to daily nutrition and health, and is expected to occur due to climate change, particularly in Mediterranean climate regions (Tita et al., 2025). Climate change poses significant challenges to global food security and cereal crop production by altering food availability and utilization. Increasing climate variability (increasing variability in temperature and precipitation) influences crop development and yield potential, with the extent of these effects dependent on phenological responses (a shift in timing of the most sensitive phases: anthesis etc.) to extreme weather conditions (Bracho-Mujica et al., 2024). Wheat researchers are closely monitoring these changes to mitigate potential risks that are projected and may occur in the future.

In Mediterranean climates, the distribution of rainfall is as critical as the total seasonal precipitation for wheat yield formation. It has been estimated that a 1% change in mean temperature during April and May results in a 1.4% decrease in wheat production in major wheat-growing regions, including Central Anatolia, the Mediterranean

region, Southeast Anatolia, West Marmara and West Black Sea of Türkiye (Demirhan and Bayraktar, 2025). Spring rainfall, in particular, exerts a pivotal role in determining yield outcomes due to its alignment with key phenological stages such as heading, flowering, and grain filling in addition to winter water budget (Passioura, 2007). It has been demonstrated that spring rainfall can serve as a buffer against terminal drought, a common constraint in Mediterranean regions where precipitation sharply declines from late spring onward. In instances where spring rainfall is inadequate, wheat crops are usually faced to residual soil moisture during the most important crucial phenological stages (Flohr et al., 2020). It has been determined that this situation has a significant impact on the effects of heat stress, particularly during the processes of anthesis and early grain filling. At these times, elevated temperatures within the canopy can have a deleterious effect on pollen viability and the subsequent reduction in grain set (Farooq et al., 2011).

Anthesis time is known to be the most important phenological developmental period in cereals, which determines the number of grains (results of sterility/fertility of spikelets) per ear and thus the yield. During this period, plants are highly sensitive to the most important abiotic (temperature and water scarcity) and biotic (insect and disease agents) stress factors. The timing and shifting in these crucial phenological stages (anthesis and post-anthesis) cause to face to stress conditions (drought and heat: >32-36°C) that result with greatly reduced grain formation and development of wheat plants resulting high yield variability. Heat stress conditions cause anther sterility by affecting pollen cell and microspore and high temperature of above 30ºC during anthesis may result with complete sterility depending on wheat genotypes (Akter and Rafiqul Islam, 2017). During the wheat growing season, 1°C increase in the average temperature value causes a decrease in wheat yield by approximately 5-8%. (Mirosavljević et al., 2024; Craufurd and Wheeler, 2009). In addition ear initiation and expansion may be affected by drought at stem elongation stage resulting as reducing plant biomass and the number of grains per ear which is limiting the higher yield potential (Pantha et al., 2024).

SPAD readings have been shown to have the potential to improve yield and quality prediction and allow for further evaluations to what extent is this relationship affected by the environment. SPAD values are also used in breeding programs for screening and selection of drought tolerant wheat cultivars. Additionally assessment of photosynthetic pigments is an important indicator of senescence related to yield and its components (Barutçular et al., 2016). It was posited that SPAD measurements represent a significant approach in the prediction of grain yield, and that generative period measurements are more efficacious than vegetative period measurements for this purpose (Monostori et al., 2016). In general, SPAD chlorophyll measurements provide essential information about the tolerance mechanism of genotypes under drought conditions. The stay-green trait in wheat is characterized by the prolonged greenness of flag leaf (after anthesis) during grain filling periods supporting dry matter accumulation by sustaining photosynthetic activity in plant's organs even under combined stress conditions (Sultana et al., 2021).

In overall, spring drought and heat stress significantly not only influence yield and its formation but also the chemical composition of wheat grains, particularly affecting protein and is quality. It has been demonstrated that the total protein content and gluten content increase by 65% and 32%, respectively, when subjected to combined heat and drought stress in comparison with control conditions (Lama et al., 2023). In addition, wheat flour color is an important in bakery products for appearance. Heat and drought stress can lead to decrease in brightness color spectrum (L*) of flour which means to appear darker. It is also plausible to observe redness (a*) and yellowness (b*) which are related to protein and pigment concentrations change in grain under combined stress conditions.

In contrast, whole wheat grain is distinguished by its significant contributions to health. It is notable for its high content of phenolic compounds and vitamins, which underscore its importance in daily nutrition and its role in promoting overall health and well-being. These health contributing compounds are mainly located in the outer layers of grain such as aleurone, testa and pericarp (Martini et al., 2015). It is evident that stress conditions result in alterations to the morphological and chemical structures of grains. Consequently, there can be considerable changes in the amount of these chemicals.

As a result, the aim of this study is to simulate the drought and heat stresses that occur in the most important important generative periods related to grain structure and yield formation of wheat to examine the changes in physiological, yield and important quality characteristics of the plant. In general terms, the objective of this study mentioned as follows;

a. To reveal the changes in flag leaf SPAD chlorophyll value and dry matter accumulation under stress conditions during the generative period,

b. To determine the level of change of important quality traits under stress conditions,

c. To examine the change in grain structure at the color level and to examine the response of the antioxidant activity and phenolic contents of the grain under stress conditions,

d. To examine the senescence of the wheat cultivars examined under limited irrigation conditions and high temperature conditions.

MATERIALS AND METHODS

Experimental design and conditions

The experiment was conducted and designed to create two distinct stress-inducing environments in semi-controlled greenhouse conditions to located at Aydın Adnan Menderes University, Faculty of Agriculture in Aydın. The commonly grown 4 bread wheat cultivars adapted to in Aegean parts of Türkiye used as plant material named Ceyhan 99, Efe, Masaccio and Osmaniyem. Experimental soil was collected from the field area at 0-30 cm depth before sowing the seeds and then analyzed for physico-chemical properties and water holding capacity (%). The experimental soil characteristics are loamy sand with slightly alkaline pH level (8.3). The organic content of the soil is low (1.6 %) while phosphorus (10.7 ppm), potassium (305 ppm), calcium and magnesium were high level in the pot experimental soil. All the materials were sown to 25 cm diameter pots according to split plot design with three replications (5 plants/pot) in 2022. In all trials, N fertilization was applied to plants at the pre-sowing, tillering and stem elongation stages at rates of 0.39, 1.15 and 0.825 kg/ha, respectively.

To adjust and simulate the environmental constraints with minimized water supply 4 irrigation levels of 25 % ($IR_{25\%}$), 50 % ($IR_{50\%}$), 75 % ($IR_{75\%}$) of the maximum water holding capacity (WHC; $IR_{100\%}$) were set in the pots by using gravimetric calculation method. For this purpose, WHC 100% was calculated as;

[WHC 100%: (Weight saturated soil-Weight dry soil)/Weight dry soil)*100] by assessing the soil moisture level by using calibrated cylinders (100 cm^3) of the fully saturated water (3 h in water for saturation and the draining of 2 h) and of the dry soil (24 h, 105ºC). The simulation of post-generative drought conditions was conducted through the application of daily irrigation to pots from the stem elongation stage (BBCH 31) to harvest of the plants. In order to induce conditions of drought and heat stress, the internal temperature of the greenhouse was hourly recorded during the generative development periods (Figure 1). During the course of the experiment in generative growth stages, which spanned 45 days, the maximum temperature recorded within the greenhouse was higher than upper limit (≥ 30º C) on 35 days. This resulted in heat stress during all of the observed generative growth periods.

Evaluated parameters and measurement details

For this study, the extended BBCH-scale for cereals were used to describe the all phenological stages of wheat plants (Meier, 2018). Agronomic, physiological, quality and antioxidant properties such as number of grains per ear (GN), ear yield (g: EY), flag leaf area (cm^2, $LeafA_{FLAG}$), SPAD chlorophyll change observations in anthesis and post-anthesis stages: SPAD measurement in anthesis ($SPAD_{ANT}$: BBCH 65), SPAD measurement in medium milk stage ($SPAD_{MILK}$: BBCH 75), SPAD measurement in soft dough stage ($SPAD_{DOU}$: BBCH 85), total biomass dry matter calculations in anthesis and post-anthesis stages: total biomass dry matter (%) in anthesis (DM_{ANT}: BBCH 65), total biomass dry matter (%) in medium milk stage (DM_{MILK}: BBCH 75), total biomass dry matter (%) in soft dough stage (DM_{DOU}: BBCH 85), grain protein, starch, fiber ratios (%), total phenolic content (µg GAE/g), total antioxidant activity (% DPPH radical scavenging) and grain color (brightness: L*, redness: a* and yellowness: b*) were investigated.

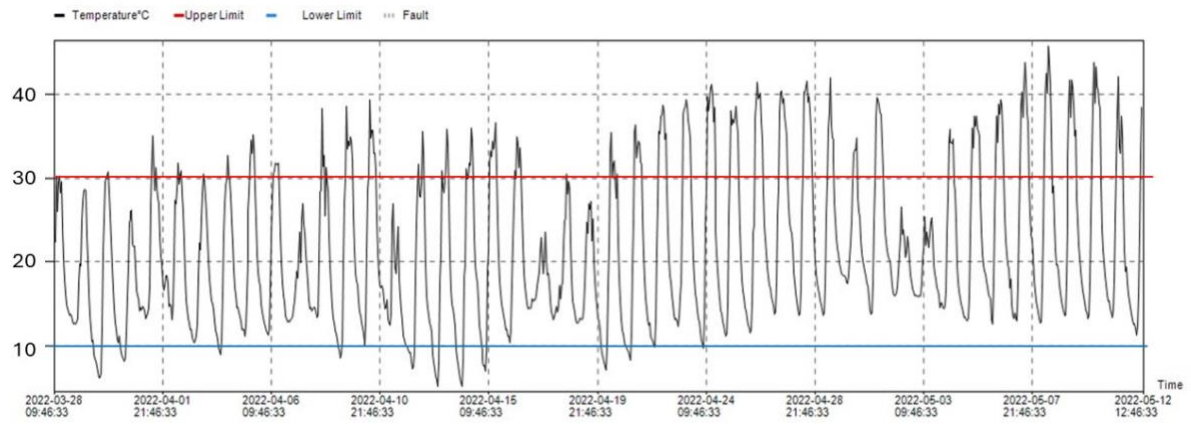


Figure 1. Daily hourly recorded temperature data during generative growth periods (Elitech Data logger; upper limit: ≥30ºC)

a)

b)

c)

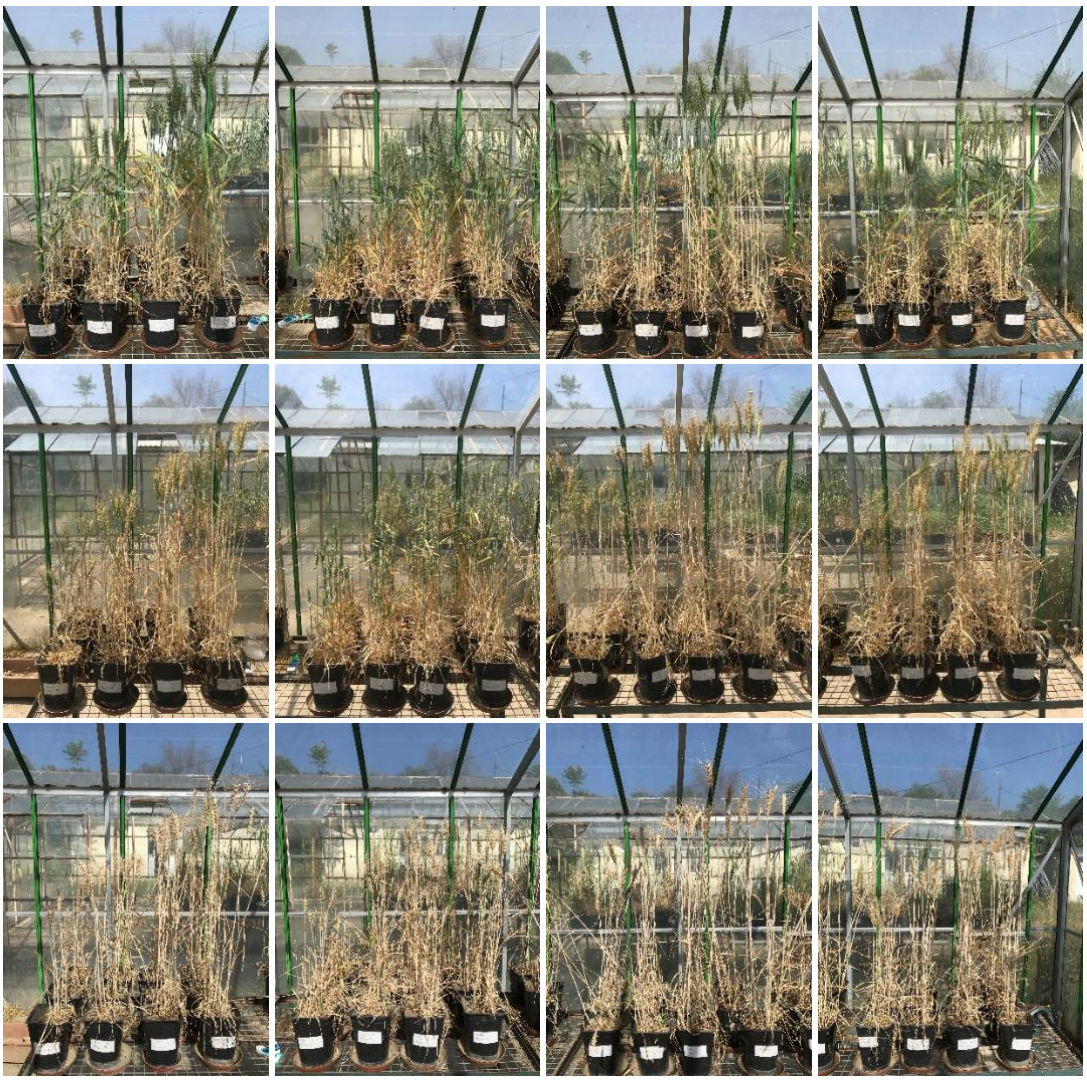


Figure 2. Images of senescence of wheat plants affected by combined stress conditions in grain filling periods (a: BBCH 75: Medium milk stage, b: BBCH 85: Soft dough stage, c: BBCH 99: Harvest; the order of the irrigation regimes from the left to the right is $IR_{25\%}$, $IR_{50\%}$, $IR_{75\%}$ and $IR_{100\%}$ and for cultivars: Ceyhan 99, Masaccio, Efe and Osmaniyem)

With the combination of heat and water stress conditions Soil Plant Analysis Development flag leaf (SPAD) chlorophyll content was measured to observe the senescence of plants during generative growth stages mentioned above. The chlorophyll measurements were conducted by using chlorophyll meter (SPAD-502, Konica Minolta, Japan). The flag leaf area measurement was performed by using LICOR (Lincoln, NE, USA) LI-3000C portable leaf area meter when flag leaf of plants fully visible (BBCH 47). Subsequent to the SPAD measurements, dry matter content measurements (24 h, 105ºC) were made by sampling randomly selected wheat plants above ground biomass (Figure 2.). After harvest the seeds were milled to whole wheat flour (0.8 mm) for quality and antioxidant analysis stored at +4ºC. The Near Infrared Reflected Spectroscopy (NIRS) method was used to analyze crude protein, starch and fiber content of wheat flour using Bruker MPA TM (Bruker, Ettlingen, Germany). The spectral grain color analysis of wheat flour was measured by using HunterLab Color Flex device (Reston, Virginia, USA). To determine total phenolic content and antioxidant activity of wheat flour acidic methanol solution (HCL/methanol/water, 1:80:10 v/v) was used and mixed with 1 g ground whole wheat flour under nitrogen gas for 1 h, then centrifuged (5000 rpm, 20 min.) then transferred to tubes and stored at +4ºC until phenol and antioxidant analysis. Folin-Ciocalteu method was used to determine total phenolic content described by Kaluza et al. (1980) and Ragaee et al. (2006) by using gallic acid as standard. For the total antioxidant activity of wheat samples, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method was used. In the last, the absorbance measurements of total phenolic content (725 nm) and antioxidant activity (517 nm) extracts were determined by Thermo Scientific spectrophotometer (Beta et al., 2005; Ma et al., 2014).

The data was subjected to analysis of variance for each parameter and all data were analyzed by using ANOVA and LSD test ($p \leq 0.05$) techniques in the "agricolae" package in R Studio v4 (de Mendiburu, 2019) statistical analysis software.

RESULTS AND DISCUSSION

ANOVA results for all parameters

According to ANOVA statistical analysis results, significant interaction ($p \leq 0.01$ and 0.05) was identified between variety and irrigation interaction (IrrixCul), with notable changes observed in anthesis and post-anthesis dry matter composition and chlorophyll concentration (SPAD), quality, and antioxidant properties except for flag leaf area and protein ratio of bread wheat varieties under varying water stress conditions. Irrigation in different WHC values in post-generative periods of wheat caused statistically significant differences ($p \leq 0.01$) in all evaluated parameters (except grain yellowness color; b*). At the same time, the cultivar factor also had a significant effect ($p \leq 0.01$ and 0.05) on all the characteristics that were analyzed (Table 1 and Table 2).

Table 1. Analysis of variance on irrigation regimes and cultivars for agronomic and physiological characteristics

		Mean squares								
Source	Df	G_N	E_Y	$LeafA_{FLAG}$	$SPAD_{ANT}$	DM_{ANT}	$SPAD_{MILK}$	DM_{MILK}	$SPAD_{DOU}$	DM_{DOU}
IR	3	695.2**	0.93**	487.6**	200.7**	119.6**	1098.0**	764.2**	1236.6**	143.8**
Cul	3	144.0**	0.37**	217.4**	404.7**	11.9ns	905.9**	719.7**	626.7**	1058.8**
IRxCul	9	21.2**	0.05**	20.3ns	63.7**	26.9*	151.8**	238.6**	74.4**	221.3**
Error	24	2.42	0.01	14.4	0.64	9.77	3.20	17.1	7.5	15.5

IR: Irrigation regimes; Cul: Cultivar; G_N: Number of grains per ear; E_Y: Ear Yield (g); $LeafA_{FLAG}$: Flag leaf area (cm^2); $SPAD_{ANT}$: SPAD chlorophyll value in anthesis stage (BBCH 65); DM_{ANT}: Total biomass dry matter (%) in anthesis stage; $SPAD_{MILK}$: SPAD chlorophyll value in medium milk stage (BBCH 75); DM_{ANT}: Total biomass dry matter (%) in medium milk stage; $SPAD_{DOU}$: SPAD chlorophyll value in soft dough stage (BBCH 85); DM_{ANT}: Total biomass dry matter (%) in soft dough stage.

Table 2. Analysis of variance on irrigation regimes and cultivars for quality and antioxidant characteristics

		Mean squares							
Source	Df	Protein	Starch	Fiber	L*	a*	b*	Phe	Aac
IR	3	47.5**	19.7**	2.65**	5.73**	0.22**	0.28ns	4055.2**	2.56**
Cul	3	6.46*	106.0**	3.79**	3.23**	0.71**	5.11**	8907.3**	6.37**
IRxCul	9	3.78ns	21.9**	0.55**	2.81**	0.29**	0.36*	4590.3**	9.19**
Error	30	1.73	1.35	0.02	0.34	0.03	0.13	43.9	0.31

Protein: Crude Protein Ratio (%); Starch: Crude starch ratio (%); Phe: Total phenolic content (µg GAE/g); Aac: Total antioxidant activity (DPPH %)

The results of ear yield formation, SPAD chlorophyll content and dry matter

The impact of heat stress, both in the pre- and post-anthesis phases, on grain number and yield traits in wheat has proven to be a subject of significant interest. The previous study demonstrates that pre-anthesis heat stress exerts a more pronounced negative effect on grain number resulted with grain abortion, while post-anthesis heat stress primarily reduces grain weight, ultimately lowering total yield (Kim et al., 2024).

From the onset of the generative development period, substantial losses occurred in yield and flag leaf area traits under varying levels of water and heat stress conditions applied to bread wheat varieties adapted to the region due to the escalation in combined stress. A subsequent analysis of the G_N revealed a broad distribution ranging from 7.16 to 31.8 across varying water holding capacity levels. A particularly notable finding was that under conditions of water stress ($IR_{25\%}$ and $IR_{50\%}$), the number of grains decreased significantly, with the highest values being attained at the $IR_{100\%}$ (26.8) doses and $IR_{75\%}$ (22.1). However, it is noteworthy that the Masaccio cultivar exhibited lower values in the majority of water treatments in comparison to other cultivars (Table 3).

The analysis of water stress conditions in terms of single ear yield revealed that the Efe cultivar exhibited superior performance in both limited irrigation and full irrigation scenarios, surpassing the other cultivars. Notably, at an irrigation level of 50% ($IR_{50\%}$), which is considered a restricted moisture condition, Efe exhibited a notably higher ear yield of 1.09 g. In contrast, a substantial decline in E_Y was observed in all varieties under limited irrigation ($IR_{25\%}$). Notably, the Ceyhan-99, Efe and Osmaniyem varieties exhibited the highest yield values at full water holding capacity ($IR_{100\%}$). At the level of all water treatments and cultivars, Ceyhan-99 reached the highest yield value of 1.110 g at the $IR_{100\%}$ dose, and Efe reached the highest yield value of 1.113 g at the $IR_{75\%}$ dose (Table 3.). It is hypothesized that the lower values of Masaccio in the G_N and E_Y compared to the other cultivars may be due to the cultivar's comparatively later and slower development. It is notable that this variety exhibits a notably high yield potential (approx. 9-10 t/ha) within the region, provided that sufficient soil moisture (with supplemental irrigation in ear formation) is available during the extended generative period.

Table 3. Number of grains per ear (G_N), ear yield (E_Y, g/ear) and flag leaf area ($LeafA_{FLAG}$, cm^2) results under combined stress conditions during post generative periods of bread wheat cultivars

		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
G_N	$IR_{25\%}$	9.61 hi	9.99 h	7.16 i	8.81 hi	8.89
	$IR_{50\%}$	18.8 e	22.6 d	15.3 fg	17.3 ef	18.5
	$IR_{75\%}$	22.8 cd	26.0 b	14.1 g	25.5 bc	22.1
	$IR_{100\%}$	31.8 a	28.0 b	19.3 e	28.1 b	26.8
	Mean C.	20.7	21.6	14.0	19.9	
Lsd IR: 1.94; Lsd C: 1.31; Lsd IR*Cul: 2.74						
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
E_Y (g)	$IR_{25\%}$	0.243 i	0.416 ghi	0.246 i	0.373 hi	0.320
	$IR_{50\%}$	0.783 cde	1.093 ab	0.463 fgh	0.586 efg	0.731
	$IR_{75\%}$	0.830 cd	1.113 a	0.553 fgh	0.893 bc	0.847
	$IR_{100\%}$	1.110 a	1.033 ab	0.663 def	1.036 ab	0.960
	Mean C.	0.741	0.914	0.481	0.722	
Lsd IR: 0.07; Lsd C: 0.11; Lsd IR*Cul: 0.20						
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
$LeafA_{FLAG}$ (cm^2)	$IR_{25\%}$	10.5	9.37	8.17	13.5	10.3 C
	$IR_{50\%}$	15.4	14.1	7.42	18.6	13.9 C
	$IR_{75\%}$	24.5	20.8	13.3	20.3	19.7 B
	$IR_{100\%}$	26.8	28.1	15.0	29.2	24.8 A
	Mean C.	19.3 A	18.1 A	11.0 B	20.4 A	
Lsd IR: 3.61; Lsd C: 3.24						

Pre-anthesis drought stress has been shown to reduce leaf area and chlorophyll content, leading to reduced photosynthetic capacity and potential yield loss. Post-anthesis drought stress also affects chlorophyll content. Ahmadi-Lajihani and Emam (2016) suggests that higher chlorophyll content under water deficit conditions is associated with increased photosynthetic rates. Maintaining stay-green duration of flag leaf with greater leaf area during grain filling contributes to improved yield under drought stress.

It was determined that the flag leaf area of wheat plants decreased significantly depending on the limited soil moisture and high temperature conditions initiated during the stem elongation (BBCH 31) to harvest period and heat stress. A marked decline in $LeafA_{FLAG}$ was observed in response to declining soil moisture levels, particularly in cases where a linear decrease occurred, commencing from the point of full water-holding capacity ($IR_{100\%}$) and progressing towards conditions of limited irrigation conditions ($IR_{50\%}$ and $IR_{25\%}$). As given in Table 3, the Masaccio cultivar had the smallest $LeafA_{FLAG}$ in addition to other yield parameters ($p \leq 0.01$).

The analysis of flag leaf SPAD chlorophyll values and dry matter changes was conducted during the anthesis and post-anthesis phenological growth periods. This analysis was undertaken to determine the senescence levels of plants and to observe dry matter accumulation in wheat plants under conditions of limited soil moisture and high temperature stress (Figure 3). It is noteworthy that, Ceyhan-99 cultivar has attained the highest SPAD values with the least chlorophyll change in all development periods under the condition of $IR_{75\%}$. In addition, studies have shown that drought-tolerant wheat varieties maintain higher leaf chlorophyll content under drought conditions than sensitive genotypes. This trait is positively correlated with higher yield under stress (Ghaffar et al. 2023).

In addition to the variation in SPAD chlorophyll, when the changes in dry matter accumulation during these developmental periods was analyzed, the cultivar Efe, which has the highest ear yield E_Y, g), caused the highest dry matter accumulation during the grain filling periods (DM_{MILK} and DM_{DOU}) and reflected the assimilates it stored positively as yield, even under water and heat stress. Furthermore, both the Ceylan-99 and Efe cultivars demonstrated the capacity to maintain their yield values, exhibiting reduced senescence and enhanced assimilate accumulation under conditions of combined stress. In general, the level of greenness of the flag leaves of the cultivars decreased significantly under restricted irrigation conditions, and they had the lowest value at $IR_{25\%}$ under water stress conditions, especially at dough maturity. At the end of this growth period, Efe exhibited the highest accumulation of dry matter at the highest water holding capacity ($IR_{100\%}$) under water and heat stress, while the Ceyhan-99 with its high level of almost ≈80% dry matter accumulation at $IR_{50\%}$ under restricted irrigation conditions, achieved significant results under both stress conditions (Figure 3.).

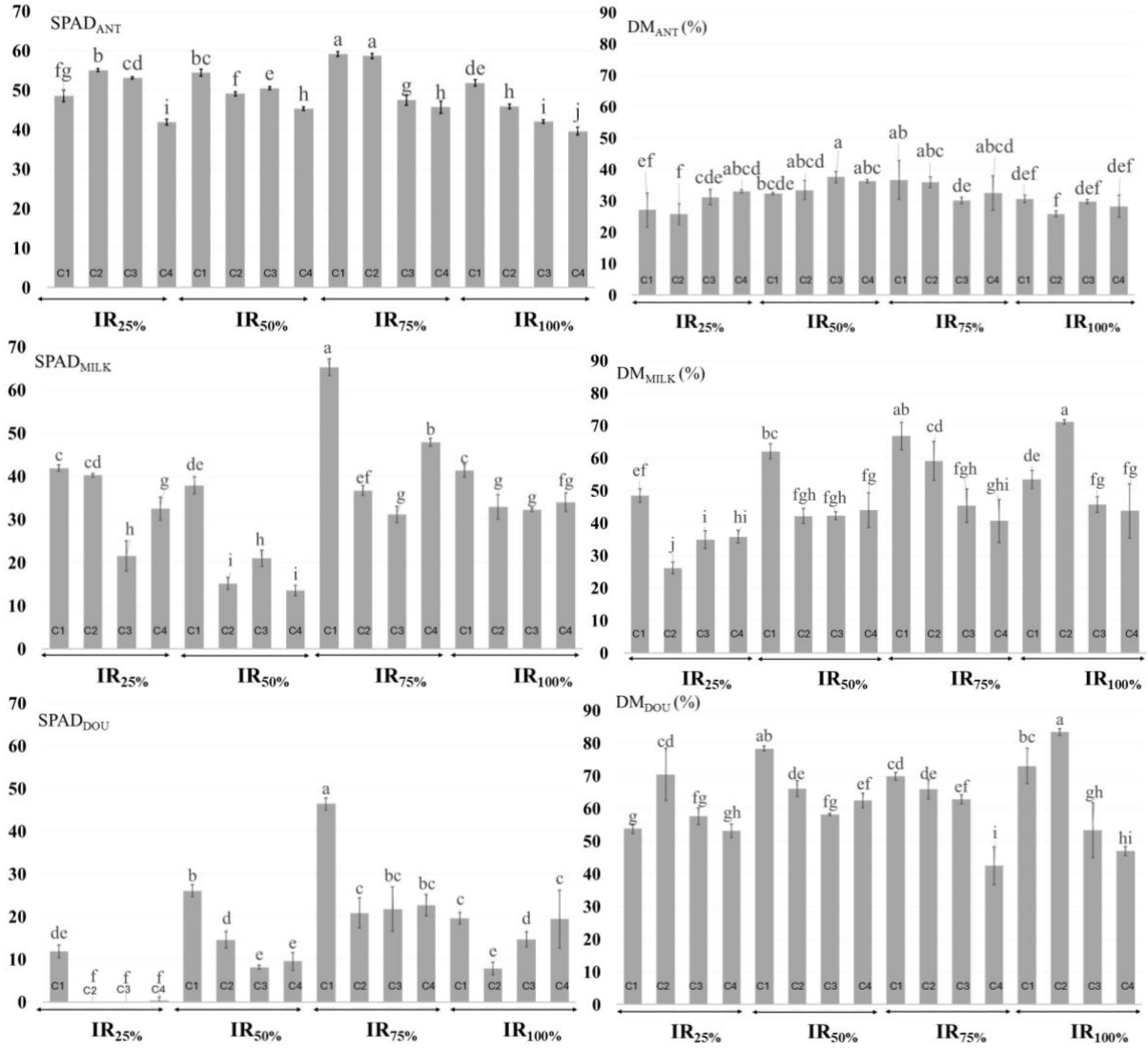


Figure 3. Flag leaf SPAD chlorophyll and dry matter content change in anthesis and post-anthesis growth periods in water and heat stress conditions (C1: Ceyhan 99, C2: Efe, C3: Masaccio, C4: Osmaniyem)

As was evidenced in previous studies, drought conditions have been demonstrated to result in a considerable decline in both chlorophyll concentration and total biomass in wheat. A study involving 40 local wheat cultivars found that drought stress led to notable decreases in shoot and root fresh and dry biomass, plant height, flag leaf area, and total chlorophyll content (SPAD). Specifically, the reduction in total chlorophyll content under drought stress was found to be 35% (Ghaffar et al. 2023).

It is imperative to maintaining the soil moisture within the (optimal range of) 75% to 100% of soil water holding capacity to ensure optimal yield in wheat during these critical developmental phases, which frequently align with the spring season. It is widely acknowledged that a decline in spring precipitation, consequent to climate change, has the potential to engender substantial reductions in yield components during these developmental phases (grain-filling) of plant growth (Nazim Ud Dowla et al., 2018).

The results of grain quality and flour color characteristics

In the context of combined stress conditions, Ceyhan 99 exhibited a notable increase in yield, chlorophyll and dry matter accumulation, while Efe demonstrated a marked enhancement in protein and fiber ratios. While the protein ratio in grain increased significantly (16.2 and 17.3%) under limited water conditions, the protein ratio decreased (14.6 and 12.7%) under sufficient moisture conditions. The protein content of wheat increased by approximately 36% under the highest restricted irrigation dose ($IR_{25\%}$). Post-anthesis heat and drought stress have been demonstrated to significantly influence the protein content and composition of wheat grains. A number of studies have reported an increase in total protein content under such stress conditions. For instance, Fernie et al. (2022) reviewed that exposure to temperatures exceeding 30°C led to a 0.78% to 0.82% increase in grain protein content for every unit increase in heat degree days above this threshold. A similar outcome was reported by another

study, which found that a 2-day heat treatment at 38°C, applied 20 days after flowering, resulted in a 5.0% and 2.7% increase in total protein content for two different wheat cultivars, compared to control conditions. Moreover, a combination of heat and drought stress has been documented to enhance total protein content by 65% and gluten content by 32% in comparison with control conditions (Lama et al., 2023). As a result, these stressors demonstrated significant positive influence on grain protein value, particularly affecting dough properties and end-use quality.

Table 4. Grain quality characteristics results under combined stress conditions during post generative periods of bread wheat cultivars

		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
Protein (%)	$IR_{25\%}$	17.0	16.2	16.9	19.1	17.3 A
	$IR_{50\%}$	18.5	15.4	16.2	14.5	16.2 A
	$IR_{75\%}$	15.6	14.3	14.7	13.8	14.6 B
	$IR_{100\%}$	14.0	12.8	12.5	11.6	12.7 C
	Mean C.	16.2 A	14.7 B	15.1 B	14.7 B	
	Lsd IR: 1.22; Lsd C: 1.12					
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
Starch (%)	$IR_{25\%}$	58.8 de	56.9 ef	59.5 cd	67.1 a	60.6
	$IR_{50\%}$	55.4 f	59.5 cd	60.1 cd	64.9 b	60.0
	$IR_{75\%}$	56.4 f	65.1 b	65.0 b	64.0 b	62.6
	$IR_{100\%}$	59.8 cd	64.4 b	61.2 c	63.5 b	62.2
	Mean C.	57.6	61.5	61.5	64.9	
	Lsd IR: 1.11; Lsd C: 0.99; Lsd IR*Cul: 1.94					
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
Fiber (%)	$IR_{25\%}$	3.38 de	4.56 a	3.77 c	2.61 g	3.58
	$IR_{50\%}$	3.79 c	3.43 d	3.34 de	2.51 gh	3.26
	$IR_{75\%}$	4.15 b	3.73 c	3.09 f	2.27 hi	3.31
	$IR_{100\%}$	3.14 ef	2.34 hi	2.36 h	2.11 i	2.48
	Mean C.	3.61	3.51	3.14	2.37	
	Lsd IR: 0.19; Lsd C: 0.11; Lsd IR*Cul: 0.24					

In general, the starch ratio values decreased in limited irrigation dose, while the Osmaniyem variety reached the highest value with 67.1% in $IR_{25\%}$ dose. It is notable that the starch ratio (55.4-67.1%), which constitutes a significant proportion of the grain, exhibits a decline under stress conditions. The Ceyhan 99 variety demonstrated notable performance in terms of yield and protein ratio, while exhibiting low starch ratio values under both limited and full irrigation conditions. Conversely, the Osmaniyem variety exhibited a contrasting response, exhibiting the lowest fiber ratio values under all water doses. The highest fiber ratio values were obtained at the highest restricted water level. Under conditions of water and temperature stress, an increase in protein and fiber ratio values was observed at 25% of soil water holding capacity level (Table 4).

Pre- and post-anthesis drought and heat stress have been demonstrated to exert a significant influence on the color attributes of wheat grains, the L* (brightness), a* (redness), and b* (yellowness) values. It can be mentioned that water and heat stress during the grain-filling period constitute significant abiotic factors that are capable of constraining both grain yield and quality in wheat. While the study primarily focuses on yield and protein content, it implies that water scarcity and heat stress can also affect grain quality parameters, which may include color attributes (Mahdavi et al. 2022). Heat stress during grain development has been reported to affect grain appearance, including color attributes. Specifically, heat stress can lead to changes in grain brightness (L*), redness (a*), and yellowness (b*), although the extent of these changes depends on the timing and severity of the stress, as well as the genotype (Hafeez et al. 2023).

An examination of the results pertaining to the L* of flour color revealed that the maximum value was attained at $IR_{50\%}$ under conditions of limited irrigation. Conversely, as the moisture content increased, the brightness value diminished. While Ceyhan-99 exhibited the lowest value on a cultivar basis, divergent results were obtained, particularly regarding interactions. In the case of limited irrigation ($IR_{50\%}$), the brightness rate increased for the Ceyhan-99, Efe and Osmaniyem cultivars, while Masaccio reached the highest values under full water conditions ($IR_{100\%}$) and restricted irrigation conditions ($IR_{25\%}$), although no clear result was obtained. It is hypothesized that there will not be sufficient level of assimilate accumulation in the grain filling stage under limited irrigation conditions and starch ratio will be less as an important factor in the increase of L* value. While this hypothesis is supported by the findings of low starch and L* ratios in Ceyhan99 under $IR_{25\%}$ conditions, the overall outcomes of the water-variety interaction are complex (Table 5).

Table 5. Bread wheat grain color (Brightness: L*, redness: a*, and yellowness: b*) results under combined stress conditions during post generative periods of bread wheat cultivars

		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
L* Brightness	$IR_{25\%}$	80.3 f	83.8 a-d	83.8 abc	81.6 f	82.4
	$IR_{50\%}$	83.8 abc	84.5 a	83.6 a-d	84.0 ab	84.0
	$IR_{75\%}$	83.4 bcd	82.3 ef	82.9 cde	83.3 bcd	83.0
	$IR_{100\%}$	82.8 de	83.6 a-d	84.4 a	83.2 b-e	83.5
	Mean C.	82.6	83.6	83.7	83.0	
Lsd IR: 0.629; Lsd C: 0.484; Lsd IR*Cul: 0.978						
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
a* Redness	$IR_{25\%}$	2.14 b-e	1.71 fg	2.09 cde	1.98 def	1.98
	$IR_{50\%}$	2.41 b	1.86 efg	1.95 def	2.19 bcd	2.10
	$IR_{75\%}$	2.32 bc	2.36 bc	1.70 fg	1.69 fg	2.02
	$IR_{100\%}$	2.92 a	1.60 g	2.34 bc	2.30 bc	2.29
	Mean C.	2.45	1.88	2.02	2.04	
Lsd IR: 0.197; Lsd C: 0.145 Lsd IR*Cul: 1.94						
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
b* Yellowness	$IR_{25\%}$	10.5 def	11.2 ab	10.2 def	10.4 def	10.6
	$IR_{50\%}$	11.5 a	11.3 ab	9.64 g	10.5 cde	10.7
	$IR_{75\%}$	11.4 a	11.7 a	9.92 fg	10.4 def	10.9
	$IR_{100\%}$	11.7 a	11.1 abc	10.1 efg	10.8 bcd	10.9
	Mean C.	11.3	11.3	9.99	10.5	
Lsd IR: 0.19; Lsd C: 0.288; Lsd IR*Cul: 0.608						

With regard to the redness (a*) value, the Ceyhan-99 variety was distinguished by its redder flour colour in comparison to the other varieties under all water conditions. The interaction between the Irri*Cul factor was not clearly determined by obtaining high/low values at full and limited irrigation doses. With regard to yellowness (b*), Ceyhan-99 exhibited elevated values in all water conditions except $IR_{25\%}$. The grain b* color value of the Ceyhan-99 was found to decrease significantly under limited water conditions. Furthermore, a comparison of Ceyhan-99 and Efe cultivars revealed that they exhibited higher values in all water conditions when compared to other cultivars. It is particularly noteworthy that the Ceyhan-99 and Efe varieties exhibited higher values in yellowness, a trait that is a significant criterion for quality (Table 5.). Although direct studies on these parameters under such stress conditions are limited, more targeted studies are required on these parameters. In summary, while direct measurements of L*, a*, and b* values under post-anthesis drought and heat stress are scarce, existing research suggests that such stress conditions can alter grain color. These changes are likely due to disruptions in pigment biosynthesis and deposition, as well as accelerated grain maturation.

The results of grain antioxidant activity and phenolic content

The total phenolic content and antioxidant activity of wheat grain, which is of significance to human health and is the most consumed daily food, demonstrated significant variation among the cultivars at differing water levels. Notably, the total phenol content and antioxidant activity exhibited their lowest values (2652 µg GAE/g, 31.6%) at full irrigation doses. Post-anthesis drought and heat stress have been demonstrated to significantly influence the accumulation of phenolic acids and antioxidant properties in wheat grains. A study examining six durum wheat cultivars under varying growth conditions found that ferulic acid was the predominant phenolic acid, ranging from 390.1 to 785.6 µg/g dry matter across all cultivars and conditions. It is of particular interest that severe drought conditions resulted in an increase in ferulic acid and total phenolic acids. Conversely, heat stress primarily enhanced the accumulation of minor individual phenolic acids rather than total phenolic acids (Laddomada et al., 2021). Osmaniyem exhibited the highest total phenol content (3431 µg GAE/g at $IR_{25\%}$ and 3381 µg GAE/g at $IR_{50\%}$) at limited irrigation doses, while the Ceyhan-99 exhibited the lowest content (2127 µg GAE/g at $IR_{100\%}$ full water holding capacity). With regard to antioxidant activity, the lowest value (31.6%) was observed at full water holding capacity. Furthermore, the Masaccio variety exhibited the highest antioxidant activity value at $IR_{50\%}$ under limited soil moisture conditions, while the Ceyhan 99 variety demonstrated the lowest antioxidant activity value (28.7%) at $IR_{100\%}$ (Table 6).

Table 6. Total phenolic acid content given in gallic acid equivalent (µg/g) and DDPH radical scavenging activity (total antioxidant: % inhibition) results under combined stress conditions during post generative periods of bread wheat cultivars

		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
Total Phenolic (µg GAE/g)	$IR_{25\%}$	2959 d	2863 d	2859 d	3431 a	3028
	$IR_{50\%}$	2173 fg	2874 d	2519 e	3381 ab	2737
	$IR_{75\%}$	2573 e	3251 c	2866 d	3239 c	2982
	$IR_{100\%}$	2127 g	2939 d	3291 bc	2250 f	2652
	Mean C.	2458	2982	2884	3075	
Lsd IR: 69.8; Lsd C: 55.0; Lsd IR*Cul: 110.5						
		Ceyhan-99	Efe	Masaccio	Osmaniyem	Mean IR.
Total Aac (DPPH %)	$IR_{25\%}$	33.2 bcd	32.2 ef	32.9 cde	32.6 def	32.7
	$IR_{50\%}$	31.1 gh	33.6 bc	34.6 a	30.0 i	32.3
	$IR_{75\%}$	32.9 cde	33.9 ab	32.1 ef	30.6 hi	32.4
	$IR_{100\%}$	28.7 j	31.7 fg	32.0 ef	34.0 ab	31.6
	Mean C.	31.5	32.9	32.9	31.8	
Lsd IR: 0.49; Lsd C: 0.48; Lsd IR*Cul: 0.93						

CONCLUSION

The drought that has occurred in recent years has had a detrimental effect on both winter and summer crops due to the spring and summer drought conditions in the Aegean region of Türkiye. This research focuses on the critical developmental stages of wheat, where climate factors notably impact yields, end-use quality and also health benefits. The evaluation of the obtained results indicated that the water and heat stress conditions applied from the stem elongation period onwards exerted a significantly negative impact on yield formation in the ear, flag leaf area, flag leaf SPAD chlorophyll change and biomass dry matter formation. However, it was determined that cultivars with optimal yield levels were more prominent than those with very high yield potential in the region, in terms of both yields, SPAD and dry matter accumulation. This finding suggests that these cultivars possess a higher tolerance for increasing stress conditions, exhibiting a lesser effect on yield characteristics and plant senescence levels. Consequently, it can be concluded that these cultivars, which also stand out in terms of some quality characteristics, can better tolerate adverse climate conditions. For instance, drought and heat stress have been demonstrated to have a detrimental effect on flag leaf chlorophyll content and dry matter accumulation in bread wheat (*Triticum aestivum* L.). However, the extent to which these effects manifest varies between genotypes, thereby suggesting that breeding programs with a focus on stress tolerance could mitigate these adverse effects.

In general terms, it has been found that the flag leaf senescence level in wheat increases and the yield potential decreases significantly under combined stress (drought and heat) levels. However, some quality, phenol and antioxidant activity characteristics have changed positively. The findings of this study, conducted under controlled conditions, indicate that the yield potential of wheat plants is significantly diminished under conditions of generative period stress. It is further understood that adverse conditions will occur in the production amount due to the effect of climate change. It is therefore concluded that conducting further studies under field conditions is of great importance in order to provide clearer information.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

There are not any conflict of interest.

Author contribution

A.Y; experiment conceptualization, methodology, data processing, investigation, writing and editing.

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Effect of PGPB on leaf nutrient status and growth of *Begonia semperflorens*

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Abstract

Bacteria are increasingly used in agriculture as eco-friendly alternatives to promote plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, hormone production, and improved nutrient uptake. This study aims to examine the effects of plant growth-promoting bacteria on the growth, quality, and nutrient content of *Begonia semperflorens*, a commercially valuable ornamental plant known for its long flowering period and vibrant blooms. In this study, one-month-old *Begonia semperflorens* seedlings were dipped into bacterial suspensions (10^8 CFU ml^{-1}) of *Pseudomonas chlororaphis* MF-1, *Bacillus megaterium* M-3, and *Agrobacterium radiobacter* A-16 for 20 minutes prior to planting. After planting, the seedlings were irrigated with bacterial suspensions as watering solution at twice at 15-day intervals. At the end of one month, plant growth and quality parameters (plant height, number of leaves, leaf area, plant fresh weight, plant dry weight, stem diameter, flower stem, plant crown width, number of blooming flowers). were evaluated, along with macro- and microelement contents (total N, P, K, Ca, Mn, Mg, and Fe) in both plant leaves and the growing medium. All bacterial treatments significantly improved plant growth and quality compared to the control group, with M-3 exhibiting the most pronounced effects, increasing plant height by 40%, leaf number by 133%, leaf area by 348%, and flower number by 61%. A-16 enhanced crown width (45%) and flower stem length (26%), while MF-1 improved plant height (43%) and flower production (51%). Additionally, M-3 increased total nitrogen in leaves by 43%, while bacterial treatments enhanced various elements in plant leaves. The results show that these bacterial isolates improve begonia plant characteristics, providing high-quality plants and offering a sustainable alternative as microbial fertilizers in ornamental plant cultivation.

Keywords: *Pseudomonas chlororaphis*, *Bacillus megaterium*, *Agrobacterium radiobacter*, *Begonia semperflorens*, Microbial fertilizer

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INTRODUCTION

Efforts to find solutions that contribute to reducing the use of chemical fertilizers in agricultural production have gained momentum within the framework of sustainable agriculture. As a sustainable substitute for heavy chemical use, biofertilizers support plant growth by releasing a variety of growth-promoting hormones and enhancing the availability of essential inorganic macro- and micronutrients (such as phosphorus solubilization, potassium solubilization, biological nitrogen fixation, zinc solubilization, and the production of hydrolytic enzymes like mannanase, phytase, etc.) (Sarıbuga et al., 2014; Karagöz et al., 2021). Therefore, studies focused on the use of microorganisms and their products to promote plant growth will contribute to the reduction of chemical fertilizer use in agricultural production and environmental pollution, thereby supporting the development of sustainable agriculture.

Scientific research has shown that a variety of microorganisms have been successfully used in organic agriculture (Aksoy and Yılmaz, 2008). Among these, bacteria are undoubtedly the most commonly used microorganisms in agriculture. Within the realm of bacteria, those found in the plant root rhizosphere play a crucial role in controlling plant diseases and pests, promoting plant growth, enhancing plant systemic resistance to stress

conditions, and facilitating the breakdown of organic and inorganic substances in the soil for easier plant uptake. (Karakurt et al., 2011; Tozlu et al., 2012; Şenol et al., 2014; Dikbas et al., 2023). Plant growth promoting bacteria (PGPB) mostly belong to the genera *Acinetobacter, Aereobacter,Alcaligenes,Agrobacterium, Artrobacter, Azospirillum, Azotobacter, Beijerinckia, Burkholderia, Bacillus, Erwinia, Enterobacter, Micrococcus, Pseudomonas, Flavobacterium, Paenibacillus Rhizobium, Rhodosprilum, Rhodobacter, Serratia* and *Xanthomonas* (Dandurand et al., 1994; Daza et al., 2000; Dadasoğlu et al., 2013). The most studied PGPB species belong to the genera *Paenibacillus, Bacillus, Pseudomonas* and *Azospirillum* (Çakmakçı et al., 2006; Sezen, 2012). Successful results have been observed in terms of plant development and yield with the use of PGPBs. (Liu et al., 1995; Altın, 2004; Wang et al., 2005; Altın and Bora, 2015; Karagoz et al., 2018; Tozlu et al., 2019; Şenol Kotan, 2020).

PGPB is widespread in agricultural and natural environments and is isolated from plants, flowers, seeds, water, soil, animals, and humans (Wang et al., 2022). It exists as both an epiphyte and an endophyte in plants (Pantoea agglomerans, 2024). PGPB exhibits beneficial antibacterial activity against phytopathogens, making it a preferred biocontrol agent and plant growth promoter (Guevarra et al., 2021). It provides many benefits to plant symbionts through direct and indirect mechanisms. It facilitates nitrogen fixation in plant roots by utilizing atmospheric nitrogen (Dobbelaere et al., 2003; Çakmakçı et al., 2005; Shridhar, 2012; Mohammadı, 2018). It also solubilizes potassium, ammonia, and phosphorus in symbiont plants, effectively serving as a natural fertilizer. Additionally, it uses bacterial siderophores to degrade heavy metals such as iron, offering bioremediation applications for soil pollution caused by heavy metals (Khan 2005; McCauley et al., 2009; Anonymous, 2024).

The ornamental plant industry is continually advancing with new technologies and cultivation methods to deliver high-quality plant material. However, challenges like rising production costs, pests, disease spread, and climate change persist (Cardoso et al., 2022). As a result, it's crucial to develop next-generation yields and establish standards for more efficient and sustainable production. This study was conducted due to the limited utilization of *Begonia semperflorens* in bacterial applications, despite its frequent use in various studies on ornamental plants. In this study, the effects of *Pseudomonas chlororaphis* MF-1, *Bacillus megaterium* M-3 and *Agrobacterium radiobacter* A-16 isolates on the growth parameters, quality traits and macro and micro element content of Begonia semperflorens plant were evaluated for the first time. The study aims to contribute to sustainable applications in ornamental plant cultivation and to reveal new bacterial isolates that will be evaluated as biofertilizer candidates especially for begonia.

MATERIALS AND METHODS

Bioagent bacteria and plant material used in the study

Pseudomonas chlororaphis MF-1*, Bacillus megaterium* M-3*, Agrobacterium radiobacter* A-16 bacteria were chosen for its biological controls and plant growth-promoting properties (Kotan et al. 2004; Karakurt et al., 2011; Tozlu et al., 2012; Senol et al. 2014; Şenol Kotan et al., 2024). The bacteria were obtained from the culture collection unit at the Plant Protection Department of Atatürk University's Faculty of Agriculture in Turkey. Begonia semperflorens, an evergreen perennial species renowned for its extended blooming period and vibrant flowers, was selected for this study due to its substantial commercial value in landscape and floral design, as well as to examine its response to plant growth-promoting bacterial isolates; the seedlings used in the experiments were sourced from a retailer in Turkey.

Calculation of viable bacterial counts

For bacterial enumeration, 10 ml serial dilutions (10^{-1}-10^{-9}) were made by transferring a 1 ml gr^{-1} sample into 9 ml of sterile peptone. Each dilution was mixed and 100 µl was spread on NA-containing petri dishes. The dilutions were inoculated into three Petri dishes each, incubated at 28°C for 24-48 hours, and bacterial counts were determined from three replicates, with the total count (CFU) calculated by multiplying the dilution factor (Anonymous, 2005).

Greenhouse studies

One-month-old seedlings were dipped into the prepared bacteria and planted in pots after waiting for 20 minutes (Figure 1). Bacteria isolates were used for bacterial treatment, and begonia seedlings were irrigated twice at 15-day intervals with bacteria-containing media. Sterile nutrient broth was used in the control application. 25 ml of bacteria solution (10^8 CFU ml^{-1}) was given to each pot depending on the application. Experimental soil peat, characterized by a particle size of 0–40 mm, a pH ($CaCl_2$) of 5.2–6.0, porosity of 95–99%, dry bulk density of <55–90 g/L, and organic matter content of 95–99%, was used as the growth medium. The seedlings were planted in pots with a volume of 0.5 L. The plants were irrigated with equal water every 2-3 days according to their water needs. The experiment was conducted from July 1 to August 1 in a greenhouse in Erzurum, Türkiye, under natural photoperiod conditions (14–15 h daylight). Average daytime and nighttime temperatures were 24–28 °C and 16–20 °C, respectively. Relative humidity was maintained at 50–70%. After a 30-day period, data regarding both plant growth and the growing medium were collected.

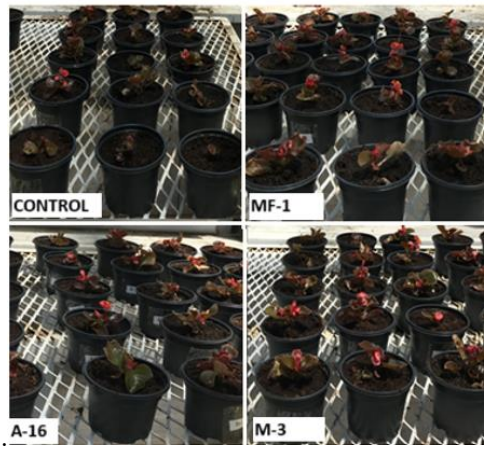


Figure 1. Potted image of one month old begonia seedlings after applications

Determination of plant growth parameters

The study evaluated several growth parameters (plant height (mm), number of leaves (leaves.plant^{-1}), leaf area (cm^2), plant fresh weight (g), plant dry weight (g), stem diameter (mm), flower stem (mm), plant crown width (mm), number of blooming flowers (number.plant^{-1})). For dry weight measurement, the leaves of the plants were placed in paper bags and dried in an oven at 80°C for 48 hours. After drying completely, their dry weights were measured using a precision balance with an accuracy of 0.001 grams (Karagöz et al., 2021).

Plant and growing medium nutrient analysis

Total nitrogen content in plant samples was measured using the micro Kjeldahl method (Bremner 1996). The determination of nutrients (P, K, Ca, Mg, Fe) in plants was carried out using an ICP OES spectrophotometer (Inductively Coupled Plasma spectrophotometer) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) as described by Karagöz et al. (2021). For the growing medium, extractable P, K, Ca, and Mg contents were determined using the Mehlich-III extraction method, as outlined in the Soil and Plant Analysis Council (2000). Elemental concentrations in the extracts were also measured using ICP-OES.

Experimental Design and Statistical Analysis

The pots used in the greenhouse studies were distributed according to the randomized block design, each treatment consisted of 5 replications, and in each replication, 3 pots were used. In total, 15 pots were used for each treatment. Data analysis was performed using analysis of variance (one way-ANOVA) in SPSS version 20.0 (SPSS Inc.), with mean differences assessed through Tukey's HSD multiple range test. Statistical significance was set at a p-value of less than 0.05. All experiments were conducted in five times, and the results are presented as the mean ± standard deviation with a 95% confidence interval (Karagöz et al., 2021).

RESULTS AND DISCUSSION

Bacterial isolates were applied to *Begonia semperflorens*, a species widely used in the ornamental plant industry, at 15-day intervals. Their effects on plant growth and quality were evaluated at the end of the experiment (Table 1). According to ANOVA results, bacterial treatments had a statistically significant effect ($p < 0.05$) on several growth and quality parameters, including plant height, number of leaves, leaf area, fresh and dry weight, stem diameter, flower stem length, crown width, and number of blooming flowers. Among the tested isolates, *B. megaterium* (M-3) showed the most notable effect, particularly on vegetative growth characteristics.

Table 1. Vegetative growth of begonia plant in response to bacteria suspension treatments

Plant Parameters	Applications			
	Control	MF-1	A-16	M-3
Plant height (mm)	89.0±20 ^{b}	127.7±8 ^{a}	117.8±14 ^{a}	125.2±16 ^{a}
Number of leaves (leaves·plant^{-1})	19±2.4 ^{c}	33±4.5 ^{b}	31±2.4 ^{b}	44.3±4.2 ^{a}
Leaf area (cm^2)	160.9±12.3 ^{c}	449.2±28.0 ^{b}	642.1±36.1^{a}	721.3±35.4 ^{a}
Plant fresh weight (g)	53.92±6.2 ^{d}	108.3±1.9 ^{c}	110.8±5.5 ^{b}	118.2±14.2 ^{a}
Plant dry weight (g)	2.1±0.4 ^{c}	3.89±0.1 ^{b}	3.73±0.2 ^{b}	3.97±0.4 ^{a}
Stem diameter (mm)	7.76±0.9 ^{b}	7.83±0.8 ^{b}	9.14±1.4 ^{a}	9.55±1.0 ^{a}
Flower stem (mm)	32.9±4.1 ^{b}	35.81±4.9 ^{a}	41.68±6.8 ^{a}	37.27±3.8 ^{a}
Plant crown width (mm)	121.6±1^{b}	159.6±18 ^{a}	177.0±14 ^{a}	161.7±15 ^{a}
Number of blooming flowers (number.plant^{-1})	4.7±2.3 ^{b}	7.1±1.6 ^{a}	7.4±1.9 ^{a}	7.6±1.8 ^{a}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$). (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

The effects of bacteria on begonia plant growth parameters were studied of this study. In begonia plants, bacterial treatments led to significant improvements in various growth parameters compared to the control. Plant height increased by 43% with the MF-1 *P. chlororaphis* isolate, 40% with the M-3 *B. megaterium* isolate, and 32% with the A-16 *A. radiobacter* isolate. The number of leaves increased by 133% with the M-3 isolate, while leaf area increased by 348% with the M-3 isolate and by 299% with the A-16 isolate. Furthermore, the leaf fresh weight increased by 119% and leaf dry weight by 89% with the M-3 isolate. Stem diameter increased by 23% with the M-3 isolate and by 17% with the A-16 isolate. Flower stem length increased by 26% with the A-16 isolate. Plant crown width increased by 45% with the A-16 isolate, 32% with the M-3 isolate, and 31% with the MF-1 isolate. The number of flowers increased by 61% with the M-3 isolate, 57% with the A-16 isolate, and 51% with the MF-1 isolate compared to the control. Bacterial applications showed significantly higher values than the control in all plant parameters considered statistically significant

In a previous study; It was determined that many bacterial formulations caused increases in both plant development and plant chlorophyll amount (Aktaş 2014). It has been noted that the bacterial strain *Pseudomonas fluorescens* positively impacts plant characteristics, including plant height, leaf surface area, and the number of flowers per plant in *chrysanthemums* and *dahlias*. (Göre & Altın, 2006), *Azospirillum* and *Bacillus* strains on *Petunia* (Hoda & Mona, 2014), *Paenibacillus polymixa*, *Achromobacter xylosoxidans*, *Pseudomonas putida*, *Pantoea agglomerans*, *Brevibacillus brevis*, *Bacillus megaterium*, *Bacillus megaterium* strains on *Cyclamen persicum* (Girgin 2019) showed an increase in flowering date, number of flowers/branches and flowering duration. In the study conducted by Şekerci & Ünlü (2023), it was stated that two different bacterial formulations using *B. subtilis* and *B. cereus* provided a two-fold increase in seedling height and number of leaves and a four-fold increase in seedling fresh weight in kalanchoe plants. In addition, PGPB applications; It has been reported that it has positive increases on plant growth parameters in species such as geranium (Mishra et al., 2010), tulip (Parlakova, 2014), jasmine (Jayamma et al., 2014), calendula (Arab et al., 2015) (Dalda Şekerci & Ünlü 2023). It was determined that all bacterial isolates used in the study also provided significant increases in plant growth parameters in begonia plants. Begonia plants at the end of the study are shown in Figure 1.



Figure 1. The effect of control and bacteria applications on plants (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

As a result of the application of bacteria to the plant, the nutrient element content in the plant leaf was examined and the results are given in Table 2. According to the plant nutrient element analysis results performed on the leaves of the plants, it was determined that there were statistically significant differences in Total N, P, Ca, K and Mg elements. It was observed that there was no statistically significant difference in Fe elements.

Table 2. Results of nutrient analysis in begonia leaves

Nutrition Elements	Applications			
	Control	MF-1	A-16	M-3
Total N (%)	2.75±0,02 ^{d}	3.06±0,03 ^{c}	3.20±0,04 ^{b}	3.95±0,06 ^{a}
P (%)	0.78±0.01 ^{d}	0.90±0.04 ^{a}	0.79±0.08 ^{c}	0.86±0.01 ^{b}
K (%)	3.86±0.06 ^{d}	4.51±0.05 ^{a}	4.16±0.02 ^{b}	4.06±0.06 ^{c}
Ca (%)	0.64±0.01 ^{b}	0.65±0.01 ^{b}	0.66±0.01 ^{a}	0.59±0.01 ^{c}
Mg (%)	0.79±0.01 ^{b}	0.80±0.01 ^{a}	0.74±0.01 ^{c}	0.68±0.01 ^{d}
Fe (ppm)	18.77±1.05^{ns}	20.07±0.39 ^{ns}	18.60±0.48 ^{ns}	18.61±0.20 ^{ns}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$), NS: Statistically insignificant result. (MF-1: *P. chlororaphis*, A-16: *A. radiobacter,* M-3: *B. megaterium*)

According to the nutrient element analysis carried out on plant leaves, the highest value for total N element was reached with M-3 isolate, the highest value for P, K and Mg elements was reached with MF-1 isolate, and the highest value for Ca element was reached with A-16 isolate. For Fe element, where no statistically significant difference was found, the highest value was again reached with MF-1 isolate. Nitrogen, which is one of the most important nutrients that plants must definitely take from their growing environment, is an important factor limiting plant growth after water (Vitousek & Howarth 1991). Total nitrogen in plant leaves was found to be 43% higher with the application of M-3 isolate and 16% higher with the application of A-16 isolate compared to the control application. In general, the sufficiency level of total nitrogen in the leaf was reported by Pritss and Handley (1998) as 2.0-2.8%, and by İbrikçi et al. (1994) as 2.50-3.20%. According to the studies, it was found that the total nitrogen amount in the leaf was sufficient and bacterial applications increased the total nitrogen amount compared to the control application. It has been shown that nitrogen-fixing bacteria provide resistance to pathogens, reduce growth period, stimulate plant development, and increase flowering, yield and/or quality (Bashan & de-Bashan 2002; Shridhar 2012; Mohammadı 2018).

Phosphorus is an important element required by plants for the formation of Adenosine Triphosphate (ATP), sugars and nucleic acids. The P element in plant leaves was found to be 15% higher in the MF-1 isolate and 10% higher in the M-3 isolate compared to the control application. Potassium increases the resistance of plants to diseases, provides seed maturation and development of the root system of the plant (Kacar & Katkat 2010). The amount of potassium in plant leaves was found to be 17% higher for the MF-1 isolate application and 8% higher for the A-16 isolate application compared to the control application. Çakmakçı et al. (2005) reported that P-solubilizing *Bacillus* RC07*, Bacillus* M-13, and *Rhodobacter* RC04, *Paenibacillus* RC05 bacteria increased N and P uptake. In addition, *Bacillus* RC07 and *Bacillus* M-13 were reported to be able to solubilize calcium phosphates. In another study, it was reported that PGPB applications increased the uptake of micronutrients as well as essential nutrients such as N, P and K by the plant (Dobbelaere et al. 2003; Çakmakçı et al. 2005; Karlıdağ et al. 2007). According to the results of the study, it can be said that the bacterial isolates used stimulate plant systemic resistance and support plant root development by increasing the uptake of potassium element into the plant. Magnesium helps the uptake of other elements, especially phosphorus, and plays a role in the activation of many enzymes (Plaster, 1992; Gardiner & Miller, 2008). The Mg element rate in plant leaves was found to be 3% higher for the MF-1 isolate application than the control application.

Iron element plays a very important role in plant respiration and photosynthesis reactions. In its deficiency, the amount of chlorophyll decreases and plant growth slows down (McCauley et al., 2009). The amount of Fe element in plant leaves was found to be 7% higher for the MF-1 isolate application compared to the control application. Iron plays an important role in the absorption of calcium and magnesium. Gardiner and Miller (2008). It was determined that PGPRs caused an increase in plant height, stem diameter, plant fresh weight, plant dry weight, root length, root dry weight, yield, fruit size and fruit number at varying rates. Khan (2005) revealed that bacteria such as *Acinetobacter* and *Pseudomonas* increased in Fe, Mg, Zn, K, Ca and P contents in the plant. The increase in Fe uptake by MF-1 may have stimulated chlorophyll synthesis and the plant growth parameters.

The nutrient element results obtained from the growing medium of the plants as a result of the application of bacterial isolates to the plant are given in Table 3.

Table 3. Results of nutrient analysis in growth medium

Nutrition Elements	Applications			
	Control	MF-1	A-16	M-3
Total N (%)	0.062±0,0006 ^{d}	0.067±0,0004 ^{c}	0.068±0,0002 ^{b}	0.072±0,0006 ^{a}
P (%)	0.14±0.006 ^{a}	0.10±0.002 ^{b}	0.06±0.002 ^{d}	0.07±0.004 ^{c}
K (%)	0.08±0.011 ^{a}	0.08±0.004 ^{a}	0.05±0.003 ^{b}	0.07±0.004 ^{a}
Ca (%)	0.69±0.03 ^{a}	0.60±0.009 ^{b}	0.55±0.007 ^{c}	0.60±0.007 ^{b}
Mg (%)	0.17±0.006 ^{a}	0.15±0.001 ^{b}	0.17±0.002 ^{a}	0.15±0.001 ^{b}
Fe (%)	0.13±0.003 ^{b}	0.12±0.002 ^{c}	0.19±0.002 ^{a}	0.13±0.001 ^{b}

*Mean values in the same column by the same letter are not significantly different from the HSD Tukey's multiple comparison test. ($P < 0.05$), NS: Statistically insignificant result.

According to the results of the nutrient element analysis in the growing medium, it was determined that there were statistically significant differences in total N, K, P, Ca, Fe and Mg elements. It was determined that the results of the total N determination in the growing medium were parallel to the total nitrogen ratio in the plant leaf. Total N element in the growing medium was found to be 17% higher in the M-3 isolate application, 9% in the A-16 isolate application and 3% in the MF-1 isolate application compared to the control application. It is thought that this situation is due to the ability of the bacterial isolates to fix nitrogen in the growing medium and to increasing its uptake into the plant at the right rate. It has been stated that PGPB bacteria can fix symbiotic or asymbiotic nitrogen, mineralize organic phosphate and other nutrient elements, and dissolve mineral phosphate and iron

(Çakmakçı et al. 2007; Karagöz et al. 2016a; Dadaşoğlu et al. 2017). It is thought that P, K, Ca and Mg elements were taken up from the soil to the plant at higher rates in bacterial applications compared to the control application and therefore they were low in the growing medium. In addition, the same situation is valid for Fe element for MF-1 and M-3 isolates. Compared to the control application in the growing medium, A-16 isolate decreased P element by 57%, K element by 37.5%, Ca element by 20%, and MF-1 and M-3 isolates decreased Mg element by 11% compared to the control application. It is known that bacteria applied to the soil also contribute significantly to the decomposition of organic matter in the soil and facilitating their uptake by the plant. It has been reported that PGPB applications increased the uptake of nutrient elements such as N, P, K, Mg and Fe (Khan; 2005; Karlıdağ et al. 2007; Karthikean, et al. 2010). Orhan et al. (2006) stated that bacterial applications significantly affected the available Mg in the soil.

CONCLUSION

Ornamental plant cultivation is increasingly turning to alternative methods to reduce dependency on chemical inputs. PGPBs, one of these alternatives, have significant potential in effectively controlling environmental and soil pollution. It was determined that MF-1 *P. chlororaphis*, A-16 *A. radiobacter* and M-3 *B. megaterium* isolates used in this study had highly positive effects on plant height, leaf number, leaf area, leaf fresh weight, leaf dry weight, stem diameter, flower stem, plant width and number of flowers opened in begonia plants compared to control application. Especially, M-3 bacterial isolate application had high total N element ratio in plant leaves and growing medium and showed the best result in many of the plant growth parameters, while MF-1 isolate application increased P, K, Mg, Fe elements in plant leaves, A-16 isolate application increased Ca element, Mg and Fe elements in plant leaves in growing medium and positively affected plant quality. According to these results, it is anticipated that the bacterial strains could serve as highly effective microbial fertilizers, particularly for begonia and other ornamental plants. There are limited studies in the literature regarding the use of such bacterial applications on plant growth parameters and the quality traits of potted ornamental plants. This highlights the value of the findings from the current study and underscores their potential contribution to future sustainable agriculture research.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declare no competing interests.

Author contribution

The author conducted the experiments, recorded and processed the data, designed tables and graphs, prepared the draft manuscript, revised and edited the final manuscript, developed and discussed and interpreted the results.

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Cytotoxic and antimicrobial analysis of biosynthesized selenium nanoparticles from *Solanum tuberosum* peels

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Abstract

In recent years, interest in the eco-friendly manufacturing of metal nanoparticles from plant extracts has surged. Nonetheless, no research has examined the combined antibacterial and anticancer properties of SeNPs synthesized with *Solanum tuberosum* (*S. tuberosum*) extract. This study involved the synthesis of selenium nanoparticles (ST-SeNPs) utilizing phytochemicals with reducing and capturing properties derived from the aqueous extract of *S. tuberosum* shell through a green synthesis approach. To determine the unique characteristics of ST-SeNPs nanoparticles, a variety of techniques were used, including scanning electron microscopy (SEM), zeta potential analysis, transmission electron microscopy (TEM), dynamic light scattering (DLS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), UV-visible (UV-Vis) spectroscopy, and energy dispersive X-ray spectrometry (EDX). The optical characteristics of ST-SeNPs were validated using UV-Vis measurement, revealing the peak absorbance at 350 nm. FTIR examination verified the presence of functional groups on the surface of the produced ST-SeNPs nanoparticles. Upon examination of the SEM results, it was concluded that the synthesized SeNPs exhibited uniform distribution and possessed a round morphology. The anticancer efficacy of the produced nanoparticles on the A549 lung cancer cell line and OVCAR-3 ovarian cancer cell line after 24 and 48 hours of exposure was assessed using the MTT test. It was established that elevated concentration inhibited cell growth. The inhibitory efficacy of SeNPs against the proliferation of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Candida albicans* (C. albicans) was assessed using the disk diffusion agar technique. The evaluated SeNPs exhibited antibacterial efficacy against bacterial and yeast cells. The results indicate that ST-SeNPs produced via green synthesis can serve as anticancer and antibacterial agents.

Keywords: *Solanum tuberosum* peels, Selenium nanoparticle, Anticancer, Antimicrobial

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INTRODUCTION

Nanotechnology is the name given to the entirety of scientific studies conducted in interdisciplinary technologies such as physics, chemistry, biology, materials science, electronics, and medicine with dimensions ranging from 1 to 100 nanometers. We can also say that this field is a set of studies conducted on a very small scale (Zambonino et al., 2021). Nanoparticles (NPs) used in the field of nanotechnology and nanoscience are unique due to their ultra-small sizes and offer significant advantages over materials such as better physical, chemical, and biological properties, larger surface area, spatial confinement, reduced defects, and higher surface energy (Chaudhary et al., 2016). The dimensions of these particles significantly influence their prospective applications across various domains, including biomedicine, nanobiotechnology, agriculture, pharmacology, and

optoelectronics, owing to attributes such as surface functionality, chemical composition, morphology, and crystallinity (Rasouli, 2019; Elahian et al., 2017). Nanoparticles can be of different types depending on their areas of use. Nanomaterials derived from metals, including silver, gold, selenium, copper, zinc, and tellurium, are referred to as metallic nanoparticles. Metallic nanoparticles can be produced by several synthesis processes, and these nanoparticles have garnered interest from researchers due to their significant attributes, including their characteristics and size specificity, particularly when made using plant extracts. The method of using plants or their parts is safe, economical, and environmentally friendly. In this method, no harmful chemicals or reducing agents are used, and the plant extract obtained here acts as a reducing agent, reducing metal ions, and thus NPs are synthesized. Synthesized NPs such as silver, selenium, copper, gold, and zinc contain many functional properties such as anti-tumor, antimicrobial, and antioxidant roles (Burlec et al. 2023).

Selenium (Se) is a crucial element utilized in medical applications due to its antioxidant, immune-modulating, antibacterial, and antifungal properties, as well as its roles in cancer prevention, growth enhancement, and reproductive improvement. Additionally, it is employed in various industrial sectors, including electronic components, solar cells, sensors, biological imaging, metallurgy, glass manufacturing, and pigment production (He et al., 2025). Nanoparticles of selenium are significant for study due to their elevated absorption rate and reduced toxicity relative to their organic and inorganic counterparts. Because of its special qualities, such as high biological activity, bioavailability, low toxicity, good particle dispersion, and large surface area, selenium nanoparticles are thought to be a promising material for many applications (Kumar and Prasad, 2021). Experimental studies have shown that SeNP can stop cancer cell proliferation and even lead to apoptosis. This effect has been shown to induce ROS-mediated necrosis in prostate cancer (Sonkusre and Cameotra, 2017), by inducing autophagy in colorectal cancer (Huang et al., 2019), and by increasing apoptosis in skin, breast, and liver cancer (Chen and Wong, 2009). Due to their low toxicity, Se-NPs have recently attracted much more attention as a promising candidate in medical diagnostics, especially in addition to anti-cancer therapy in medical applications, with their excellent antioxidant, antibacterial, antiviral, antifungal, sensor, and anti-inflammatory biological properties. SeNPs have proven to be effective against a variety of bacteria and dangerous fungi (Lin et al., 2021; Abbas et al., 2021). Moreover, Se-NPs exhibit physical, chemical, electrical, optical, magnetic, semiconducting, catalytic, and biological properties (Ansari et al., 2024).

SeNPs have attracted great attention worldwide due to their wide applications in therapeutics (Park et al., 2025). The promising role of SeNPs in the stabilization of the immune system and the activation of the defense response makes this nanomaterial advantageous. Green synthesis applications, which are easy to synthesize, lower cost, have a wide spectrum of materials (organisms) used, and are an environmentally friendly method for obtaining SeNPs, have become more preferred (Roy et al., 2025). SeNPs synthesized by the green synthesis method are attracting great interest from scientists with their current studies due to their biocompatibility, low toxicity, and strong biological activities, including antitumor and antimicrobial effects (Chen et al., 2025).

Potatoes is one of the most consumed products worldwide. Potato peels are generally considered waste, which constitutes a significant portion of kitchen waste. Potato peels can also be an important food in animal nutrition (Kowalczewski et al., 2022). The extract of potato peels contains a large amount of components with anticancer, antioxidant, antibacterial, anti-inflammatory, antiseptic, and antiapoptotic properties. The extract of potato peels contains bioactive components such as gallic acid, caffeic acid, chlorogenic acid, and neochlorogenic acid, which help in anticancer, antioxidant, and antibacterial activities (Jimenez-Champi et al., 2023).

This study aims to contribute to the waste management system by using potato peels, which are considered agricultural waste. In the present study, environmentally friendly SeNPs were synthesized from waste potato peels, and the characterization of SeNPs and their antimicrobial and antiproliferative effects on cancerous cells were investigated. For this purpose, SeNPs, which have low cost, easy synthesis, biocatalytic, and photocatalytic advantages, were used on A549 (human lung carcinoma cell line) and OVCAR-3 (human ovarian carcinoma cell line) cells and human pathogenic bacterial strains (*S. aureus* and *E. coli*) and yeast (*C. albicans*).

MATERIALS AND METHODS

Since the manuscript is an experimental study, it does not require any ethical committee decision.

Material

S. tuberosum was collected from kitchen waste. To remove the contamination on the collected potato waste, it was first washed thoroughly with tap water and then with pure water. The cleaned waste potatoes were dried in the shade in the laboratory.

Preparation of Potato Peels Aqueous Extract

After the dried potato waste was ground into powder in a grinder, it was stored in suitable containers for both synthesis and characterization studies. To prepare the extract from 30 g of powdered potato waste, it was boiled in 500 mL of deionized water at 85 °C on a magnetic stirrer for 60 minutes and obtained. The acquired aqueous extract was stored in a cool, dark location for three hours. The aqueous potato waste was separated from the

extract by filtering the liquid with Whatman filter paper no. 1. The extract derived from the trash was preserved in bottles within a refrigerator maintained at 4 °C for subsequent utilization.

Synthesis of ST-SE-NPs

For the preparation of SeNPs from *S. tuberosum* shell aqueous extract, the green synthesis method was used to prepare a mixture by taking 1 mL of the solution containing 10 mM selenium and 9 mL of the potato extract. The pH of the combination was subsequently measured and recorded. The reaction mixture was maintained in a dark environment using a rotary shaker at 75 °C for 3 hours at 200 rpm. The alteration in the color of the reaction mixture was noted. The reaction mixture was thereafter incubated at 37 °C for 96 hours. The complete color change of the reaction mixture at the end of the interval indicated the formation of nanoparticles.

Characterization of Biosynthesised Nanoparticles

SeNPs were studied by UV-Vis spectrophotometry, Fourier Transform Infrared Spectroscopy, zeta potential analysis, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectrometry (EDX).

Determination of Cytotoxic Activity

The cytotoxic activities of SeNPs were applied to A549 and ovarian carcinoma OVCAR-3 cancer cell lines by modifying the MTT method (Mosmann, 1983). Cisplatin was used as a positive control in cytotoxicity experiments. Cisplatin was dissolved with DMSO, and intermediate stock solutions were prepared with cell culture media. Cell lines (A549) were cultured in Dulbecco's modified medium (DMEM), 10% fetal bovine serum (FBS), 1% glutamax, and 1% penicillin-streptomycin medium. Cells were seeded at a density of 5 x 10^3 cells/well in 96-well plates containing 90 µL of medium per sterile well. Two separate microplates were used to facilitate two separate incubation periods of 24 and 48 hours for both cell lines. Cells were allowed to adhere to the bottom of the microplates for 24 hours. Plates were incubated for 24 hours in a humidified environment containing 37°C and 5% carbon dioxide (CO_2), and the cells were given the drug candidate compound ML-SeNP (600, 300, and 100 g/mL). When the cells reached approximately 80-90% confluence, they were removed from the flasks and counted by the hemocytometric method. For A549 and OVCAR-3 cell lines (5×10^3) and RPE-1 (10×10^3) cell lines, they were injected in triplicate into 96-well plates, and various doses were applied to the seeded plates the next day. Ultrapure water was added to the control group cells. MTT assay determined changes in cell viability 24 and 48 hours after this application. 10 µL of the MTT (5 mg/mL) solution we prepared was applied to each well-containing cell and then incubated for exactly 3 hours at 37 ºC in a 5% CO_2 humidified environment. After the time was up, it was removed, and 100 microliters of DMSO was added to each well. It was then shaken gently and then left for 20 minutes. Subsequently, the optical density (OD) values in the wells were measured using a UV-vis spectrophotometer (Multiskan GO, Thermofisher, USA) as applied by İpek et al. (2023). The absorbance values from the control wells were determined as the average. Then, the cells were evaluated according to the 100% viability principle. The absorbance values obtained from the wells treated with ML-SeNP were normalized to the control absorbance value. This value was accepted as % viability. MTT analysis was performed at three different times. Statistical evaluation of viability data was performed using the GraphPad Prism 8 package program. Two-way analysis of variance was applied in repeated measurements to evaluate the effect of the applied tests on cell viability.

Antimicrobial Activity

E. coli, *S. aureus*, and *C. albicans* were chosen for antimicrobial evaluation. Cultures were acquired from the Microbiology Laboratory of Inonu University Medical Faculty Hospital. These bacteria and fungi were selected due to their propensity to induce common diseases in people and their resistance to current antibiotics.

RESULTS AND DISCUSSION

UV-visible spectroscopy of SeNPs was measured between 300 and 800 nm (Figure 1). Strong absorption peaks were observed approximately in the 330 nm region, indicating that selenite was reduced to SeNPs with the potato extract. When the obtained graph was examined, it was consistent with the UV-visible spectroscopic results of SeNPs in the literature (Baran et al., 2024).

The FTIR spectrum of potato (*S. tuberosum*) displayed a broad OH band at 3280 cm^{-1} (Akpomie, 2021). The infrared absorption bands for C–H stretching in alkenes and alkanes were observed at 2914 and 2847 cm^{-1}, respectively, whereas the C≡C group displayed absorption bands at 2113 and 1990 cm^{-1} (Ezekoye et al., 2020; Akpomie, 2021). The absorption peak at 1636 cm^{-1} is ascribed to the carbonyl C=O stretching (Akpomie, 2021; Trinh, 2020). The absorption band at 1013 cm^{-1} is attributed to the C–O stretching vibration (Figure 2) (Chukwuemeka-Okorie et al., 2018). The peaks at 1509 and 1364 cm^{-1} are attributed to the O-H bending of the carboxylate group. The peak at 987 cm^{-1} signifies the C-N stretching associated with the amine functional group. The absorption peaks of SeNPs correspond to the stretching, bending, and vibration frequencies of specific organic functional groups, such as NH_2, COOH, CH_2, and CO, as documented in previous studies (Ullah et al., 2021). Ren et al. (2013); Liu et al. (2012).

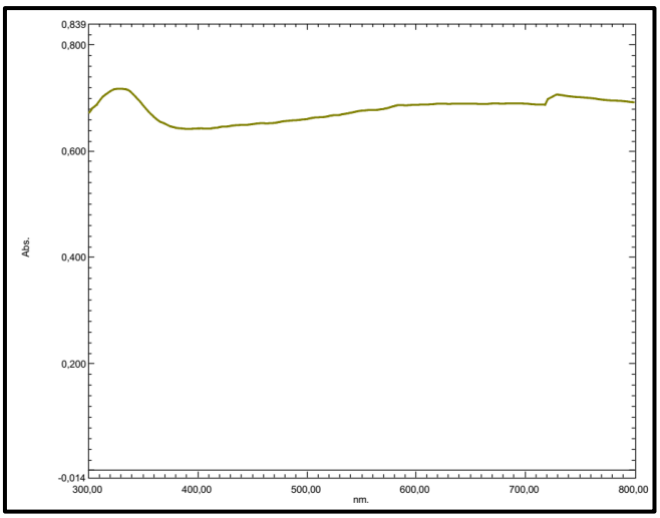


Figure 1. UV-visible spectroscopic results of ST-SeNP nanoparticles

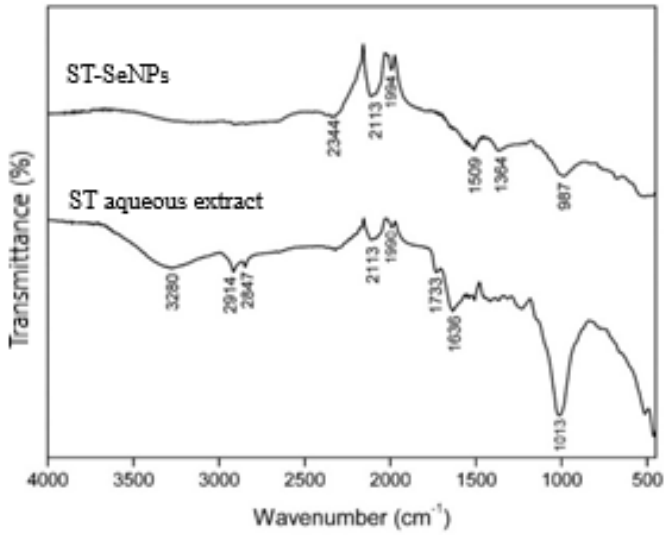


Figure 2. FTIR spectrum of synthesized ST-SeNPs and potato aqueous peel extract

The SEM image of the synthesized ST-SeNPs (Figure 3) was captured at a power setting of 20 kV and a magnification of 20.000X in high vacuum mode, with a scale of 1000 nm. The synthesized selenium nanoparticles exhibited uniformity and smoothness, with an average particle size ranging from 30 to 100 nm. Selenium nanoparticles exhibit a hexagonal structure. The average size of green synthesis selenium nanoparticles (SeNPs) is marginally larger than that produced by chemical methods (Ramamurthy et al. 2013).

Figure 4 illustrates the detection of selenium (Se) peak at approximately 1.5 and 11.2 keV, confirming the reduction of sodium selenite to selenium nanoparticles as indicated by EDX analysis. Upon examination of the figure, the elemental analysis reveals that the Se element occupies the highest rank, with a weight percentage of 88.43% (Baskar et al., 2019).

The XRD examination of selenium in the produced SeNPs reveals three prominent peaks across the spectrum at 2θ values between 5 and 80 (Figure 6). The diffraction at 2θ values of 23.460, 29.661, and 43.620 corresponds to the (100), (101), and (102) planes of the face-centered cubic selenium structure, respectively (Khiralla and El-Deeb 2015).

The synthesis of selenium nanoparticles, as analyzed in Figure 5 via TEM, indicates that Se-NPs exhibit a nearly spherical morphology at the nanometer scale, with an average diameter of 32.97 ± 15.77 nm (Boroumand et al., 2019).

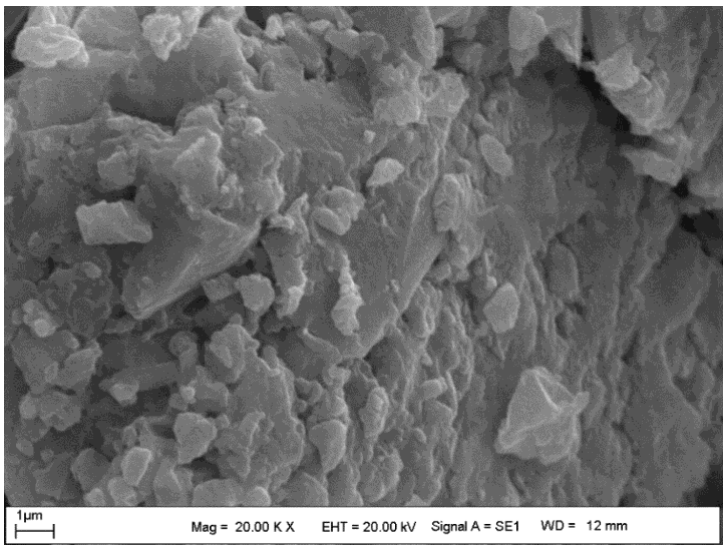


Figure 3. SEM image of synthesized selenium nanoparticles

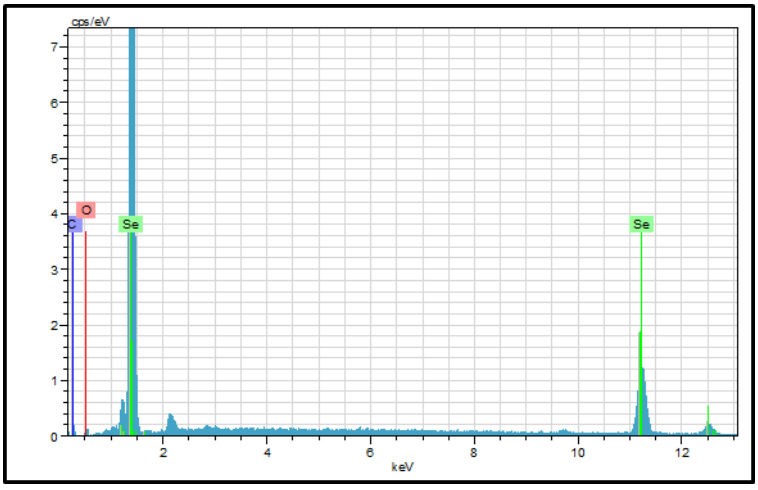


Figure 4. EDX spectrum of selenium nanoparticles.

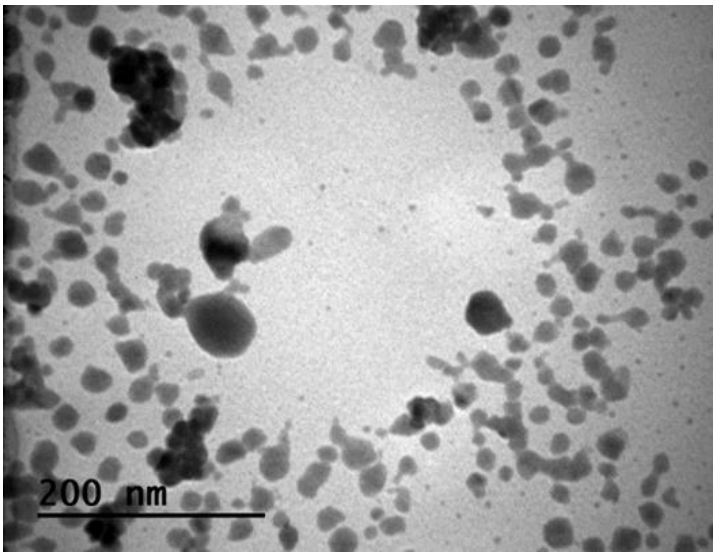


Figure 5. TEM image of SeNPs at 200 nm

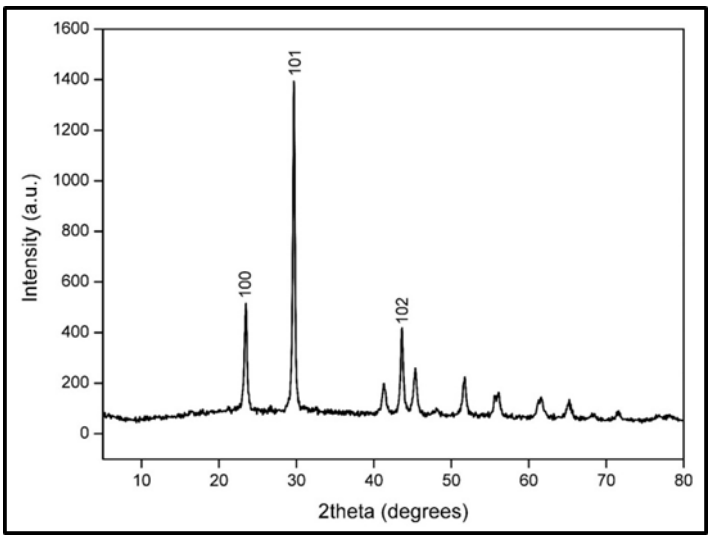


Figure 6. XRD spectrum of selenium nanoparticles

Figure 7 illustrates that the average hydrodynamic diameter of Se-NPs, as measured by DLS, was approximately 98 nm. The size distributions of SeNPs were assessed through dynamic light scattering, with results presented in Figure 7. The average particle size of SeNPs was approximately 98 nm, suggesting a homogeneous particle size distribution in the synthesized SeNPs.

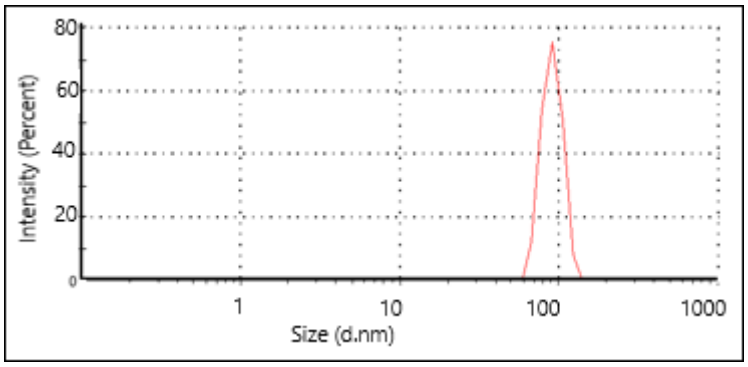


Figure 7. DLS images of synthesized SeNPs.

Anticancer Effect of ST-SENPs

Figure 8 illustrates the in vitro cytotoxicity of SeNPs at various doses and their impact on the growth of A549 and OVCAR-3 cells, as assessed by the MTT assay. The potential mechanism of SeNPs' anti-proliferative activity involves the induction of oxidative stress by the generation of reactive oxygen species (ROS), resulting in DNA damage or apoptosis via the activation of caspases or apoptotic proteins, and may potentially induce cell cycle arrest. This claim is also supported by the research of Menon and Shanmugam (2020). SeNPs were used at varying concentrations (1, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/mL) to treat A549 and OVCAR-3 cells at 24 and 48 hours. The inhibitory concentration (IC50) value for 50% cell death of biosynthesized SeNPs against A549 cells was determined to be 2000 µg/mL at both 24 and 48 hours. The inhibitory concentration (IC_{50}) of the produced SeNPs against OVCAR-3 cells, indicating 50% cell mortality, was established at 2000 µg/mL at both 24 and 48 hours. However, the cytotoxicity test of the synthesized SeNPs against A549 and OVCAR-3 cells did not show significant cytotoxicity at low concentrations, and as the concentration increased at 24 and 48 hours, cytotoxicity also increased (Rajasekar and Kuppusamy, 2021). We obtained the most effective cytotoxicity in the OVCAR-3 cell line with a concentration of 2000 µg/mL after 24 hours, and in the A549 cell line, we obtained a concentration of 2000 µg/mL again after 48 hours of incubation. Menon and Shanmugam (2020) conducted an experimental study with SeNPs in the A549 cell line, and Toubhans et al. (2020) Their experimental research on OVCAR-3 cell line cytotoxicity supports our study.

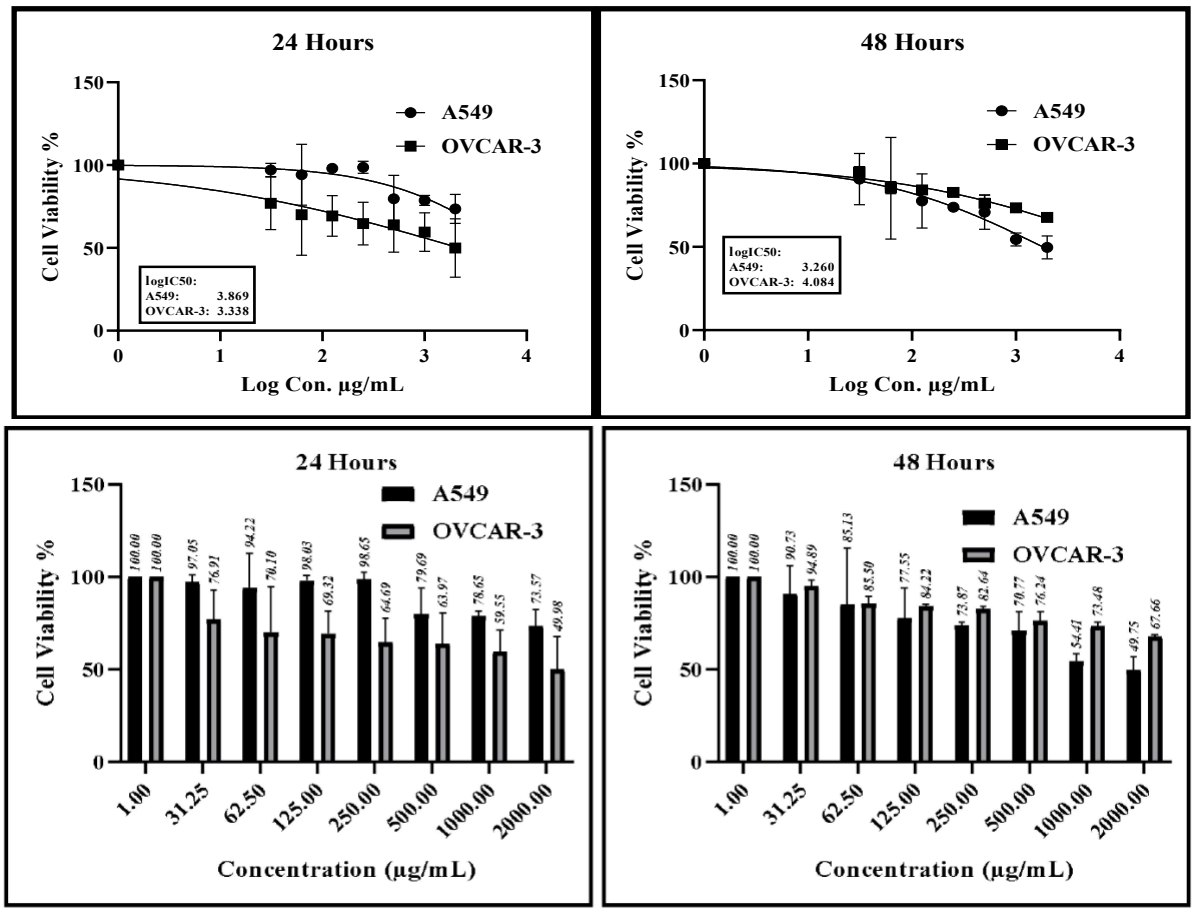


Figure 8. MTT assay for relative cell viabilities of A549 cells and OVCAR-3 cells incubated with biosynthesized ST-SeNPs for 24 and 48 h.

Numerous studies have shown that the generation of reactive oxygen species (ROS) plays a role in the apoptotic process and is strongly associated with the mitochondrial electron transport chain. Typically, selenium nanoparticles (Se-NPs) produce excessive reactive oxygen species (ROS) to induce apoptosis by inhibiting the electron transport chain. As a result, high intracellular ROS production triggered by Se-NPs can induce apoptosis and cell cycle arrest, thereby promoting cancer cell death. Moreover, apoptosis may exert cytotoxic effects on cancer cells via extrinsic or intrinsic pathways (Menon et al., 2018).

In general, cancer is one of the deadly diseases that requires careful and selective treatment planned after a meticulous diagnosis. There are common treatment methods for cancer, such as chemotherapy, radiotherapy, and immunotherapy. However, these methods can also create a lot of exposure, especially in terms of resistance to radiotherapy and chemotherapy options and toxic side effects. For these reasons, there is a need to develop new cancer treatments. (Xia et al. 2024). Selenium nanoparticles increase cell apoptosis, and when used together with anticancer drugs, they increase the effectiveness of the drugs and show an anticancer effect (Kumar and Prasad, 2021). Various anticancer agents, including metals, polymers, biomolecular materials, and semiconductors, have been used for cancer treatment. Among these functional anticancer agents, selenium nanoparticles have also gained great importance as anticancer biomaterials. SeNPs have less toxicity than organic and inorganic selenium compounds, and the high activity of bio-SeNPs has been used in many therapeutic applications, such as anticancer (Xiao et al., 2023).

Antimicrobial Activity of ST-SENPs

Selenium atom is an element recognized for its exceptional antioxidant properties. This property influences cellular function in both adaptive and innate immunity and demonstrates selenium's ability to regulate bacterial infections. Selenium compounds exhibit notable antibacterial activity against various bacterial infections. (Medina and Webster 2018, Tran et al. 2015). As a new type of selenium that shows improved biological activities compared to traditional selenium compounds, SeNPs have been extensively demonstrated to have broad-spectrum antibacterial properties. In our analysis, SeNPs demonstrated antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and fungi (Figure 9). The disk diffusion method demonstrated that SeNPs exhibited greater antimicrobial activity against *E. coli* (5 mm) and *C. albicans* compared to *S. aureus*

(8 mm) (Figure 9). The microorganism to which sensitivity was highest was identified as *C. albicans*. In some studies, conducted with SeNPs, similar to our study, it has shown antibacterial activity in S. aureus and E. coli bacteria (Vahdati and Tohidi Moghadam 2020, Tran et al., 2016). Similarly, in their study with *C. albicans*, Safaei et al. (2022) showed that the produced nanoparticles significantly prevented fungal growth under optimum conditions.

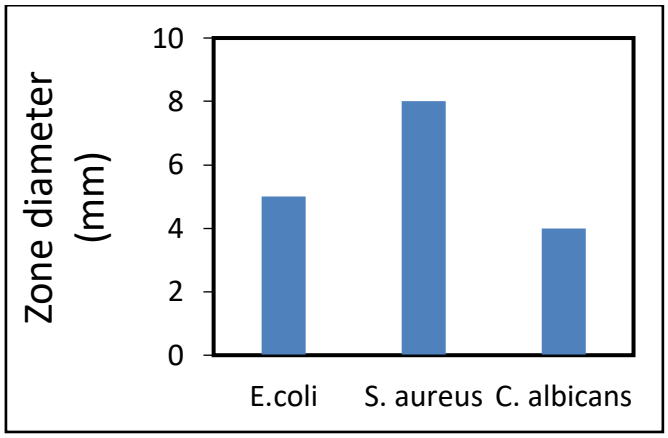


Figure 9. Antimicrobial activity of Se-NPs against *E. coli*, *S. aureus*, and *C. albicans*.

Metal nanoparticles demonstrate antimicrobial properties due to their distinctive characteristics, such as a high surface-to-volume ratio, specific surface area, quantum effects, enhanced surface reactivity, and unique chemical and physical properties. Research indicates that nanoparticles can eliminate resistant infections via interactions with the thiol (-SH) protein group. This interaction alters cell morphology and gene expression, influences membrane permeability, disrupts mitochondrial membrane integrity, induces DNA damage, and generates oxidative stress (Safaei et al. 2022, Truong et al. 2021).

CONCLUSION

In this study, the cytotoxic activity of SeNPs synthesized with potato peels on A549 cells and OVCAR-3 cell lines, as well as their antimicrobial effects against gram-negative *E. coli*, gram-positive *S. aureus* bacteria, and *C. albicans* yeast, were analyzed. ST-SeNPs showed potential as anticancer and antimicrobial agents and could serve as alternative treatments or adjuvants for bacterial infections, thus reducing the burden on current antibiotics and their applications. Consequently, it was concluded that reducing exposure to conventional antibiotics could help reduce rapid antimicrobial resistance, which is a significant effort considering that the resistance rate currently exceeds the rate of new drug development. The obtained findings encourage the use of metal nanoparticle systems exhibiting cytotoxic and antimicrobial properties. Further studies are required to comprehensively elucidate the mechanisms and effects of cytotoxicity and antibacterial properties associated with these metal nanoparticle systems.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text and Table are original and that they have not been published before.

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SCoT marker-based evaluation of genetic diversity in selected walnut (*Juglans regia* L.) accessions

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Abstract

This study investigates the genetic diversity among different walnut (*Juglans regia* L.) genotypes using the SCoT (Start Codon Targeted) marker system. A total of 136 bands were obtained from 15 markers, of which 106 were polymorphic, with an average of 9.07 bands per primer. The SCoT markers yielded a polymorphism rate of 77.9%. In order to interpret the genetic distance among *Juglans regia* L. genotypes, a UPGMA dendrogram was constructed using MVSP 3.22 software. According to the resulting dendrogram, the lowest similarity (0.593) was observed between samples W6 and W26, while the highest similarity (0.970) was observed between W1 and W4, followed by 0.962 between W4 and W5. Principal Component Analysis (PCA) was also conducted using MVSP 3.22. The PCA results revealed a homogeneous distribution and wide variation. The UPGMA dendrogram and PCA analysis were consistent with each other. The SCoT analyses conducted on walnut genotypes provide highly valuable information for assessing the level of genetic diversity, understanding population structure and selecting superior individuals. Such studies contribute to the conservation of genetic resources and support the development of new cultivars with high yield, disease resistance and strong adaptation to climate change in future breeding programs. Based on this study, SCoT analyses demonstrate that the Sivas province constitutes an important genetic diversity reservoir and that walnut genotypes possess rich genetic variation.

Keywords: *Juglans regia* L., SCoT marker, Genetic diversity

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INTRODUCTION

Walnut (*Juglans regia* L.), a member of the *Juglandaceae* family, is a significant fruit species distinguished by its broad ecological adaptability and high economic value (Martínez et al., 2010). Believed to have originated in Central Asia, this species is now widely cultivated across various geographical regions around the world. Particularly notable for its wide variation in morphological and agronomic traits such as fruit shape, size, shell thickness and yield, walnut exhibits a high degree of genetic diversity. This diversity is of great importance for local adaptation, disease tolerance and the development of high-quality products (Wani et al., 2016; Hayes et al., 2016). Walnuts are consumed both fresh and dried, as well as in processed forms (e.g., walnut oil, walnut paste), making them a valuable commodity in both domestic markets and international trade (Delaviz et al., 2017; Bernard et al., 2018). In recent years, global trends toward healthy diets have led to a significant increase in walnut production and consumption. Nutritional analysis of walnuts reveals that they are a rich source of high-quality vegetable oils (up to 60%), proteins (15–20%), dietary fiber, vitamins (particularly vitamin E and B-group vitamins) and minerals (such as potassium, magnesium and phosphorus). Furthermore, walnuts contain considerable amounts of antioxidant compounds (including flavonoids, polyphenols and tocopherols), which contribute to reducing oxidative stress and preventing chronic diseases. Owing to these properties, walnuts are considered protective against cardiovascular diseases, diabetes and certain types of cancer (Vischi et al., 2017; Güney et al., 2021; Shah et al., 2023).

Globally, walnut cultivation represents a major agricultural sector and Türkiye holds a significant position in terms of both production volume and richness of genetic resources (Yıldız et al., 2021). Local walnut varieties that have evolved through natural selection and farmer selection over centuries in various regions of Türkiye possess high adaptive capacity against biotic and abiotic stress factors, owing to their genetic diversity. These local varieties serve as valuable genetic resources for modern breeding programs due to their internal quality traits and resilience (Yıldız et al., 2021). The conservation of local genotypes is crucial for sustainable walnut cultivation and the preservation of biological diversity. In addition, these genotypes offer a competitive edge in both local and international markets due to their unique flavor and aroma profiles (Tabasi et al., 2020; Yıldız et al., 2021; Xie et al., 2025). Preserving the genetic diversity of local walnut (*Juglans regia* L.) populations is essential not only for the sustainability of agricultural biodiversity but also for transmitting genetic resources to future generations and enhancing agricultural resilience against environmental challenges such as climate change. In this context, molecular characterization of local walnut genotypes plays a critical role in both conservation efforts and the identification of genetic material for breeding. Today, molecular marker technologies are widely used for the evaluation of plant genetic resources. Among these, the SCoT (Start Codon Targeted) markers have gained prominence in recent years, as they target coding regions, offering high reproducibility and polymorphism rates, thus serving as an effective tool for analyzing genetic diversity (El-Sheikh et al., 2020; Mahammadi et al., 2021; Zhang et al., 2024). SCoT markers are designed based on the universal ATG start codons found in plant genomes and provide information directly associated with functional regions of the genetic structure. Compared to random markers such as ISSR and RAPD, SCoT markers offer greater specificity and reliability and they are advantageous in reflecting genetic differences in phylogenetic relationships among varieties (Collard ve Mackill, 2009). SCoT analyses conducted on walnut genotypes provide valuable insights into genetic diversity, population structure and the identification of superior individuals. Such studies contribute to the conservation of genetic resources and support the development of new cultivars with high yield potential, disease resistance and enhanced adaptability to climate change (Pan et al., 2024; Zarinkolah et al., 2024). In this study, the genetic diversity of *Juglans regia* L. germplasm, locally grown for many years in the Divriği district of Sivas Province, was investigated using SCoT marker groups. This research aims to reveal the genetic structure of local walnut varieties in the region and to contribute to their conservation and use in future breeding programs.

MATERIALS AND METHODS

Plant Materials

Fresh leaves from 32 different walnut (*Juglans regia* L.) samples, belonging to germplasm that has long been established and adapted to the natural flora of the Divriği district in Sivas, were collected. Information regarding the walnut samples is provided in Table 1.

Genomic DNA Isolation and PCR

DNA extraction from the leaves of walnut samples was performed using the CTAB protocol as described by Doyle and Doyle (1990). The concentrations of stock DNA were measured using a MaestroNano Pro spectrophotometer (MN913A, MaestroGen) and the DNA samples were subsequently diluted to a final concentration of 5 ng/µL. The SCoT primers selected for PCR amplification and the corresponding PCR cycling conditions are provided in Table 2. The PCR reaction mixture consisted of 4 µL of DNA (20 ng), 1 µL of primer, 10 µL of PCR master mix (Eco Tech, Cat No: ET5) and 10 µL of dH_2O. For electrophoresis of the PCR products, a 2% agarose gel prepared in Tris-borate-EDTA buffer was used. DNA bands were visualized under UV light. A total of 36 SCoT primers were initially screened across the samples and 15 primers exhibiting polymorphic patterns were selected for further analysis (Table 2).

Statistical Analysis

The bands obtained from the PCR analysis were scored as 1 for presence and 0 for absence. A dendrogram based on Jaccard's similarity coefficients was generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in MVSP 3.22 software (Kovach, 2007). Application of MVSP 3.22 to the SCoT molecular data enabled the construction of a genetic similarity matrix. In addition, Principal Component Analysis (PCA) was performed using MVSP 3.22 to assess the genetic relationships among walnut populations.

RESULTS AND DISCUSSION

To determine the genetic relationships among walnut samples, 36 SCoT primers were initially screened in 10 samples and 15 primers exhibiting polymorphism were selected and applied to all samples.

A total of 136 bands were obtained from the 15 markers, of which 106 were polymorphic, with an average of 9.07 bands per primer. The SCoT markers yielded a polymorphism rate of 77.9%. In the UPGMA dendrogram constructed based on SCoT data, 32 samples were clustered according to their genetic distance matrix (Figure 1). According to the dendrogram, the lowest similarity (0.593) was observed between samples W6 and W26, while the highest similarity was observed between W1 and W4 (0.970), followed by W4 and W5 (0.962) (Figure 1, Table 3).

Table 1. Details of walnut populations used in the study

Genotype Code	Taxonomy	Province	Province	Longitude	Latitude	Altitude (m)
W1	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W2	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W3	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W4	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W5	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W6	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W7	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W8	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W9	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W10	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W11	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W12	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W13	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W14	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W15	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W16	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W17	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W18	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W19	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W20	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W21	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W22	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W23	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W24	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W25	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W26	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W27	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W28	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W29	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W30	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W31	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W32	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150

Table 2. SCoT primers used for PCR amplification

SCoT Primers Code	DNA Sequences (5'-3')	Tm ºC	PCR Amplification
SCoT-6	CAACAATGGCTACCACGC	56	94 ºC - 3 min 94 ºC - 1 min 48–55 ºC 1 min 72 ºC - 1 min 72 ºC - 8 min 35 cycles
SCoT-14	ACGACATGGCGACCACGC	61	
SCoT-15	ACGACATGGCGACCGCGA	61	
SCoT-16	ACCATGGCTACCACCGAC	58	
SCoT-17	ACCATGGCTACCACCGAG	58	
SCoT-18	ACCATGGCTACCACCGCC	61	
SCoT-19	ACCATGGCTACCACCGGC	61	
SCoT-21	ACGACATGGCGACCCACA	58	
SCoT-23	CACCATGGCTACCACCAG	58	
SCoT-26	ACCATGGCTACCACCGTC	58	
SCoT-28	CCATGGCTACCACCGCCA	61	
SCoT-31	CCATGGCTACCACCGCCT	61	
SCoT-32	CCATGGCTACCACCGCAC	61	
SCoT-34	ACCATGGCTACCACCGCA	58	
SCoT-35	CATGGCTACCACCGGCCC	63	

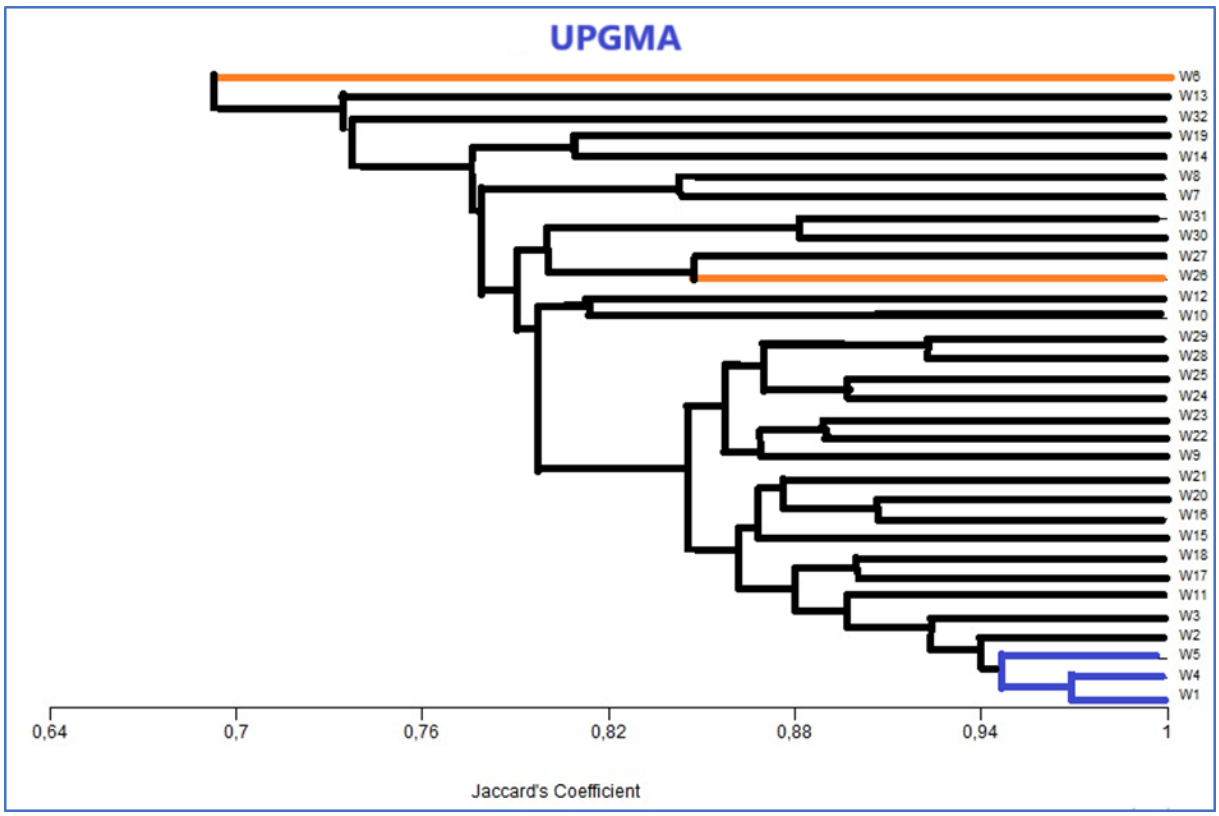


Figure 1. UPGMA Dendrogram of Genetic Similarity Between SCoT Markers and 32 Walnut Genotypes.

Table 3. Pairwise Genetic Distance Matrix Obtained from 15 SCoT Primers.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32
W1	1,000																															
W2	0,947	1,000																														
W3	0,939	0,931	1,000																													
W4	**0,970**	0,947	0,924	1,000																												
W5	0,933	0,925	0,902	**0,962**	1,000																											
W6	0,695	0,674	0,688	0,708	0,713	1,000																										
W7	0,776	0,756	0,785	0,789	0,782	0,754	1,000																									
W8	0,828	0,821	0,811	0,856	0,863	0,754	0,843	1,000																								
W9	0,853	0,846	0,850	0,853	0,846	0,672	0,797	0,837	1,000																							
W10	0,787	0,793	0,782	0,800	0,806	0,680	0,768	0,781	0,836	1,000																						
W11	0,909	0,902	0,864	0,924	0,887	0,698	0,756	0,795	0,807	0,808	1,000																					
W12	0,778	0,770	0,786	0,791	0,784	0,669	0,758	0,718	0,813	0,813	0,785	1,000																				
W13	0,735	0,714	0,702	0,748	0,727	0,690	0,711	0,712	0,727	0,710	0,768	0,771	1,000																			
W14	0,789	0,769	0,758	0,817	0,795	0,739	0,770	0,813	0,756	0,768	0,769	0,772	0,739	1,000																		
W15	0,885	0,835	0,853	0,870	0,835	0,754	0,798	0,811	0,823	0,781	0,823	0,772	0,798	0,813	1,000																	
W16	0,902	0,866	0,856	0,902	0,866	0,718	0,775	0,829	0,827	0,814	0,841	0,805	0,732	0,817	0,888	1,000																
W17	0,902	0,895	0,857	0,902	0,881	0,720	0,750	0,803	0,801	0,788	0,870	0,792	0,762	0,791	0,859	0,891	1,000															
W18	0,896	0,888	0,851	0,896	0,888	0,688	0,758	0,797	0,822	0,769	0,836	0,800	0,756	0,785	0,824	0,856	0,900	1,000														
W19	0,799	0,778	0,780	0,812	0,778	0,720	0,752	0,752	0,752	0,778	0,765	0,768	0,736	0,810	0,823	0,841	0,814	0,808	1,000													
W20	0,902	0,881	0,843	0,888	0,867	0,706	0,763	0,789	0,842	0,815	0,842	0,806	0,721	0,791	0,859	0,906	0,878	0,871	0,843	1,000												
W21	0,873	0,880	0,856	0,859	0,838	0,677	0,775	0,815	0,855	0,814	0,813	0,791	0,732	0,775	0,858	0,862	0,848	0,870	0,827	0,891	1,000											
W22	0,857	0,836	0,854	0,830	0,836	0,659	0,731	0,785	0,882	0,769	0,784	0,787	0,742	0,718	0,841	0,803	0,805	0,826	0,740	0,818	0,859	1,000										
W23	0,874	0,881	0,843	0,860	0,867	0,693	0,763	0,803	0,856	0,829	0,828	0,820	0,762	0,791	0,831	0,848	0,864	0,885	0,786	0,878	0,877	0,890	1,000									
W24	0,837	0,830	0,806	0,837	0,816	0,694	0,738	0,752	0,875	0,791	0,818	0,824	0,764	0,738	0,835	0,838	0,826	0,833	0,789	0,840	0,824	0,836	0,883	1,000								
W25	0,873	0,852	0,842	0,859	0,838	0,651	0,735	0,748	0,827	0,786	0,841	0,791	0,719	0,722	0,815	0,847	0,821	0,842	0,771	0,835	0,820	0,831	0,863	0,897	1,000							
W26	0,759	0,752	0,754	0,746	0,713	**0,593**	0,683	0,672	0,752	0,709	0,738	0,783	0,692	0,696	0,766	0,772	0,733	0,781	0,720	0,733	0,772	0,782	0,760	0,790	0,844	1,000						
W27	0,821	0,813	0,789	0,821	0,787	0,635	0,734	0,735	0,802	0,746	0,788	0,792	0,732	0,748	0,775	0,808	0,782	0,831	0,758	0,795	0,808	0,791	0,837	0,827	0,836	0,847	1,000					
W28	0,867	0,846	0,878	0,867	0,859	0,698	0,811	0,795	0,877	0,780	0,835	0,841	0,740	0,742	0,823	0,841	0,815	0,836	0,779	0,815	0,827	0,853	0,870	0,875	0,869	0,794	0,858	1,000				
W29	0,910	0,889	0,880	0,910	0,903	0,677	0,786	0,812	0,851	0,811	0,865	0,815	0,731	0,759	0,840	0,900	0,872	0,852	0,795	0,872	0,857	0,855	0,872	0,863	0,871	0,783	0,846	0,922	1,000			
W30	0,806	0,812	0,802	0,820	0,812	0,700	0,774	0,787	0,786	0,772	0,786	0,790	0,744	0,774	0,831	0,792	0,754	0,788	0,756	0,767	0,792	0,775	0,822	0,811	0,806	0,785	0,837	0,857	0,817	1,000		
W31	0,793	0,799	0,774	0,806	0,799	0,672	0,774	0,773	0,773	0,800	0,786	0,790	0,744	0,760	0,802	0,792	0,780	0,774	0,742	0,767	0,792	0,762	0,836	0,811	0,792	0,756	0,823	0,814	0,817	0,882	1,000	
W32	0,742	0,735	0,764	0,756	0,775	0,714	0,719	0,734	0,721	0,704	0,734	0,736	0,672	0,705	0,748	0,754	0,715	0,710	0,702	0,729	0,687	0,722	0,742	0,758	0,754	0,729	0,754	0,776	0,752	0,767	0,782	1,000

PCA (Principal Component Analysis) is an important tool in genetic diversity studies because this analysis helps us better understand the genetic structure of different genotypes and allows us to extract meaningful patterns from large datasets. PCA groups genetic materials around specific components or key traits. This is particularly useful for visually revealing similarities and differences between different species, varieties, or populations. By using PCA plots, genetically similar genotypes can be placed in the same cluster, while larger distances can be shown between different genotypes. By identifying the main components in genetic data, it facilitates the grouping of genotypes with similar traits. Such analyses are useful in understanding genetic relationships between species or varieties. PCA helps identify genetic resources that need to be conserved by analyzing genetic diversity between different species or populations. Therefore, PCA is a valuable tool for genetic diversity studies because it analyzes large amounts of data meaningfully, uncovers relationships and provides information that can be used in areas such as biodiversity conservation, breeding and genetic resource management (Table 3, Figure 2) (Nachimuthu et al., 2014). Based on the SCoT molecular data, a PCA plot as shown in Figure 2 was obtained using the MVSP 3.22 program. The analysis revealed a broad range of variation. The UPGMA dendrogram and PCA analysis were

consistent with each other. The genetically closest and most distant genotypes were highlighted in the PCA plot. PCA is widely used as an effective dimensionality reduction method for multivariate datasets (Mishra et al., 2017). Numerous studies have been conducted by researchers to determine the genetic diversity of different walnut genotypes. The findings obtained from this study align with similar studies. Tabasi et al. (2020) investigated the relationship between genetic distance and geographical distance in 20 Iranian walnut populations, including 3 wild and 17 cultivated populations, by using the discriminatory power of SCoT markers. The results showed that SCoT markers have a strong discriminatory power and can effectively distinguish between the studied populations. This marker can be used for evaluating genetic diversity, identifying genotypes and DNA fingerprinting of Iranian walnut populations. Güney et al. (2021) used 45 SSR markers to determine the genetic diversity of 91 *Juglans regia* L. genotypes and identified 390 alleles. The average PIC value was calculated to be 0.68 and the genetic distances were divided into five main groups using UPGMA, PCoA and Structure-based clustering methods. Tenche-Constantinescu et al. (2024) created an inventory of 150 trees from five different populations and molecular analyses revealed an average polymorphism rate of 49.44% using SCoT markers. Zarinkolah et al. (2025) obtained a total of 166 bands using 16 SCoT primers, with 2 polymorphic bands from the SCoT-19 primer and 19 polymorphic bands from the SCoT-15 primer. The average PIC value was 0.30 and PCA and AMOVA results revealed a relatively high level of genetic diversity among the studied genotypes. Sağbaş et al. (2023) used 20 SCoT markers in hawthorn genotypes and found PIC values ranging from 0.251 to 0.297. Jalilian et al. (2018) used twelve SCoT primers in pear samples and calculated an average PIC value of 0.58. Seresht et al. (2023) used SCoT markers in 50 pomegranate genotypes, with a PIC value of 0.39. In line with the results of similar studies, it can be concluded that SCoT markers can provide valuable information regarding the genetic relationships among walnut genotypes, which can be utilized in future walnut breeding and conservation programs.

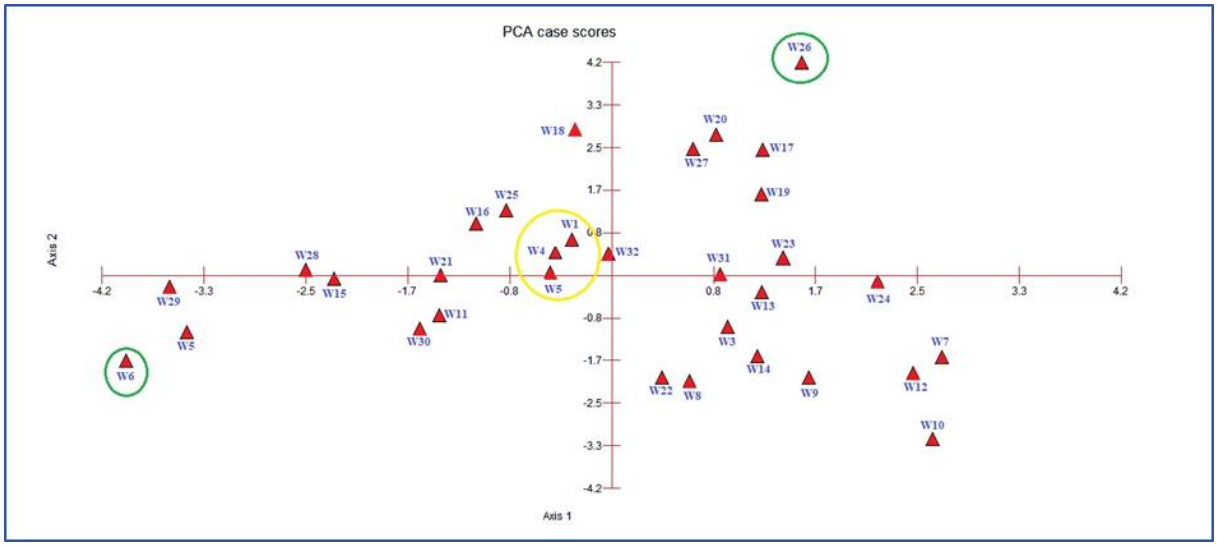


Figure 2. According to SCoT Markers Pricipal Component Analyses of 32 Walnut Populations Using MVSP 3.22 Software (Kovach Computing Services, Pentraeth, Wales, UK)

CONCLUSION

In this study, the genetic diversity of some *Juglans regia* L. germplasm was analyzed using SCoT marker groups. A polymorphism rate of 77.9% was obtained from 136 bands generated by 15 markers. The lowest genetic similarity (0.593) was observed between the W6 and W26 samples, while the highest genetic similarity (0.970) was found between the W1-W4 samples and (0.962) between the W4-W5 samples. Principal Component Analysis (PCA) revealed a homogeneous distribution and wide variation among the genotypes. The UPGMA dendrogram and PCA analysis were consistent with each other. The SCoT analysis conducted on walnut genotypes provides valuable information for determining genetic diversity levels, understanding population structure and selecting individuals with superior traits. Such studies contribute not only to the conservation of genetic resources but also to the development of new cultivars with high yield, disease resistance and enhanced adaptation to climate change in future breeding programs. This study concludes that SCoT markers can provide useful insights into the diversity among walnut genotypes and that these insights could be applied in future walnut breeding and conservation programs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest.

Author contribution

Methodology, G.O. and Y.C.; formal analysis, G.O. and Y.C.; investigation, Y.C.; resources, Y.C.; writing-original draft preparation, G.O. and Y.C.; writing-review and editing, Y.C.; All authors have read and agreed to the published version of the manuscript.

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Forage yield and nutritional quality determination of huser plateau pasture (Rize, Türkiye)

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Abstract

This study evaluated the forage yield and nutritional quality of Huser Plateau pasture in Rize, Türkiye, over the 2023-2024 growing seasons. Utilizing quadrat sampling and Near-Infrared Reflectance Spectroscopy (NIRS), we determined an average dry yield of 102 kg/da. The average crude protein (CP) content was 16.29%, indicating substantial forage potential. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) averaged 29.74% and 56.67%, respectively, revealing temporal fluctuations in digestibility. Calculated parameters indicated moderate to high forage quality, with Digestible Dry Matter (DDM) at 65.73%, Dry Matter Intake (DMI) at 2.19%, and Relative Feed Value (RFV) at 112.29. Digestible Energy (DE) was 3.08 Mcal/kg, and Metabolizable Energy (ME) was 2.53 Mcal/kg. Mineral analysis showed adequate phosphorus (0.32%) and potassium (1.91%) levels, while calcium (1.30%) and magnesium (0.23%) ratios varied between years. The Ca/P ratio averaged 4.25, and the K/(Ca+Mg) ratio averaged 1.46. These findings highlight the Huser Plateau pasture's significant forage potential, suggesting that strategic management practices are essential to mitigate seasonal variations and optimize livestock productivity.

Keywords: Forage quality, Huser plateau, Nutrient composition, Rize, Yield

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INTRODUCTION

Pastures and meadows constitute indispensable roughage sources for animal nutrition, holding a pivotal position within livestock farming operations (Aydın & Uzun, 2005). These ecosystems serve as critical reservoirs of genetic resources for cultivated flora, provide vital habitats for wildlife, and perform essential ecological functions, including the preservation of biodiversity and the mitigation of soil erosion (Çomaklı & Menteşe, 1999; Carlier et al., 2005). In Turkey, approximately 70% of livestock production is dependent on pastures and meadows, which contribute substantially to the annual roughage requirements of livestock, particularly with respect to essential nutrients such as crude protein and starch (Gökkuş, 1994; Okatan & Yüksek, 1997; Çınar *et al.,* 2005; Çomaklı, 2018).

Studies on pasture and meadow vegetation are primarily conducted for two main purposes. The first is to gather data on the quantitative and qualitative characteristics of pasture and meadow areas in regions where vegetation information is limited. The second aim is to observe the effects of methods and their impacts on vegetation in pasture and meadow improvement and management practices (Cerit and Altın, 1999; Ağın and Kökten, 2013; Seydoşoğlu *et al.,* 2018; Seydoşoğlu and Kökten, 2018; 2019).

When examining studies on pastures conducted in various regions of Turkey; Tutar (2017) aimed to compare the botanical composition and yield of four different aspects of a natural pasture in Ormanardı Village, Central District of Bingöl Province. The highest crude protein content (12.9%) was observed in the southern aspect, which was statistically similar to the western and eastern aspects. Crude protein yield ranged from 2.3 to 16.7 kg/da, with significant differences among aspects. Crude ash, ADF, NDF, digestible dry matter, dry matter intake, and relative feed value ranged from 6.8% to 8.3%, 34.8% to 37.4%, 52.5% to 62.7%, 59.7% to 61.7%, 1.92 to 2.08%, and 91.8 to 109.4, respectively. The pasture contained 58 different taxa belonging to 49 genera from 21 families. Çaçan et

al. (2014) conducted a study in two different natural pasture areas, one protected and one grazed. In the protected area, dry herbage yield was 203.70 kg/da, fresh herbage yield was 781.28 kg/da, and the botanical composition consisted of 38.33% grasses, 31.94% legumes, and 29.73% other families. The pasture quality degree was 4.34, and the pasture condition was medium. The crude protein, ADF, and NDF contents were 19.69%, 29.48%, and 43.31%, respectively. In the grazed area, dry herbage yield was 106.85 kg/da, fresh herbage yield was 288.68 kg/da, and the botanical composition consisted of 26.53% grasses, 23.65% legumes, and 49.80% other families. The pasture quality degree was 3.39, and the pasture condition was poor. The crude protein, ADF, and NDF contents were 15.40%, 37.76%, and 50.86%, respectively. Tanrıverdi (2019) evaluated the botanical composition and yield of a natural pasture area in Kıyıbaşı Village, Central District of Muş Province, according to aspects. The highest crude protein content (14.37%) was observed in the eastern aspect, which was statistically similar to the southern and western aspects. Crude protein yield ranged from 3.31 to 7.68 kg/da, with significant differences among aspects. Crude ash, NDF, ADF, digestible dry matter, dry matter intake, and relative feed value ranged from 6.4% to 8.7%, 57.0% to 59.8%, 21.2% to 34.0%, 62.4% to 72.3%, 2.00 to 2.10%, and 102.0 to 112.5, respectively. A research conducted by Tutar and Kökten (2019) in Ormanardi Village, Central District, Bingöl Province, located in the Eastern Anatolia Region of Turkey, aimed to compare the yield and quality of a natural pasture's four different aspects. Hay yield varied between 23.2 and 129.3 kg da-1 across the pasture aspects, showing statistical significance ($p<0.01$). The grazing capacity of the pasture was calculated as 18.4 Animal Units (AU). The study also determined that CP yield and CP ratio ranged from 2.3-16.7 and 9.9-12.9%, respectively. ADF, NDF, DMI, DDMI, and RFV were found to be 34.8-37.4%, 52.5-62.7%, 1.92-2.08%, 59.7-61.7%, and 91.8-109.4, respectively.

The primary objective of this research is to evaluate the forage resources and overall ecological integrity of Huser Pasture, situated in Rize, Türkiye, through a detailed analysis of forage yield and nutritional characteristics. It is anticipated that the results of this investigation will furnish critical data for the implementation of sustainable livestock management strategies within the local context. Moreover, this study seeks to elucidate the pasture's ecological and productive attributes, thereby establishing a baseline for subsequent research and facilitating evidence-based decision-making in regional agricultural practices.

MATERIALS AND METHODS

This research was conducted in Huser Plateau pasture, situated within the Çamlıhemşin district of Rize province, a region renowned for its exceptional natural beauty and rich biodiversity within the Eastern Black Sea Region of Türkiye. The study site is located at an approximate altitude of 2300 meters above sea level, approximately 12 km from the district center. The research period encompassed the years 2023 and 2024, allowing for the observation of seasonal variations in vegetation. The geographical location of the study area is illustrated in Figure 1, providing a regional context. Representative photographs of the site's characteristic features, including topography, vegetation cover, and dominant plant communities, are presented in Figure 2, offering a visual representation of the study environment.

Soil samples were collected from representative locations within the Huser Plateau pasture to characterize the physicochemical properties of the soil. These samples were analyzed to determine key parameters relevant to pasture productivity and plant nutrition. The analysis revealed the following characteristics: soil saturation was measured at 64.9%, indicating a medium level of water retention. The soil pH was 4.59, classifying it as moderately acidic. The total salt content was low at 0.11%, while the lime content was also low at 0.10%. The organic matter content was 3.19%, considered sufficient for supporting plant growth. Available phosphorus (P_2O_5) was measured at 6.57 kg/da, indicating a medium level, and available potassium (K_2O) was 56.29 kg/da, classified as sufficient. Analysis of long-term meteorological records obtained from the Turkish State Meteorological Service (MGM) indicates an average annual temperature of 14.5 °C and a substantial annual precipitation total of 2300 mm for Rize province (Anonymous, 2025).



Figure 1. Location of the study area on the map (Google Earth)



Figure 2. Some photos taken from the study area

Plant material was collected from twelve distinct locations within Huser Plateau pasture during July of both 2023 and 2024, coinciding with the period of peak biomass production. This sampling strategy aimed to capture the seasonal variation in vegetation composition and nutritional quality. At each location, vegetation was harvested at ground level using 50 × 50 cm quadrats, ensuring representative sampling of the plant community. Immediately after harvesting, fresh weights of the collected samples were measured in situ using a portable precision balance to minimize moisture loss prior to further processing. Subsequently, samples were oven-dried at 70 °C for 48 hours to achieve a constant dry weight. These dry weights were then used to calculate yield per unit area (kg/da). The dried plant material was ground and homogenized using a mill equipped with a 1 mm sieve to ensure sample uniformity for subsequent chemical analyses.

The concentrations of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent protein (ADP), and the minerals phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined using Near-Infrared Reflectance Spectroscopy (NIRS) with a Foss NIR Systems Model 6500 Win ISI II v1.5 instrument. NIRS was selected for its rapid, non-destructive, and cost-effective analysis of multiple constituents, allowing for efficient processing of a large number of samples.

Several key nutritional parameters were calculated based on the ADF and NDF values using established equations from the literature. Digestible dry matter (DDM) was calculated using the equation: DDM = 88.9 – (0.779 × %ADF) (Oddy *et al.,* 1983). Dry matter intake (DMI) was estimated using the equation: DMI = 120 / (%NDF) (Sheaffer *et al.,* 1995). Relative feed value (RFV) was calculated as: RFV = (DDM × DMI) / 1.29 (Sheaffer *et al.,* 1995). Digestible energy (DE) was estimated using the equation: DE = 0.27 + 0.0428 × (%DDM) (Fonnesbeck *et al.,* 1984), and metabolizable energy (ME) was calculated as: ME = 0.821 × DE (Mcal/kg) (Khalil *et al.,* 1986). These calculated parameters provide valuable estimates of forage quality and potential animal performance.

Furthermore, the ratios of Ca/P and K/(Ca+Mg) were calculated to assess the balance of macro elements, which is crucial for animal health and metabolic functions. Maintaining appropriate mineral ratios is essential for preventing nutritional imbalances and optimizing animal productivity.

Descriptive statistical analyses were performed on the collected data for all examined parameters. The JMP statistical software package was utilized to calculate descriptive statistics, including means, standard deviations, and ranges for each parameter. This statistical approach provides a comprehensive overview of the data and allows for comparisons between sampling years and locations.

RESULTS AND DISCUSSION

The nutrient composition of grass samples collected from Huser Plateau pasture during the two-year study period (2023-2024) is summarized in Table 1. These data provide a comprehensive assessment of the pasture's nutritional quality and reveal potential interannual variations in nutrient concentrations.

Table 1. Yield and nutritional value of Huser Plateau pasture

Features Analyzed	1. Year	2. Year	Average
Fresh Herbage Yield (FHY) (kg/da)	428±21.47	516±33.51	472
Dry Herbage Yield (DHY) (kg/da)	88±7.26	116±10.64	102
Crude Protein (CP) (%)	16.95±3.01	15.63±2.21	16.29
Acid Detergent Fiber (ADF) (%)	32.56±2.52	26.92±1.63	29.74
Neutral Detergent Fiber (NDF) (%)	66.99±4.41	46.34±3.78	56.67
Acid Detergent Protein (ADP) (%)	0.97±0.06	0.77±0.04	0.87
Digestible Dry Matter (DDM)	63.54±1.97	67.93±1.27	65.73
Dry Matter Intake (DMI)	1.79±0.12	2.59±0.22	2.19
Relative Feed Value (RFV)	88.23±8.97	136.36±14.15	112.29
Digestible Energy (DE) (Mcal/kg)	2.99±0.08	3.18±0.05	3.08
Metabolic Energy (ME) (Mcal/kg)	2.45±0.07	2.61±0.04	2.53
Phosphorus (P) (%)	0.34±0.06	0.29±0.04	0.32
Potassium (K) (%)	2.18±0.15	1.64±0.12	1.91
Calcium (Ca) (%)	0.93±0.08	1.67±0.23	1.30
Magnesium (Mg) (%)	0.11±0.04	0.34±0.12	0.23
Ca/P	2.74±0.28	5.76±0.42	4.25
K/(Ca+Mg)	2.10±0.11	0.82±0.19	1.46

The nutrient composition and yield characteristics of forage samples collected from Huser Plateau pasture during the 2023-2024 growing season are presented in Table 1. Significant interannual variations were observed in several key parameters. Fresh herbage yield (FHY) averaged 472 kg/da across the two years, with a notable increase from 428 ± 21.47 kg/da in 2023 to 516 ± 33.51 kg/da in 2024. Dry herbage yield (DHY) also exhibited a similar trend, increasing from 88 ± 7.26 kg/da in 2023 to 116 ± 10.64 kg/da in 2024, resulting in a two-year average of 102 kg/da. Crude protein (CP) content averaged 16.29%, with slightly higher values observed in 2023 (16.95 ± 3.01%) compared to 2024 (15.63 ± 2.21%). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents showed a marked decrease from 2023 (32.56 ± 2.52% and 66.99 ± 4.41%, respectively) to 2024 (26.92 ± 1.63% and 46.34 ± 3.78%, respectively), resulting in two-year averages of 29.74% for ADF and 56.67% for NDF. Acid detergent protein (ADP) remained relatively stable across the two years, averaging 0.87%. Consequently, calculated nutritional parameters, including digestible dry matter (DDM), dry matter intake (DMI), and relative feed value (RFV), showed substantial improvement in 2024. DDM increased from 63.54 ± 1.97% in 2023 to 67.93 ± 1.27% in 2024 (average: 65.73%), while DMI increased from 1.79 ± 0.12% to 2.59 ± 0.22% (average: 2.19%). Notably, RFV exhibited a considerable increase from 88.23 ± 8.97 in 2023 to 136.36 ± 14.15 in 2024 (average: 112.29). Digestible energy (DE) and metabolizable energy (ME) also showed slight increases in 2024, averaging 3.08 Mcal/kg and 2.53 Mcal/kg, respectively, over the two years. Mineral analysis revealed average concentrations of 0.32% for phosphorus (P), 1.91% for potassium (K), 1.30% for calcium (Ca), and 0.23% for magnesium (Mg). The Ca/P ratio increased substantially from 2.74 ± 0.28 in 2023 to 5.76 ± 0.42 in 2024 (average: 4.25), while the K/(Ca+Mg) ratio decreased from 2.10 ± 0.11 to 0.82 ± 0.19 (average: 1.46).

This study investigated the forage yield and nutritional characteristics of Huser Plateau pasture over two growing seasons (2023-2024), revealing significant interannual variability in several key parameters (Table 1). The observed increase in fresh and dry matter yields from 2023 to 2024 could be attributed to variations in climatic conditions, particularly precipitation and temperature patterns, between the two years. These factors play a crucial role in plant growth, development, and biomass accumulation.

The average crude protein (CP) content of Huser Plateau pasture (16.29%) falls within the range reported in other studies conducted in Türkiye. For instance, Kılıç (2018) reported a similar CP content of 16.6% in Beypınarı pasture (Trabzon), while Şahinoğlu (2010) found CP values ranging from 16.33% to 18.64% in Koşu village pasture (Samsun). These findings suggest that Huser Plateau pasture provides a comparable level of protein to other pastures in the region, adequate for supporting livestock nutrition. However, Güllap (2010) reported lower CP values (8.26-13.12%) in Erzurum pastures, and Parlak *et al.* (2015) found a range of 9.10-13.18% in Çanakkale pastures, highlighting the influence of geographical location, altitude, and plant species composition on forage CP content.

A notable finding of this study was the substantial decrease in ADF and NDF concentrations from 2023 to 2024. This decrease is reflected in the increased DDM, DMI, and RFV values observed in 2024. Lower fiber content generally translates to improved forage digestibility and intake by livestock. The average ADF and NDF values of Huser Plateau (29.74% and 56.67%, respectively) are comparable to those reported by Şahinoğlu (2010)

(ADF: 29.82-31.99%; NDF: 46.39-55.21%) and Aydın and Başbağ (2017) (ADF: 29.78%; NDF: 47.76%). However, Nadir (2010) reported lower ADF (24.38-26.84%) and NDF (34.59-36.32%) values and consequently much higher RFV values (174.96 to 189.77), indicating potentially higher forage quality in the studied area. These differences could be attributed to variations in plant species composition, growth stage at harvest, and environmental factors.

The mineral content of Huser Plateau pasture also showed interannual variation. The average P, K, Ca, and Mg concentrations were within the range reported in other studies (Şahinoğlu, 2010; Aydın and Başbağ, 2017), suggesting adequate mineral supply for livestock. The Ca/P ratio, which is crucial for bone development and other metabolic functions, increased substantially from 2023 to 2024. The K/(Ca+Mg) ratio, another important indicator of mineral balance, decreased in 2024. These shifts in mineral ratios warrant further investigation to understand their implications for animal health and productivity.

The observed differences in forage quality and yield among studies highlight the importance of site-specific management practices. Factors such as grazing intensity, fertilization, and weed control can significantly influence pasture productivity and nutritional value.

CONCLUSION

This study, conducted in the Huser Plateau pasture of Rize, Türkiye, over the 2023-2024 growing seasons, aimed to assess the forage yield and nutritional quality of this high-altitude grassland. Utilizing quadrat sampling and NIRS analysis, we determined an average dry herbage yield of 102 kg/da, with a crude protein content of 16.29%, highlighting the pasture's potential as a valuable forage resource. The analysis revealed that while the pasture provided adequate crude protein, the variations in ADF and NDF levels between the two years, resulting in an average NDF of 56.67% and ADF of 29.74%, suggest temporal fluctuations in forage digestibility. Calculated parameters such as DDM (65.73%), DMI (2.19%), and RFV (112.29) indicated moderate to high forage quality, further supported by digestible and metabolizable energy values of 3.08 Mcal/kg and 2.53 Mcal/kg, respectively. Mineral analysis showed adequate phosphorus and potassium levels, though calcium and magnesium ratios varied significantly between years, underscoring the importance of monitoring mineral balance for livestock nutrition. Overall, Huser Plateau pasture demonstrates substantial forage potential, but strategic management practices are necessary to address seasonal variations and ensure optimal livestock productivity.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

The author read and approved the final manuscript.

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The influence of rootstock and biostimulants on the dynamics of antioxidant defense mechanisms under salt stress in potted Öküzgözü grapevines (*Vitis vinifera* L.)

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Abstract

Salt stress has become an increasingly serious threat to viticulture due to global climate change and a lack of irrigation resources. Salt accumulated in the soil causes ionic imbalance, osmotic stress and consequently oxidative stress in grapevines, negatively affecting the morphological, physiological and biochemical processes of the plant. The defense responses developed by the plant against these stress conditions may differ largely depending on the rootstock material used and the supporting external applications. In this context, in the study, two different levels of salt stress (Salt 1: 150 mM, Salt 2: 150 mM+150 mM) were applied on three different rootstocks (Ownroot, 1103P and 140Ru) of potted Öküzgözü (*Vitis vinifera* L.) and the effects of biostimulants (*Saccharomyces cerevisiae* and *Ascophyllum nodosum*) on the antioxidant system were investigated under salt stress conditions in 2023 vegetation period. The results showed that rootstocks developed different responses to salt stress and the severity of these responses varied depending on the type of treatment. Total phenolic compound level increased the most in high salt stress and biostimulants balanced this stress. Antioxidant activity, similar to total phenolic compounds and as expected, reached the maximum level at high salt stress and both parameters were highest on the 1103P rootstock. SOD level decreased with increasing salt stress and the highest SOD activity was measured in the control group 140Ru vines. CAT activity reached the highest level in 1103P at high salt stress. CAT and APX activities increased to higher levels with the second salt treatment than the first. The decrease in SOD enzyme more effectively manifested the use of biostimulants against stress, while CAT and APX activities showed an upward trend with the use of biostimulants, while CAT and APX activities showed an upward trend with the use of biostimulants.

Keywords: Antioxidant enzyme, *Saccharomyces cerevisiae*, *Ascophyllum nodosum*, *Vitis vinifera*, 140Ru, 1103P

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INTRODUCTION

The grapevine (*Vitis vinifera* L.), which has thousands of years of agricultural history, shows a high level of ecological adaptability. Although different grape varieties and rootstocks vary in their level of resistance to environmental stress conditions, their ability to adapt to various climatic changes makes this species of strategic importance for global agriculture (Deng et al., 2021; Zhou et al., 2022). Vineyards demonstrate their multifaceted economic importance not only through the production of table, dried and wine grapes, but also through their rich content of biological compounds that can be utilized in various sectors such as food, pharmaceuticals, cosmetics, fertilizers, energy production and functional materials (Baroi et al., 2022).

Climate change, especially temperature increase, is a major challenge for crop production worldwide due to reduced water resources and increased soil salinization (Nikolaou et al., 2021).

Under saline soil conditions, high salt concentrations lead to severe reductions in plant growth and yield. Salt stress triggers the accumulation of reactive oxygen species (ROS), causing damage to cellular structure and impairment of physiological functions, which significantly negatively affects vine yield and quality in viticulture (Meggio et al., 2014; Wei et al., 2023). It is estimated that about 20% of irrigated land worldwide is affected by salinity, which poses a significant threat to agriculture, especially grapevine cultivation (Huo, 2023).

Plants have evolved several defense mechanisms that confer tissue resilience to survive in saline environments (Parida and Das, 2005; Munns et al., 2006). These mechanisms include ion exclusion, vacuolar storage, osmotic compensation (e.g. proline accumulation) and enhancement of enzymatic and non-enzymatic antioxidant defenses (Demiral and Türkan, 2005; Cramer et al., 2007).

In plants, reactive oxygen species such as O_2^- and H_2O_2 are produced during normal metabolic processes (Lacuesta et al., 1997). However, ROS production increases under stress conditions and this balance is controlled by the antioxidant systems of the plant (Ashraf, 2009). It is known that plants with high antioxidant levels are more resistant to salt stress than those with low levels. Major enzymatic defense systems in plants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). The cooperation and balanced functioning of these enzymes, especially the interaction between APX and CAT, is critical in limiting the accumulation of toxic ROS (Özden et al., 2009). While CAT, together with SOD, constitutes the most effective defense mechanism (Scandalios, 1993), APX plays an important role in intracellular detoxification by inactivating H_2O_2 through ascorbate (Asada and Takahashi, 1987). Studies have revealed that there are inter-cultivar differences in the antioxidant defense responses of plants under saline conditions (Silveira et al., 2001).

Grapevine is traditionally a non-irrigated crop (Cifre et al. 2005), but in arid climates irrigation strategies are essential to maintain yield and fruit quality (Chaves et al., 2010). Salinity is therefore increased when poor quality irrigation water is used (e.g. borehole water containing significant amounts of salt) and/or when insufficient salt leaching occurs, for example when low irrigation rates are applied, which is typical during drought (Rengasamy, 2006).

In viticulture, soil salinity increases by accumulating in the root zone due to factors such as irrigation water with high salt content, water loss through evaporation and poor drainage (Várallyay, 1994; Phogat et al., 2020; Zhou-Tsang et al., 2021). The detrimental effects of salt stress on plants are mainly mediated by two mechanisms: osmotic stress and ion toxicity. An excess of Na^+ and Cl^- ions disrupts the osmotic balance between the root and soil, limiting water uptake (Abbasi et al., 2016; Raghda'a Ali Al-Khafajy et al., 2020); at the same time, excessive accumulation of these ions leads to ion toxicity by inhibiting the uptake of essential nutrients (Al-Abbassi et al., 2022)

Vines are grown on rootstocks that regulate the interactions between vines and the environment in terms of nutrient uptake, salinity tolerance and water stress tolerance, as well as phylloxera resistance. Rootstocks derived from wild *Vitis* species differ in their ability to exclude Cl^- and Na^+ ions, which determines their level of salinity tolerance. While *V. berlandieri* has a high exclusion capacity, this ability is reduced in rootstocks crossed with *V. vinifera*; therefore, hybrid rootstocks such as 41B show more limited tolerance to salt (Fisarakis et al., 2001).

Biostimulants are agronomic products that have become very important in agriculture because they are formulated with substances that help plants adapt to different environmental conditions and can stimulate physiological and biochemical processes (Wu et al., 2024). Biostimulants are materials that positively affect plant growth, nutrition, product quality and yield; which are applied to plants from leaves, soil or seeds in order to increase the resistance of plants against abiotic stress, which may contain organic or inorganic compounds, microorganisms, some of which also have soil structure regulating effects (Yılmaz and Şensoy, 2021).

The main group of plant biostimulants are humic acid and fulvic acid, protein hydrolysates and other N containing compounds, seaweed extracts and botanical preparations, chitosan and other biopolymers, inorganic compounds, beneficial fungi and bacteria (Du Jardin, 2015). Besides non-microbial biostimulants, microbial biostimulants have also recently been used in agriculture. Microbial inoculants mostly include plant growth-promoting rhizobacteria (PGPR) and endophytic fungi such as arbuscular mycorrhizal fungi and trichoderma and are used as biostimulants (De Pascale et al., 2017).

Seaweed extracts are a large group of macroscopic, multicellular seaweeds that can be brown, red and green. These substrates contain organic matter and fertilizer nutrients. They can improve yield and quality by increasing plant growth, photosynthetic activity and tolerance to biotic and abiotic stresses (Sharma et al., 2014; Bulgari et al., 2019). Seaweeds contain a variety of active minerals and organic compounds that contribute to the growth and development of plants, as well as various hormones (Battacharyya et al., 2015). Especially those from *Ascophyllum nodosum* and *Sargassum spp*. have been effective in conferring resistance to abiotic stresses in plants. These extracts reduce damage due to salt and temperature stresses by lowering leaf osmotic potential and support antioxidant metabolism and vitality (Anjos et al., 2020; El Boukhari et al., 2023).

Although not used as intensively as seaweeds, yeast-based biostimulants have proven to be effective against different plant diseases and have been commercialized as resistance inducers in the context of sustainable

agriculture (Reglinski et al., 1994; Tumpa and Khokon, 2020; Shahzadi et al., 2022). The mechanisms of yeasts as plant protection agents include production of volatile compounds, lethal toxins and lytic enzymes, competition for space and nutrients, mycoparasitism (Kowalska et al., 2022), and induction of plant immunity by triggering complex signaling cascades resulting in activation of complementary defense pathways and Induced Systemic Resistance (ISR) (Moon et al., 2015; Narusaka et al., 2015; Lee at al., 2017).

In our previous study (Karaman et al., 2024), we applied a single dose of 150 mM NaCl to Nero D'Avola cultivar on its own roots and observed the effects of yeast and seaweed In this study, we aimed to determine of the effects of *Ascophyllum nodosum* and *Saccharomyces cerevisiae* as biostimulants against low (150mM) and high salt stress (150 mM + 150 mM) and to compare the antioxidative responses in Öküzgözü cultivar on different roots (ownroot, 1103P, 140Ru).

MATERIALS AND METHODS

Plant Material

The plant material of the research was 2-year-old potted vines of Öküzgözü (*Vitis vinifera* L.) variety in the greenhouse of Ankara University, Faculty of Agriculture, Department of Horticulture. The vines consisted of groups of 16 vines each on their own roots, grafted on 140Ru rootstock (*Vitis berlandieri* × *Vitis rupestris*) and grafted on 1103P rootstock (*Vitis berlandieri* × *Vitis rupestris*). The pots had a volume of 15 liters and were filled with a medium consisting of peat, perlite, coccopite and sand (1:1:1:1, v/v).

Treatments

The Control group consisted of 4 vines on their own roots, grafted on 1103P and 140Ru. Only 200 mL of water was sprayed on the leaves of the Control group on the application dates. Salt+*Saccharomyces cerevisae* vines consisting of 12 vines were sprayed with 400 mg/200 mL *Saccharomyces cerevisiae* yeast (Zymaflore FX 10, Laffrot, France) and Salt+*Ascophyllum nodosum* vines consisting of 12 vines were sprayed with 0.4 mL /200 mL *Ascophyllum nodosum* moss (Searius, Gübretaş, Turkey) on 10.07.2023 (BBCH75). The application dose of biostimulants has been determined according to Karaman et al. (2024). On 17.07.2023, 150 mM of Salt 1 (Salt+*Saccharomyces cerevisae* 1, Salt+*Ascophyllum nodosum* 1) and on 24.07.2023, 150 mM of Salt 2 (Salt+Saccharomyces cerevisae 2, Salt+*Ascophyllum nodosum* 2) were applied to Salt group vines consisting of 12 vines and Salt+*Saccharomyces cerevisae* and Salt+*Ascophyllum nodosum* group vines.

Leaf sampling

Leaf samples were taken from the lower 4th node of each grapevine, 1 week after each salt application. The collected leaves were cleaned with distilled water to remove residues. The leaves were then frozen in liquid nitrogen and stored at -40°C until analysed.

Total phenolic compounds assay

Total phenolic compound levels (TPC) of leaf samples were measured according to Singletton and Rossi (1965). 500 mg of liquid nitrogen-frozen leaf samples were crushed in a homogenizer (Ultra-Turrax T25-Germany) by adding 5mL of methanol for 5 minutes. They were then kept in an ultrasonic bath (Jeiotech US-Korea) for 30 min, followed by centrifugation at 10000 rpm and 4°C for 10 min (Sigma 3K30-Germany). The supernatant was passed through 0.45 µm polyvinylidene difluoride filters (Millipores-USA). To determine the total phenolic compound level, 7.5 mL of distilled water was added to the tubes, followed by 100 µL of leaf extract. 500 µL of Folin Ciocalteu was added and kept for 3 minutes, 1 mL of 20% saturated sodium carbonate solution was added at the end of the time and the final volume was completed with 10 mL of pure water and kept in the dark for 1 hour. At the end of 1 hour, the absorbances at 765 nm were recorded with a Shimadzu UV 1208 model UV VIS spectrophotometer (Japan). The results were calculated as mg GAE/kg by calculating the absorbance values of the stock solutions prepared from gallic acid standards at concentrations of 500-5000 ppm and the equation obtained from this graph (R^2=0.998).

Antioxidant activity assay

The radical scavenging activities of grapevine leaf extracts were evaluated by DPPH assay (Brand-Williams et al., 1995). The antioxidant activities of the extracts were expressed as the effective concentration (EC_{50}), which is the concentration (mg/mL) of the extract required to reduce the absorbance of DPPH● solution by 50%.

Antioxidant enzyme assays

All antioxidant enzymatic measurements were performed at 0-4°C. Frozen leaf samples (0.5 g) were homogenized in a homogenizer (Ultra-Turrax T25-Germany) in 5 mL 100 mM sodium phosphate buffer (pH 7.5) containing 100 mM Na-phosphate, 0.5 mM EDTA-Na2 and 1 mM ascorbic acid. The homogenized samples were centrifuged at 8000 g for 30 minutes (Sigma 3K30-Germany). The supernatant was analyzed by spectrophotometer at 20°C for superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzyme assays (Şahin et al., 2024). Catalase activity was measured by the decrease in absorbance at 240 nm over 1 minute following the decomposition of H_2O_2 (Çakmak et al., 1993). Superoxide dismutase activity was measured using the nitroblue tetrazolium (NBT) method. The inhibition of the photochemical reduction of NBT was measured spectrophotometrically at 560 nm (Gong et al., 2005). Ascorbate peroxidase activity was determined by measuring

the decrease in ascorbate and the change in absorbance at 290 nm (Nakano & Asada, 1981). Enzyme activities were expressed as U/g.

Data analyses

The study was carried out according to the randomized block design with three replications. Statistical analyses of the data obtained were carried out using JMP 13.2.0 package software (SAS Institute Inc., Cary, NC) ; the significance of differences between treatments was evaluated at $p \leq 0.01$ significance level by subjecting all samples to the Tukey multiple comparison test. Principal component analysis (PCA) was performed to determine the interaction between all treatments and rootstocks and between parameters related to oxidative stress. All these analyses were performed using the relevant statistical software.

RESULTS

Total phenolics

Phenolic compounds are important components of plant defense responses to environmental stresses and their synthesis levels are generally increased under stress conditions. In this study, the effects of two different salt stresses and two different biostimulants (*S. cerevisiae* and *A. nodosum*) on phenolic compound accumulation were evaluated on ownroot, 1103P and 140Ru rootstocks of Öküzgözü (*Vitis vinifera* L.) cultivar (Figure 1). In uninoculated grapevines, the phenolic compound level in the control group was measured as 4658 mg GAE/kg. Low dose salt treatment (Salt 1) increased this value by 61% to 7502 mg GAE/kg. High dose salt treatment (Salt 2) provided the highest increase in phenolic compound content and reached 12956 mg GAE/kg with an increase of 177% compared to the control group. This result indicates that salt stress activates phenolic metabolism more strongly in a dose-dependent manner. *S. cerevisiae* biostimulant applied together with Salt 1 increased the phenolic content to 6736 mg GAE/kg, which is lower than Salt 1 but 44% higher than the control group. For the same salt dose, *A. nodosum* application decreased the phenolic content to 4524 mg GAE/kg and remained below the control group. This suggests that *A. nodosum* had a negative or insufficient effect on phenolic compound production in this combination. On the other hand, *S. cerevisiae* biostimulant applied together with high dose salt (Salt 2) resulted in a very high level of phenolic accumulation with 13220 mg GAE/kg, which means an increase of approximately 184% compared to the control group. In the second treatment with *A. nodosum* (Salt + *A. nodosum* 2), the level of 12028 mg GAE/kg was reached, which means an increase of approximately 158% compared to the control group. In this context, it can be said that biostimulant applications strongly support phenolic metabolism, especially in high dose salt stress. Similar trends were observed in 1103P rootstock. While the control group was 5692 mg GAE/kg, Salt 1 treatment increased this value by 4.3% to 5936 mg GAE/kg. However, phenolic accumulation increased dramatically mainly at high dose salt stress; Salt 2 treatment increased by approximately 177% compared to the control group with 15744 mg GAE/kg. When biostimulant treatments were evaluated, a decrease to 5100 mg GAE/kg was observed in the combination of low dose salt and *S. cerevisiae*, which is about 10% below the control group. The application with *A. nodosum* gave a result close to the control level with 5782 mg GAE/kg. *S. cerevisiae* and *A. nodosum* applied together with high dose salt produced phenolic content of 6828 mg GAE/kg and 13544 mg GAE/kg, respectively. It was observed that *A. nodosum* was especially effective in the second application with high salt doses. In particular, A. nodosum was found to be highly effective in the second application with high doses of salt, increasing the phenolic compound content by 138%. In 140Ru rootstock, the control group was 4968 mg GAE/kg in terms of phenolic content. Salt 1 application provided a very low increase of 5058 mg GAE/kg, remaining below the control level. On the other hand, the Salt 2 application shows an increase of approximately 163% with 13076 mg GAE/kg. In biostimulant treatments, the combination of low dose salt and *S. cerevisiae* increased phenolic content to 5848 mg GAE/kg, 17.7% higher than the control group. *A. nodosum* provided a similar result (21% increase) with 6036 mg GAE/kg. *S. cerevisiae* applied with high doses of salt produced phenolic content of 11168 mg GAE/kg, while *A. nodosum* remained at a lower level with 9184 mg GAE/kg. These results indicate that *A. nodosum* was the most effective biostimulant against high salt stress in 140Ru rootstock, while both biostimulants showed limited effect at low doses. In general, salt stress increased phenolic compound content in all rootstocks. This increase was more pronounced at higher salt doses, confirming once again that phenolic metabolism is an important biochemical indicator of stress responses. The effect of biostimulant treatments differed depending on the dose and rootstock type. The most remarkable results were observed in *S. cerevisiae* and *A. nodosum* treatments in combination with high doses of salt on ownroot and 1103P rootstock. These findings suggest that biostimulants can enhance the phenolic response to salt stress, especially in appropriate combinations.

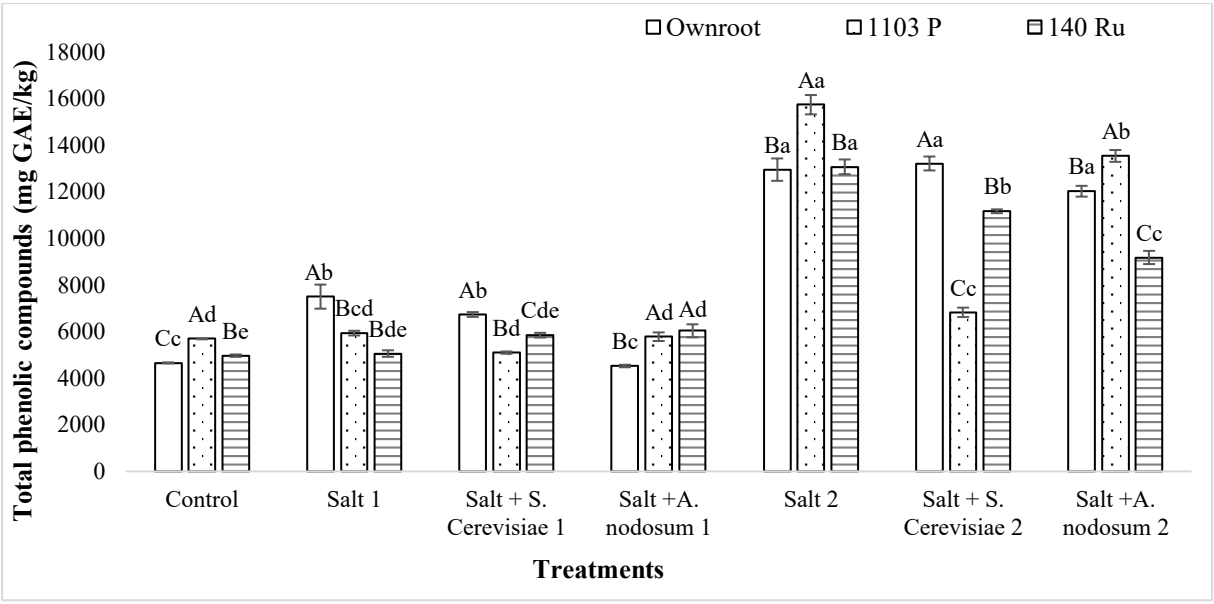


Figure 1. Total phenolic compound contents of leaves according to treatments. Each value represents the mean ± standard error of three replicates. Means indicated by different letters in the graph are significantly different ($p \leq 0.01$). Uppercase letters indicate difference between rootstocks and lowercase letters indicate difference between treatments. Vertical bars correspond to standard error.

EC_{50}

Since EC_{50} value is an inversely proportional measure reflecting antioxidant capacity, low EC_{50} values mean high antioxidant activity. As shown in Figure 2, while the EC_{50} value of the control group was 0.3310 mg/mL in ownroot grapevines, this value decreased by 24% to 0.2511 mg/mL with low dose salt application, and decreased by 65% to 0.1147 mg/mL with high salt application. This indicates that salt stress significantly increases antioxidant defense in a dose-dependent manner. In particular, the EC_{50} value of 0.0457 mg/mL obtained with Salt 2 + *S. cerevisiae* 2 treatment stood out as the lowest value among all groups and revealed that this biostimulant application most strongly promoted the antioxidant response against salt stress in ungrafted vines. In 1103P rootstock, the control group exhibited a basal level with an EC_{50} value of 0.3305 mg/mL. Salt treatments significantly reduced this value; 43% reduction was recorded with Salt 1 and 82% with Salt 2. However, the effect of biostimulant treatments was limited in this rootstock. Especially in Salt 2 + *S. cerevisiae* 2 treatment, the EC_{50} value increased to 0.4982 mg/mL, suggesting that antioxidant activity was suppressed. Similarly, in other biostimulant combinations, EC_{550} values close to or higher than the control group were obtained, indicating that the intrinsic defense capacity of 1103P rootstock is naturally active rather than supported by biostimulants. In 140Ru rootstock, the EC_{50} value of the control group was the highest at 0.3789 mg/mL, which represents the lowest basal antioxidant activity among the rootstocks. Salt treatments significantly decreased this value; in Salt 2 treatment, EC_{50} value decreased to 0.1424 mg/mL and antioxidant activity increased significantly. However, in biostimulant treatments, especially in Salt + *A. nodosum* 2 combination, EC_{50} increased to 0.3398 mg/mL, indicating that antioxidant activity was suppressed. In contrast, the combination of low doses of salt and *A. nodosum* (Salt 1 + *A. nodosum* 1) reduced the EC_{50} value to 0.2430 mg/mL increasing antioxidant capacity compared to the control group. In this context, it seems that biostimulant applications against high salt stress in 140Ru rootstock provided limited benefit and in some cases even weakened the defense response. Overall, the strongest antioxidant response was obtained with the second application of *S. cerevisiae* against high salt stress in ownroot vines. On the other hand, 1103P and 140Ru rootstocks could generate strong antioxidant responses even without biostimulant application, especially against high salt stress; however, biostimulant applications did not always provide the expected positive contribution in these rootstocks. These differences suggest that the physiological and biochemical defense capacities of rootstocks play a decisive role in biostimulant efficacy.

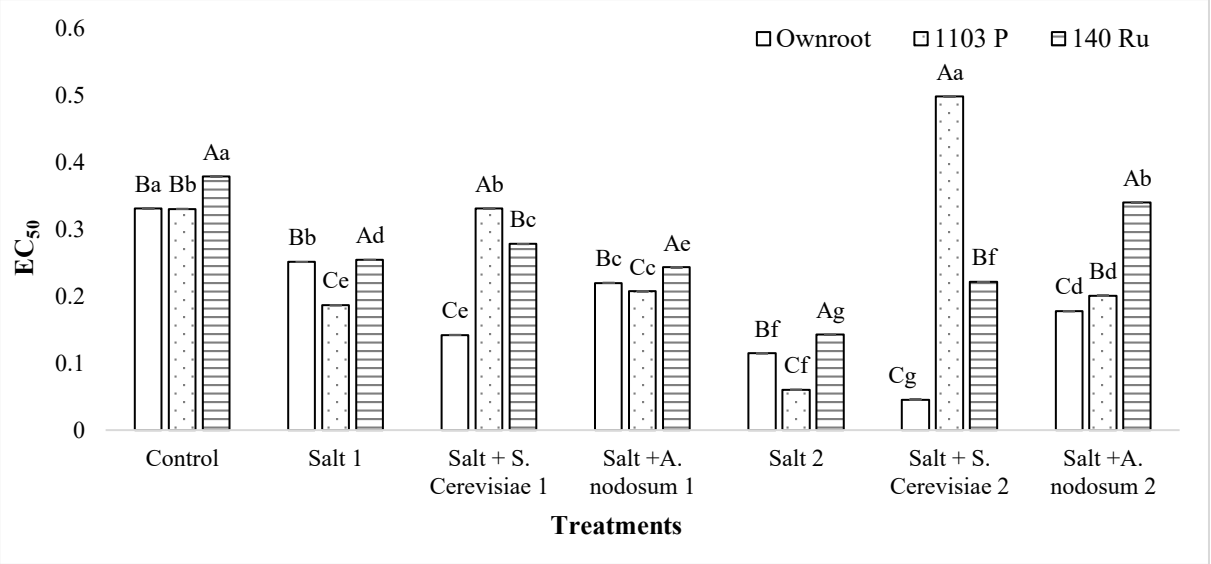


Figure 2. EC_{50} levels of leaves according to treatments. Each value represents the mean ± standard error of three replicates. Means indicated by different letters in the graph are significantly different ($p \leq 0.01$). Uppercase letters indicate difference between rootstocks and lowercase letters indicate difference between treatments. Vertical bars correspond to standard error.

Superoxide dismutase (SOD)

The SOD enzyme is involved in the detoxification of reactive oxygen species under oxidative stress conditions and reflects the defense capacity of the plant under stress. Therefore, increasing the stress level usually leads to an increase in SOD activity, whereas reducing the stress through biostimulants may lead to a decrease in enzyme levels. As can be seen from Figure 3, SOD activity in ungrafted grapevines, which was 482.778 U/g in the control group, remained almost constant (481.444 U/g) at the low level of salt stress (Salt 1), suggesting that the low salt level did not create an additional oxidative stress load in the ungrafted plant. However, the enzyme level decreased to 68.474 U/g in Salt 2 treatment, indicating that plant defense was suppressed under very high stress conditions. The increase in SOD activity to 86.889 and 97.000 U/g with the second applications of *S. cerevisiae* and *A. nodosum*, respectively, indicates that biostimulants can partially support the enzymatic response of the plant under high stress conditions. On the other hand, at low salt level, SOD activity decreased to 361.167 U/g with *S. cerevisiae* 1st application and 192.778 U/g with *A. nodosum*, indicating that these biostimulants reduce the enzyme production requirement of the plant by reducing the stress level. In 1103P rootstock, while SOD activity in the control group was 360.167 U/g, this value decreased by 42% to 207.714 U/g with low salt application. However, the activity increased to 480.444 U/g with *S. cerevisiae* application, indicating that this biostimulant is effectively combating oxidative stress by triggering the enzymatic defense system. In high salt stress, SOD level decreased to 118.061 U/g; this value increased to 187.571 U/g with *S. cerevisiae*, again supporting the protective effect of this microorganism. In *A. nodosum* treatment, the activity was close to the control level with 363.167 U/g under low salt; however, the lowest value of 67.140 U/g was observed at high salt dose. This indicates that *A. nodosum* was ineffective enough in the 1103P rootstock under high stress conditions. In 140Ru rootstock, the SOD activity in the control group had the highest basal value of 582.333 U/g, indicating that the natural defense system of this rootstock is more active compared to other rootstocks. At the low salt stress level, this value decreased by 45% to 320.407 U/g, indicating that stress suppresses the defense system. Biostimulant treatments, especially with *S. cerevisiae*, increased SOD activity again up to 482.778 U/g, indicating that this treatment balanced the defense system by reducing the stress level. Under high salt conditions (Salt 2), SOD level decreased to 130.333 U/g; however, *S. cerevisiae* (107.778 U/g) and *A. nodosum* (117.727 U/g) treatments were able to compensate this decrease to a limited extent. It is understood that the effects of both biostimulants were limited under high dose salt stress, but *S. cerevisiae* was more effective in maintaining enzymatic balance, especially under low stress conditions. In general, it was observed that high salt stress suppressed SOD enzyme activity in all rootstocks, but *S. cerevisiae* application contributed to the fight against oxidative stress by reducing this suppression especially at low stress levels. While the most dramatic enzyme decreases were observed in ungrafted plants, 140Ru rootstock stood out with its stronger basal defense capacity, while 1103P responded more positively to *S. cerevisiae* treatment. These findings suggest that rootstock selection and biostimulant applications are critical for managing of plant defense systems under salt stress conditions.

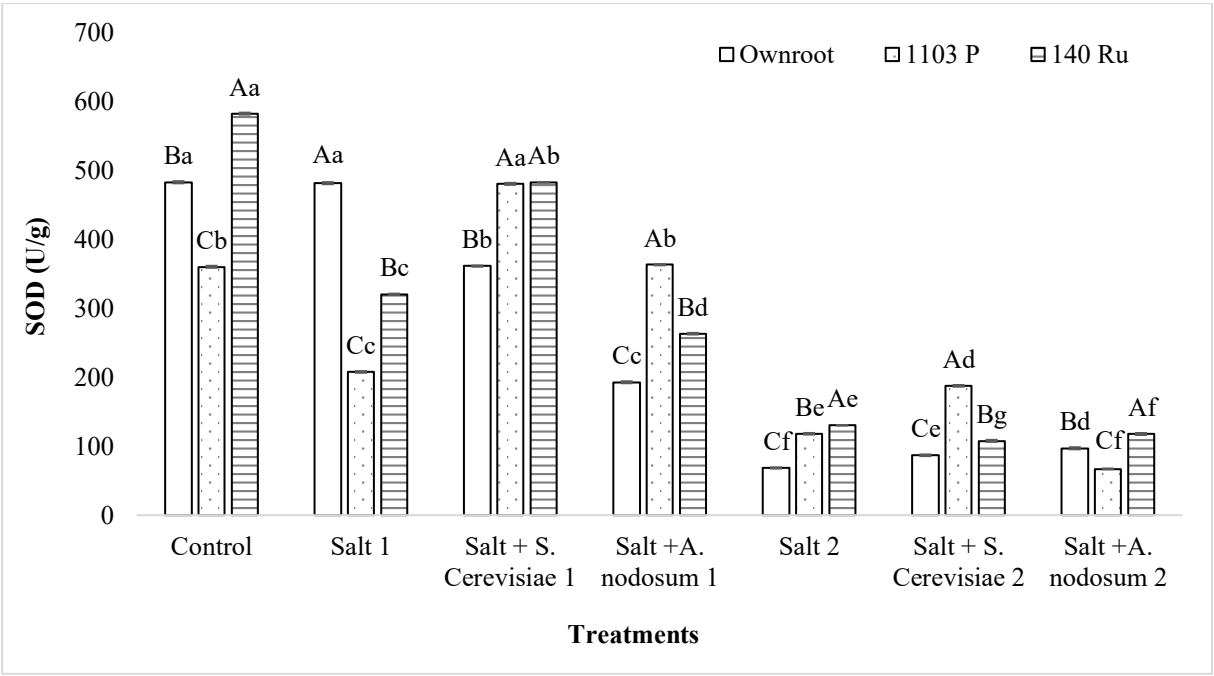


Figure 3. Superoxide dismutase (SOD) levels of leaves according to treatments. Each value represents the mean ± standard error of three replicates. Means indicated by different letters in the graph are significantly different ($p \leq 0.01$). Uppercase letters indicate difference between rootstocks and lowercase letters indicate difference between treatments. Vertical bars correspond to standard error.

Catalase (CAT)

Catalase (CAT) enzyme is an essential antioxidant defense component that prevents the harmful effects of oxidative stress by degrading reactive oxygen species (ROS) into water and oxygen in plant cells. Especially under environmental stress conditions, an increase in catalase activity is an indication that the plant responds to oxidative stress (Gill and Tuteja, 2010). Salt stress leads to oxidative damage by increasing ROS production in plants and therefore the enzyme catalase plays a central role in defense systems under stress (Mittler, 2002). According to the data obtained, low salt stress treatment (Salt 1) did not cause any change in vines on their own roots compared to the control group, and the enzyme level remained constant (Figure 4). However, catalase activity increased by 266.7% in *S. cerevisiae* treatment. Similarly, *A. nodosum* treatment showed a more limited but positive effect with an increase of 13.3%. The most remarkable finding was observed in high salt stress (Salt 2) and *S. cerevisiae* 2 treatments. High dose salt stress resulted in a 393.3% increase in catalase activity. However, the *S. cerevisiae* 2 treatment exceeded even this value, showing the most dramatic increase with a 660% increase. Finally, the *A. nodosum* 2 treatment produced an increase of 120%. On 1103P rootstock, Salt 1 treatment increased catalase activity by 150% compared to the control. This increase suggests that the plant showed a moderate stress response. On the other hand, a 50% decrease was observed in *S. Cerevisiae* treatment. This indicates that yeast treatment alone may not have promoted an adequate defense response to salt stress. In the *A. nodosum* treatment, the catalase level remained constant. Another remarkable finding was observed in the high salt treatment (Salt 2), which resulted in an extremely high increase of 1056.3% in catalase activity. This increase indicates that high levels of salt stress significantly increase the amount of reactive oxygen species (ROS) in the cell and therefore catalase enzymes are intensively activated. However, *S. cerevisiae* 2 treatment also significantly increased by 106.3%. *A. nodosum* 2 treatment produced a smaller increase (12.5%). In 140 Ru rootstock, a significant 72.7% decrease in catalase activity was observed with Salt 1 treatment compared to the control group. This decrease suggests that the antioxidative defense response of 140 Ru rootstock may be weak at low salt concentrations. Similarly, a 27.3% decrease was observed in *A. nodosum* treatment. However, this situation was reversed in *S. Cerevisiae* treatment and a slight increase of 3% was realized compared to the control group. This increase indicates that yeast application may have triggered the antioxidant defense to some extent. Catalase activity increased by 96.9% with Salt 2 treatment. This increase indicates that high salt concentration creates a stronger oxidative stress and the catalase enzyme level increased significantly in response. *S. Cerevisiae* 2 treatment also strengthened the defense mechanism with an increase of 42.4%. The highest catalase activity was observed in *A. nodosum* 2 treatment and this group showed an increase of 151.5% compared to the control group. This result suggests that seaweed application especially seaweed application promotes an effective antioxidative response against high salt stress.

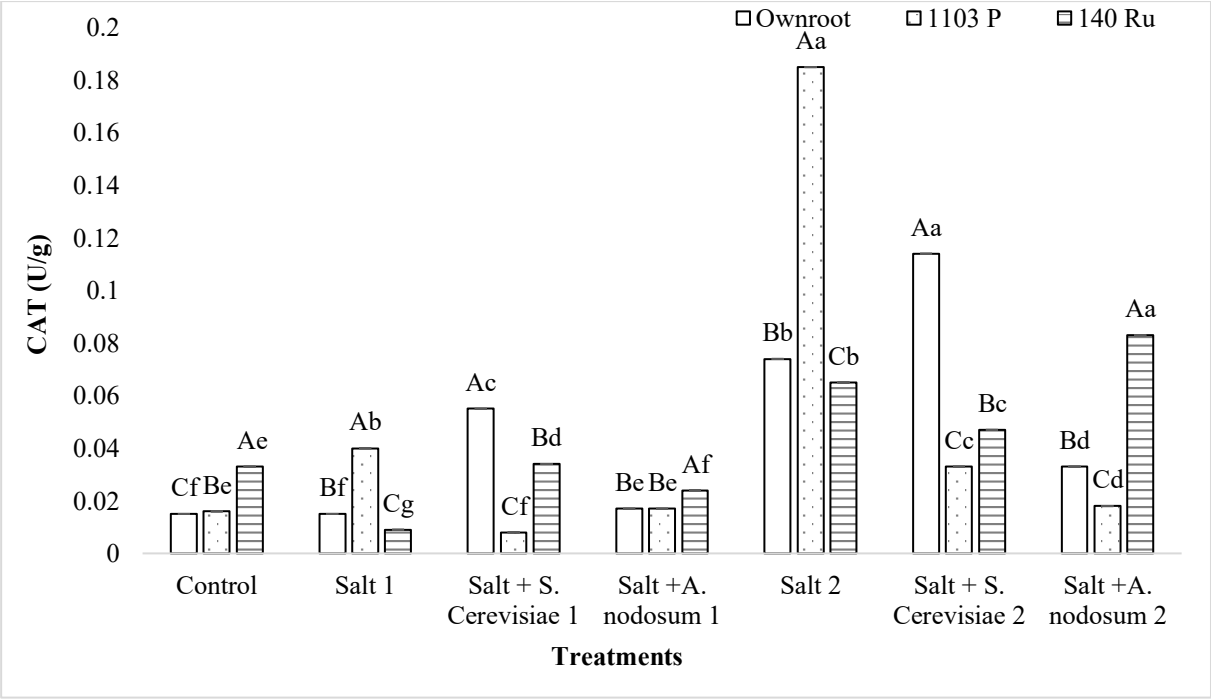


Figure 4. Catalase (CAT) levels of leaves according to treatments. Each value represents the mean ± standard error of three replicates. Means indicated by different letters in the graph are significantly different ($p \leq 0.01$). Uppercase letters indicate difference between rootstocks and lowercase letters indicate difference between treatments. Vertical bars correspond to standard error.

Ascorbate peroxidase (APX)

While the increase in APX activity under stress conditions indicates that the plant tries to limit oxidative damage; biostimulant applications may play a regulatory role in enzymatic activity by reducing this stress level (Mittler, 2002; Gill & Tuteja, 2010). In this study, when APX enzyme activity was evaluated in ownroot, 1103P and 140Ru rootstocks, 0.197, 2.125 and 0.214 U/g protein values were determined in the control groups, respectively (Figure 5). In ownroot plants, low dose salt treatment (Salt 1) increased APX activity by about 18% to 0.232 U/g. This increase indicates that the plant senses oxidative stress and initiates enzymatic defense. High dose salt stress (Salt 2) increased APX level by about 878% to 1.928 U/g, indicating that the plant showed maximum enzymatic response to severe stress. The second applications with *S. cerevisiae* and *A. nodosum* biostimulants reduced this high activity to 0.875 and 0.911 U/g, respectively, thereby significantly improving the stress level by approximately 54–52%. These results suggest that both biostimulants were effective in regulating APX activity in ungrafted plants, but *A. nodosum* showed a higher effect. The high APX activity (2.125 U/g) in 1103P rootstock in the control group indicates that the basal defense capacity of this rootstock is quite strong. However, this value decreased by 79% to 0.446 U/g in low salt stress and decreased by 83% to 0.357 U/g in high salt stress. These decreases indicate that 1103P has difficulty in maintaining APX activity in the face of salt stress. Biostimulant treatments reversed this situation: Salt 2 + *S. cerevisiae* 2 treatment increased the activity to 0.911 U/g and *A. nodosum* 2 to 0.768 U/g. These rates correspond to 155% and 115% increases in enzyme level, respectively. Therefore, *S. cerevisiae* treatment stands out as the biostimulant that best supports the APX activity of this rootstock. In 140Ru rootstock, the control group showed an APX activity of 0.214 U/g. With Salt 1 treatment, this value decreased by 24% to 0.161 U/g, but increased to 1.357 U/g under high salt stress, i.e. an increase of approximately 535%. This increase indicates that 140Ru also gives an active defense response under stress. The second treatment of *S. cerevisiae* further alleviated the stress effect by increasing this value to 1.536 U/g. This represents an increase of approximately 618% compared to the control group. Similarly, the application of *A. nodosum* made a significant contribution, reaching a value of 0.893 U/g. However, the response of this rootstock to biostimulants was stronger in favor of *A. nodosum*. In conclusion, APX enzyme formed an important defense response against salt stress in all rootstocks and the highest activity increases were observed especially in high dose salt treatments. Biostimulant applications alleviated the stress by regulating this excessive enzymatic defense and tended to normalize APX levels. Especially in ungrafted plants and 1103P rootstock, *A. nodosum* and 140Ru rootstock, *S. cerevisiae* were the most successful biostimulants.

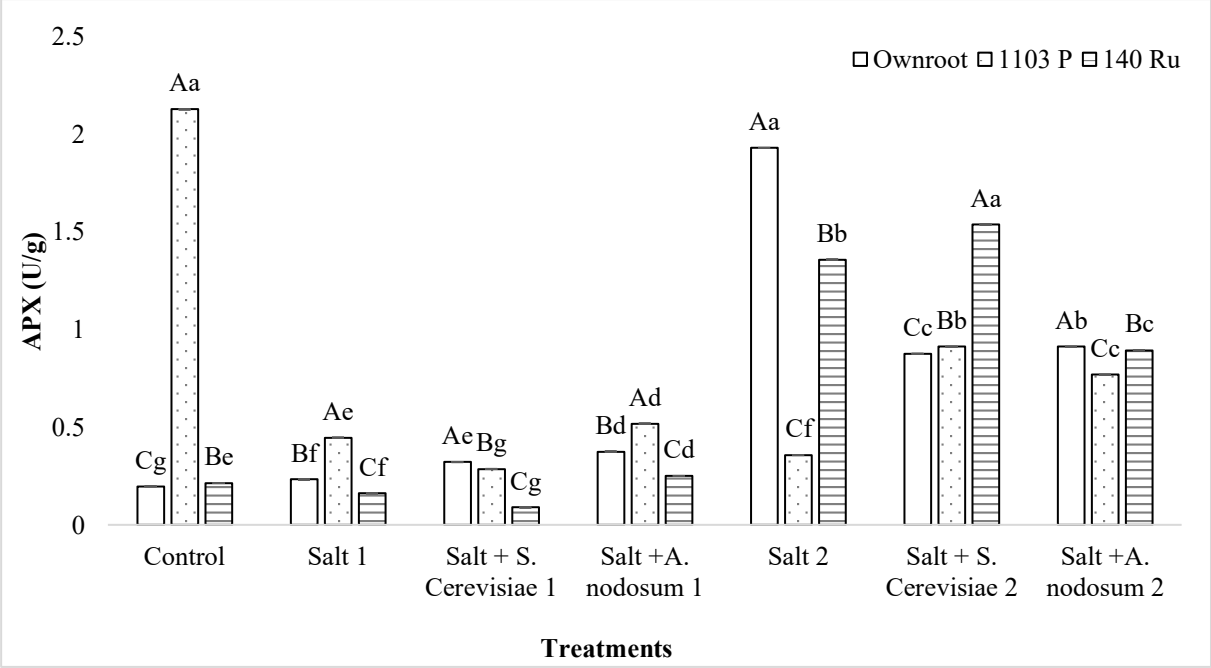


Figure 5. Ascorbate peroxidase (APX) levels of leaves according to treatments. Each value represents the mean ± standard error of three replicates. Means indicated by different letters in the graph are significantly different ($p \leq 0.01$). Uppercase letters indicate difference between rootstocks and lowercase letters indicate difference between treatments. Vertical bars correspond to standard error.

PCA analysis of all treatments and rootstocks

Principal Component Analysis (PCA) was performed to evaluate the antioxidant defense responses of 21 different treatments induced by different rootstocks (Ownroot, 1103P and 140Ru), two levels of salt stress (Salt 1 and Salt 2) and biostimulant treatments (*Saccharomyces cerevisiae* and *Ascophyllum nodosum*) were presented in Figure 6. According to the PCA results, 60.7% of the total variance was explained by the first component (PC1) and 20.6% by the second component (PC2); these two components together represented 81.3% of the variance, reflecting the majority of the variables in the analysis. When vectorial loadings were evaluated, total phenolic compound content (TPC) and catalase (CAT) activity had a positive effect on PC1, while EC_{50} and superoxide dismutase (SOD) showed negative loadings. APX (ascorbate peroxidase) positively contributed mostly on the PC2 axis. Among the treatments in the lower right quadrant of the graph, 14, especially the groups in which 1103P rootstock was applied under high salt conditions, stood out with high total phenolic content and catalase activity. This indicates that 1103P rootstock developed a more effective defense response against salt stress. Especially treatment 14 (Salt 2/1103P) and treatment 16 (Salt 2/Ownroot/*S. cerevisiae*) were the groups with the highest antioxidant capacity. The treatments in which APX activity was dominant were located in the upper right part of the graph and it was observed that treatments 18 (Salt 2/140Ru/S. *cerevisiae*), 20 (Salt 2/1103P/*A. nodosum*) and 21 (Salt 2/140Ru/*A. nodosum*) exhibited high ascorbate peroxidase activity. This reveals that an effective defense response developed in these groups, especially against H_2O_2 detoxification. In contrast, treatments 1, 3, 6, 8 and 9, located on the left side of the PCA plot and close to the EC_{50} and SOD vectors, were characterized by lower antioxidant capacity and higher oxidative stress indicators. Treatments located around the center (e.g. 5, 10, 11, 12), on the other hand, exhibited more balanced and average responses in terms of both antioxidant capacity and enzymatic defense. In conclusion, this analysis revealed that antioxidant defense systems differed depending on the applied rootstock and stress conditions. In particular, *S. cerevisiae* and *A. nodosum* biostimulants applied under high salt conditions increased the capacity of plants to cope with oxidative stress by stimulating total phenolic compound synthesis and enzymatic defense systems. The PCA graph clearly revealed these differences with its multidimensional structure and was evaluated as an effective tool in understanding the dynamics of antioxidant defense systems.

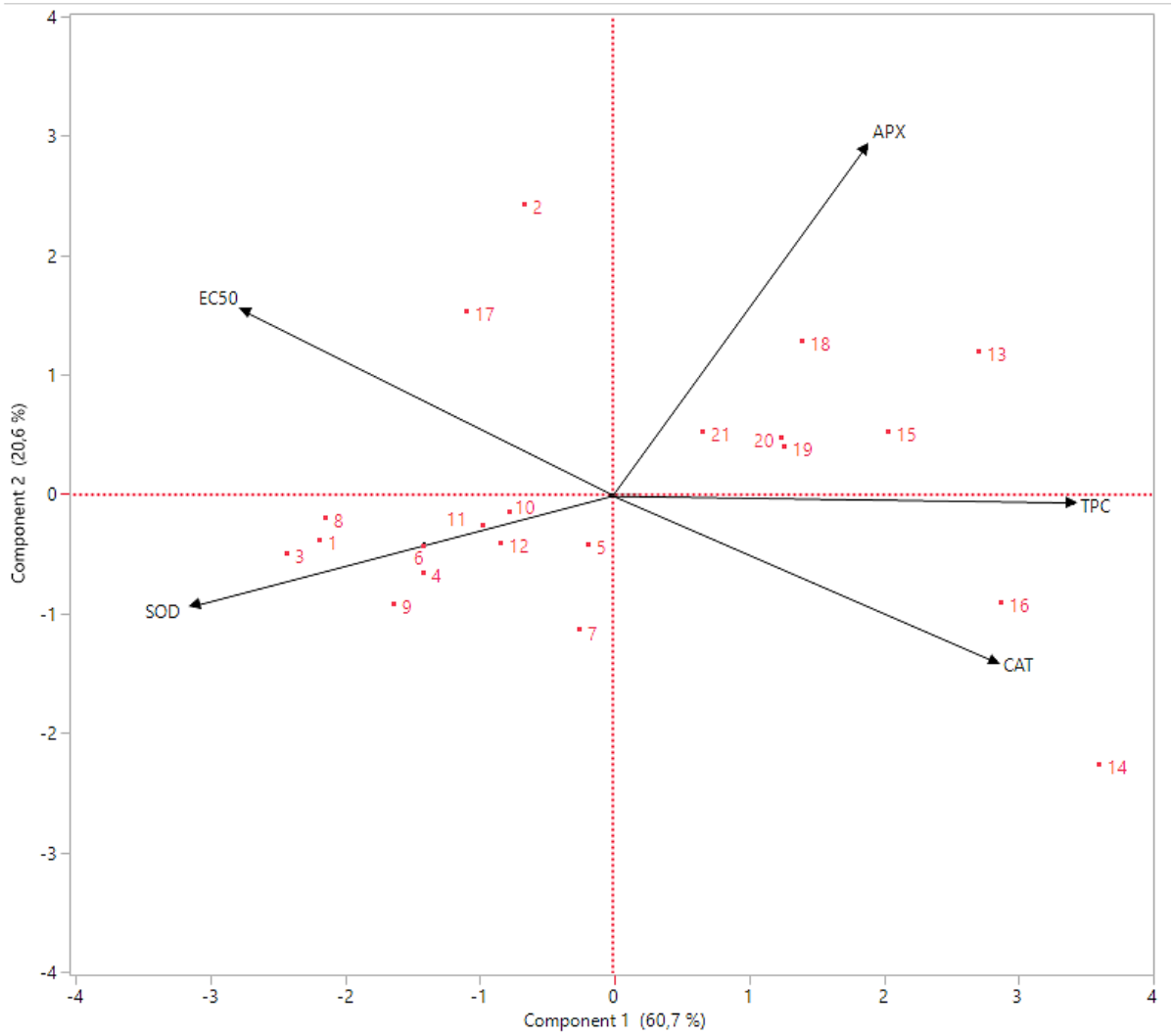


Figure 6. PCA biplot of all treatments and rootstocks.
1: Control/Ownroot, 2: Control/1103P, 3:Control /140Ru, 4: Salt 1/Ownroot, 5: Salt 1/1103P, 6: Salt 1/140Ru, 7: Salt 1/ Ownroot/*S. Cerevisae*, 8: Salt 1/ 1103P/*S. Cerevisae*, 9: Salt 1/ 140Ru/*S. Cerevisae*, 10: Salt 1/ Ownroot/ *A. Nodosum*, 11: Salt 1/ 1103P/ *A. Nodosum*, 12: Salt 1/ 140Ru/ *A. Nodosum*, 13: Salt 2/Ownroot, 14: Salt 2/1103P, 15: Salt 2/ 140Ru, 16: Salt 2/ Ownroot/*S. Cerevisae*, 17: Salt 2/ 1103P/*S. Cerevisae*, 18: Salt 2/ 1140Ru/*S. Cerevisae*, 19: Salt 2/ Ownroot/ *A. Nodosum*, 20: Salt 2/ 1103P/ *A. Nodosum*, 21: Salt 2/ 140Ru/ *A. Nodosum*.

DISCUSSION

It has been well documented in previous studies that when plants are exposed to adverse environmental conditions such as extreme temperature, heavy metals, drought, water availability, air pollutants, nutrient deficiency or salt stress, the production of ROS such as $^1O_2$, O_2^-, H_2O_2 and OH● increases (Gill and Tuteja, 2010; Choudhury et al., 2017; Wang et al., 2024). To protect themselves against these toxic oxygen intermediates, plant cells and organelles such as chloroplasts, mitochondria and peroxisomes employ antioxidant defense systems. SOD is a potent metalloenzyme common in all aerobic organisms and forms the first line of defense against oxidative stress in plants by inactivating reactive oxygen species (ROS), which increase due to environmental stresses (Gill and Tuteja, 2010). Catalase (CAT) is a high turnover rate antioxidant enzyme with a tetrameric structure that plays a critical role in the elimination of oxidative stress in plants by converting hydrogen peroxide (H_2O_2), especially formed in peroxisomes, into water and oxygen with high efficiency (Polidoros and Scandalio, 1999). APX is known to detoxify H_2O_2 into water and oxygen during stress. APX plays an important role in the salinity tolerance of plants since it has higher activity in salt tolerant species (Sudha and Ravishankar, 2002). In this study, as the dose of salt stress increased, the levels of enzymes other than SOD increased as expected. Under normal conditions, an increase in SOD activity is expected in plants exposed to salt stress because SOD forms the first line of defense by converting superoxide radical into hydrogen peroxide and oxygen. However, in some studies, similar to our results, SOD activity remained unexpectedly low or did not increase under high salt

conditions. Hernández et al. (1994) showed that high salt stress suppressed the activity of different SOD isozymes to different degrees in *Vigna unguiculata*. They emphasized that mitochondrial Mn-SOD was particularly sensitive to salt stress and that in leaves, mitochondrial Mn-SOD activity was significantly reduced (up to 35% and 60%, respectively) at 35 mM and 100 mM NaCl concentrations. The researchers have reported that SOD enzyme will break down superoxide to produce H_2O_2 and H_2O_2 will be cleared by APX/CAT. It has been suggested that when high levels of H_2O_2 are formed, this molecule may act as a signal, triggering feedback mechanisms that prevent SOD from working further, and H_2O_2 formed as a result of SOD activity may decrease the expression of SOD genes (Qu et al., 2010). The rootstock used in grafted vines can determine the distribution of salt within the plant and the severity of stress. Salt-resistant rootstocks can reduce the stress level in the leaves of the grafted cultivar by retaining Na^+ ions in the roots or transmitting them in a more controlled manner. For example, under salt stress, the amount of Na and Cl accumulated in the leaves of vines grafted on some rootstocks is lower than on others. If the rootstock partially blocked the salt and allowed less of it to pass to the leaves, superoxide production in the leaf tissue may also have been relatively low. In this case, APX and CAT may still increase (to scavenge H_2O_2, albeit to a lesser extent), but there may not have been a significant increase in SOD activity because superoxide levels were not very high. In addition, rootstock-induced hormonal/signaling effects may also alter antioxidant enzyme profiles; some rootstocks may have transmitted signals that promote other enzymes instead of SOD (Şahin, 2009). In the results of the study, SOD was the most abundant antioxidant enzyme in accordance with previous studies (Aazami et al., 2023; Gajjar et al., 2023). Similar to this study, Desoky et al. (2019) found lower levels of antioxidant enzymes in biostimulant-treated groups against salt stress compared to untreated groups.

Total phenolic compounds and antioxidant activity in grapevine (*Vitis vinifera* L.) leaves may show significant changes due to environmental stresses and agricultural treatments. Recent studies have focused on the effects of salt stress, different rootstocks and biostimulant treatments on phenolic accumulation and antioxidant defense mechanisms in plants. Plants are exposed to various abiotic stresses throughout their life cycle. Their interactions with changing environmental conditions have been an important driving force behind the emergence of certain natural products (Lattanzio, 2013). When a plant is exposed to abiotic stress, a number of genes are turned on or off, resulting in increased levels of various metabolites and proteins, some of which may be responsible for providing a certain degree of defense against these stresses (Ahmad et al., 2008; Jaleel et al., 2009). The accumulation of phenolics in plant tissues is considered an adaptive response of plants to adverse environmental conditions, thereby enhancing evolutionary fitness (Naikoo et al., 2019). A large proportion of arable land is facing abiotic stresses (drought, salinity, cold, heat, heavy metal toxicity, UV radiation, etc.), which are expected to increase due to climate change (Sharma et al., 2019). Salt stress can significantly affect secondary metabolite production in plants. Many studies show that salinity increases phenolic and flavonoid compound levels in plants by activating the phenylpropanoid pathway (Zargoosh et al., 2019,). The ionic and osmotic stress that occurs when the plant transports the salt taken by the roots to organs such as leaves can trigger the synthesis of phenolic compounds for defense. Indeed, it has been reported that leaf flavonoid and phenol content increases under increasing salt stress conditions. This is due to the fact that phenolic compounds are part of the non-enzymatic antioxidant defense in plants; phenolics together with molecules such as proline and ascorbic acid are mechanisms that neutralize reactive oxygen species (ROS) (Gajjar et al., 2023). In this study on salt stress, with increasing salinity, leaf total phenolic compound concentration increased in stressed plants, thus this increase was concentration dependent and the highest total phenol concentration was observed in vines treated with 150+150 mM NaCl. Salt-induced oxidative stress may induce a kind of protective response by promoting the accumulation of phenolic substances in plant cells. For example, increased production of phenolic compounds (especially stilbenes and flavonoids) has been observed in salt-treated grapevine cell cultures, leading to an increase in total antioxidant capacity (Almagro et al., 2022). In this study, salt treatment was reported to increase phenylalanine ammonia lyase (PAL) enzyme activity, resulting in more phenolics synthesized. Similarly, in another study, total phenolic content in leaves of Merlot and Cabernet Franc cultivars treated with salt for 60 days increased by 59-70% compared to control plants (Nikolaou et al., 2021). This finding indicates that salt stress strongly induces phenolic accumulation in grapevines. In parallel with the increase in phenolic matter, the DPPH radical scavenging capacity of leaf extracts also increases. The literature shows a generally positive correlation between total phenolic content measured by Folin-Ciocalteu and DPPH antioxidant activity, similar to our results (Pérez, et al, 2023). Biostimulant-treated vines showed lower levels of total phenolic compounds than vines under salt stress alone, suggesting that this may act as an adaptation mechanism to overcome salinity-induced oxidative stress (Jamalian et al., 2013; Karimi and Ershadi, 2015). The importance of flavonoids stems from their role in non-enzymatic defense systems (Apel and Hirt, 2004). Similarly, previous studies reported that total phenolic compound content increased after salt stress as in this research (Karaman et al., 2024; Mohammadkhani and Abbaspour, 2018; Karimi et al., 2022).

The rootstock iIn grafted plants such as grapevines, used can significantly influence the physiological and biochemical responses of the variety. To understand why different rootstocks give different results in grapevines under salt stress, the salt tolerance mechanisms provided by the rootstocks and traits such as vigor should be considered (Klimek et al., 2022).

In our results, although the total phenolic compound content increased in the biostimulant groups as the stress dose increased, the EC_{50} value was the highest in the second salt treatment, but not as high as expected in the high salt + biostimulant groups. Although total phenolics and anthocyanins were lower in some cultivars with 1103P rootstock, antioxidant capacity was reported to be high in previous studies (Bouza et al., 2024). This may be related to the composition and type of phenolic compounds, i.e. different rootstocks affect not only the amount of phenolics but also the type of phenolics (e.g. flavonols vs. tannins with stronger antioxidant effect) that accumulate.

The mechanism by which yeast-derived biostimulants increase phenolic compounds is explained by stimulating the phenylpropanoid pathway as an immune response in the plant. In viticulture, in a study focusing on the effect of foliar applied yeast derivatives on grape quality components, it was found that total phenolic content increased significantly in theTempranillo grape variety (Portu et al., 2016). Similarly, Monteiro et al. (2023) showed that *Ascophyllum nodosum* treatment significantly increased phenolic compound contents in *Touriga Franca* grape variety under drought stress conditions by applying different concentrations before vintage.

As a result, under salt stress, the conversion of superoxide radicals to H_2O_2 in leaf tissues was initially achieved by an increase in SOD activity; however, due to the degradative effect of elevated NaCl concentrations on metal cofactors, SOD activity was downregulated and the detoxification of H_2O_2 accumulation was undertaken by CAT and APX enzymes. Rootstock differences in tolerance were found to determine the severity of this defense response: 1103P rootstock exhibited a balanced ROS management, while ion exclusion was accelerated and CAT and APX levels were more sharply elevated by 140Ru rootstock; ungrafted Öküzgözü was subjected to the most intense oxidative stress as a result of high ion accumulation. Biostimulant treatments were shown to enhance CAT-APX expression through *Saccharomyces cerevisiae* extract with a "priming" effect. In contrast, *Ascophyllum nodosum* extract supported the entire SOD-CAT-APX defense network by providing osmotic buffering and modulating ROS signaling. Thus, it was concluded that the increase in CAT and APX activities observed in parallel with the increase in phenolic defense and the relative decrease in SOD is a reflection of negative feedback mechanisms due to high NaCl dose and measurement timing in accordance with the literature.

CONCLUSION

This study aimed to reveal the decisive role of rootstock selection and biostimulant applications in managing antioxidative defense mechanisms in *Vitis vinifera* grapevines under salt stress. The severity and direction of the biochemical responses developed by different rootstocks to salt stress varied significantly depending on their genotypic tolerance levels. Significant increases in total phenolic compound content and catalase activity were observed under high salt stress, whereas a general suppression of superoxide dismutase activity occurred. In particular, rootstock 1103P activated phenolic metabolism more strongly and maintained enzymatic defense more effectively under high salt conditions, indicating its greater capacity to maintain ion homeostasis and manage oxidative stress. Biostimulant treatments modulated plant defense against salt stress in different ways and these effects varied depending on the treatment dose and the type of rootstock used. In ungrafted vines, *S. cerevisiae* application was the most effective biostimulant because it in increased the antioxidant activity. In 1103P and 140Ru rootstocks, the effect of biostimulants was limited, and in some cases, it was observed that intrinsic defense mechanisms could work effectively without biostimulant support. In general, biostimulant applications have the potential to increase phenolic compound production and antioxidative enzyme activities of plants against salt stress, but the success of this effect is closely related to the severity of stress and the rootstock used. In this context, selecting appropriate rootstock-biostimulant combinations is strategically important in developing sustainable viticultural practices against salt stress. Further research is needed in this area to demonstrate the performance and contributions of different rootstocks and biostimulant applications under salt stress conditions.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text and Table are original and that they have not been published before.

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Physicochemical and functional quality characteristics of fresh, dried, and chip forms of 'fuji' apple: insights into variable relationships

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Abstract

This study investigated the physicochemical and functional quality characteristics of fresh, dried, and chip forms of 'Fuji' apples, focusing on bioactive compound retention, color stability, and sugar composition. Drying, a widely used preservation method significantly affects these attributes by altering phenolic content, antioxidant capacity, and vitamin C levels. Fresh apples exhibited the highest total phenolic content and antioxidant activity while drying processes led to reductions due to thermal degradation and enzymatic oxidation. In contrast, anthocyanin content increased in apple chips, suggesting improved pigment extraction. Significant color changes and browning effects were observed in dried apples and apple chips, primarily due to enzymatic browning. Sugar composition varied among apple products, with higher fructose levels in apple chips and stable sucrose content across all forms. Principal component analysis (PCA) highlighted distinctions between fresh and processed apples based on chemical composition and processing effects. These findings emphasize the need for optimized drying techniques to minimize nutrient loss while maintaining desirable sensory and functional properties. This study provides valuable insights into the impact of drying on apple quality, contributing to the development of nutritionally superior dried fruit products.

Keywords: Drying, Apple products, Phenolic compounds, Antioxidant activity, Sugar composition, Color stability

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INTRODUCTION

Drying is one of the most effective and widely used preservation methods for reducing the moisture content of fresh fruits and vegetables, thus lowering water activity and extending their shelf life (Zhang et al., 2010; Eminoğlu et al., 2019). This process helps maintain the stability and safety of the products and meets the demand for fruits and vegetables year-round, irrespective of seasonality. Moreover, dried products are highly advantageous for transportation and storage, as they take up less space, making them more efficient for distribution (Hou et al., 2020). Dried fruits provide health benefits and help reduce non-communicable diseases, including cardiovascular conditions (Alasalvar et al., 2020). As consumer preference shifts toward natural, minimally processed products, the dried fruit sector has continuously developed, with dried fruits taking on an increasingly prominent role in the food market (Ghinea et al., 2022).

Despite the numerous benefits of drying, the process has its challenges. Drying fruits and vegetables can lead to significant changes in their biochemical composition, particularly the degradation of bioactive compounds such as phenolic acids and flavonoids (Aghbashlo et al., 2010; Kahraman et al., 2021). These compounds are known for their antioxidant properties and play an essential role in the health benefits associated with dried fruits. Furthermore, drying can also affect the final product's texture, color, and sensory characteristics, influencing consumer acceptance (Krokida et al., 2003). The challenges of minimizing the loss of these bioactive components during the drying process are critical for ensuring that the nutritional value of dried fruits remains close to that of

fresh produce (Lutz et al., 2015). In addition, optimizing drying techniques to minimize the degradation of these compounds is essential for enhancing the quality of dried fruit while maintaining their health properties.

Apple (*Malus* × *domestica* Borkh.), one of the most widely produced and consumed fruits globally, is rich in antioxidants, particularly polyphenolic compounds, which have been shown to have numerous health properties (Dumanoğlu et al., 2018). Apples are an important source of dietary fiber, vitamins, and antioxidants, making them a valuable component of a healthy diet. While apples are commonly consumed fresh, they are also processed into various products, including juices, jams, jellies, vinegar, purees, and dried apples. The increasing popularity of dried apple products can be attributed to their convenience, long shelf life, and health benefits for their phenolic content (Herranz et al., 2019; Ghinea et al., 2022). However, the drying process itself can lead to a significant reduction in these bioactive compounds, primarily due to thermal degradation and enzymatic reactions, making it essential to explore methods that can help preserve the phenolic content in dried apple products (Aktaş et al., 2013). Several drying techniques, such as air drying, freeze-drying, and microwave drying, have been explored to minimize nutrient loss while improving the quality of dried apples (Zhou et al., 2020).

Given the growing demand for high quality dried apple products and the need to preserve their antioxidant properties, this study aimed to evaluate the effects of drying on the quality parameters of 'Fuji' apples. This research would contribute to understanding the variation in phenolic compounds and other antioxidant properties of different dried apple products, providing valuable insights into how drying affects the nutritional quality of apples. In addition to the antioxidant properties, this study evaluated other critical quality parameters, such as color and sugar composition, which are essential factors influencing consumer acceptance. By assessing these parameters, the research aims to enhance the value of dried apple products in the global market and support their role in promoting health and well-being, meeting the growing demand for functional foods that offer both convenience and nutritional benefits.

MATERIALS AND METHODS

Plant material

'Fuji' apples (*Malus* × *domestica* Borkh.) were obtained from the dwarf apple collection orchard of Ankara University, Faculty of Agriculture, Department of Horticulture.

Sample preparation and drying method

Apples were harvested at the commercial maturity stage in October 2021 based on titratable acidity, soluble solid content, and starch index. The harvested apples were transported to the laboratory, washed with tap water to remove surface contaminants, and wiped dry with blotting paper. Randomly selected apples were sliced using an apple slicer, seeds were removed, and the apples were cut into uniform cubes (1.5 cm × 1.5 cm). The apple cubes were immersed in a 2% citric acid solution for 1 minute to prevent enzymatic browning. The treated samples were arranged in a single layer on stainless steel trays and dried in an oven at 70°C until they reached two distinct moisture levels (10% for dried apple and 5% for apple chip). Quality analyses were conducted on fresh, dried, and apple chips.

Quality parameter assessments

Color analysis and browning index

Color measurements were performed using a colorimeter (Minolta CR-400, Konica Minolta, Japan) based on the CIE L*, a*, b* color space system. The color difference (ΔE^*) and browning index (BI) were calculated using the following equations (Bal et al., 2011):

Equation (1) - Color Difference (ΔE^*):

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

Where: $\Delta L^*, \Delta a^*, \Delta b^*$represent the differences in lightness, red-green and yellow-blue color values between the fresh apple samples with dried apple and apple chip samples. Typically, the higher the ΔE^*, the more noticeable the color change.

Equation (2) - Browning Index (BI):

$$\mathrm{BI} = \frac{100(\mathrm{x} - 0.31)}{0.17}$$

Equation (3) - x Calculation:

$$\mathrm{x} = \frac{at^* + 1.75Lt^*}{5.645Lt^* + a0^* - 3.012bt^*}$$

Where: $a0^*$represents the a value of the fresh apple samples. Lt, at and bt are L, a, b values of dried apple and apple chip samples.

Extraction procedure for spectrophotometric analysis

Extraction was performed following the method described by Colombo et al. (2019). A 2 g sample, ground with liquid nitrogen, was homogenized in 3 mL methanol/water (1:1, v/v) using a homogenizer (IKA-Labortechnik, Ultra-turrax T25, Germany) for 3 minutes. The homogenized samples were sonicated for 15 minutes and centrifuged (Sigma 3K30, Germany) at 14,000 rpm. The supernatant was collected, and the extraction process

was repeated with the pellet. The pooled extracts were filtered through 0.45 µm PVDF filters, adjusted to a final volume of 10 mL, and stored at +4°C in darkness for further spectrophotometric analysis.

Total phenolic content

The total phenolic content (TPC) of apples was determined using the Folin–Ciocalteu assay with some modifications (Singleton and Rossi, 1965). One hundred microliters (100 µL) of extract, 900 µL of double-deionised water, and 5 mL of Folin–Ciocalteu reagent were vortexed in a test tube. After incubation for 3 minutes at room temperature, 4 mL of saturated sodium carbonate solution (75 g L^{-1}) was added. The mixture was then allowed to stand for an additional 90 minutes at room temperature. The absorbance of the samples was measured at 765 nm using a UV–VIS spectrophotometer (Shimadzu UV-1208, Japan), and results were expressed as mg gallic acid equivalent (GAE) per kg of sample.

Antioxidant capacity

The antioxidant capacity of apple samples was assessed using the Trolox Equivalent Antioxidant Capacity (TEAC) assay, following the method described by Re et al. (1999). The assay utilized the ABTS radical solution, prepared by mixing ABTS diammonium salt with 2.45 mM potassium persulfate and allowing it to react in the dark for 12–16 hours at room temperature. During analysis, the solution was maintained at +4°C. The radical solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to absorb 0.700 ± 0.010 at 734 nm. Inhibition rates were calculated using the equation:

$$\text{Inhibition rate (\%)} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}}$$

The procedure was repeated for 20 and 30 µL sample volumes. Percentage inhibition values (10, 20, 30 µL) were used to generate a regression curve and its equation. The same steps were applied to the trolox standard Analyses were carried out with a UV-VIS spectrophotometer (UV-1208, Shimadzu, Japan), and results are reported as µmol trolox g^{-1}.

Total anthocyanin content

Total anthocyanin content was determined using the pH differential method (Giusti and Wrolstad, 2001) and expressed as mg cyanidin-3-glucoside per kg of sample. Absorbance was measured at 520 and 700 nm, and total anthocyanin content was calculated using the equation:

$$\text{Total anthocyanin (mg/kg)} = \frac{\text{(A) x (MW) x (DF) x 1000}}{\text{(ε) x (L)}}$$

Where:

A = Difference in absorbance between pH 1.0 and 4.5

MW = Molecular weight of cyanidin-3-glucoside

DF = Dilution factor

ε = Molar extinction coefficient

L = Pathlength (cm)

Titratable acidity

Ten grams of the homogenized sample were rehydrated with distilled water at 4 °C overnight. The mixture was then homogenized using a homogenizer (IKA-Labortechnik, Ultra-turrax T25, Germany) for 3 minutes. The resulting homogenate was filtered through cheesecloth, and the filtrate was used for titratable acidity analysis. Titratable acidity was determined using an automatic titrator (Mettler Toledo DL50 Graphix) and expressed as % malic acid.

Vitamin C content

Ten grams of fruit were homogenized in a metaphosphoric acid solution, centrifuged, and filtered (0.45 µm). The filtrate was analyzed using the high-pressure liquid chromatography (HPLC) (Shimadzu LC 10 AT VP, Japan) equipped with a Phenomenex Luna C18 column. The mobile phase A: 100% acetonitrile B: 2% acetic acid (A:B; 20:80) at a flow rate of 1 mL min^{-1}, with the column oven maintained at 25°C. Quantification of peaks obtained at a wavelength of 245 nm using a DAD detector was performed externally based on a standard of L-ascorbic acid.

Sugar content

Five grams of fruit were homogenized in double-deionized water to measure sugar content and kept at room temperature in a shaking incubator for 30 minutes. The mixture was then centrifuged for 15 minutes. The supernatant was filtered and analyzed using HPLC (Shimadzu LC-2030, Japan) equipped with a refractive index detector. A Phenomenex Rezex RCM-Monosaccharide column (300 x 7.8 mm) was used, with double-deionized water as the carrier phase at a 0.6 mL min^{-1} flow rate to separate the sugars. Standards for sucrose, glucose, and fructose were utilized to identify and quantify the individual sugar peaks.

Statistical Analysis

The experiment followed a completely randomized design, with all measurements performed in triplicate. Data were analyzed using ANOVA with Minitab 17 software (Minitab Inc., State College, PA, USA). Post hoc comparisons were conducted using Tukey's test at a significance level of $P \leq 0.05$, performed with MSTAT-C software (Michigan State University, East Lansing, MI, USA). Principal component and correlation coefficients analyses were also carried out using JMP 13.2.0 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The results show that all quality parameters except titratable acidity are statistically different for all three apple products. These findings align with previous studies demonstrating that processing methods influence apples' physicochemical and bioactive properties (Wolfe et al., 2003; Plaza et al., 2012).

The highest total phenolic content was observed in fresh apples, followed by dried apples and apple chips, with 672.0, 598.3 and 471.7 mg GAE kg^{-1}, respectively (Table 1). This trend can be attributed to the degradation of phenolic compounds during thermal processing, as heat exposure has been reported to cause oxidative degradation and polymerization of phenolic substances (Friedman, 1997). Additionally, enzymatic oxidation occurring in cut or processed apples may contribute to phenolic degradation, particularly in apple chips, where extensive heat exposure accelerates compound breakdown (Vinson et al., 2001).

Similarly, antioxidant activity values were higher in fresh apples (3.47 µmol trolox g^{-1}) than in other processed products, with the lowest activity observed in apple chips (1.12 µmol trolox g^{-1}) (Table 1). This aligns with findings from Leontowicz et al. (2007), who reported that fresh apples exhibited superior antioxidant activity due to intact polyphenolic compounds. Processing techniques such as drying and frying have been found to reduce the concentration of phenolic substances due to thermal degradation and enzymatic oxidation (Zhou et al., 2020). The correlation between total phenolic content and antioxidant activity has been well established, indicating that the loss of polyphenols during processing directly affects the antioxidative capacity of apple products (Pellegrini et al., 2010). However, the degree of degradation varies depending on the severity of heat treatment, drying conditions, and storage factors, and this should be investigated further in future research.

When examined in terms of anthocyanin content, the highest value was observed in apple chips (5.56 mg kg^{-1}), while the lowest anthocyanin content was found in fresh apples (1.22 mg kg^{-1}) (Table 1). This suggests that the thermal processing of chip apples may enhance anthocyanin extraction or concentration, potentially due to cell wall breakdown and increased pigment availability, as reported by Patras et al. (2010). Additionally, heat treatments can induce the release of bound anthocyanins, making them more bioavailable (Sui et al., 2014). However, prolonged or excessive heat exposure may degrade anthocyanins, leading to color changes and reduced bioactivity. In contrast, fresh apples typically retain their native anthocyanin profile, but the lack of external stressors like heat or enzymatic activity may result in comparatively lower anthocyanin levels (Tsao and Yang, 2003).

The highest vitamin C content was observed in fresh apples (9.89 mg $100g^{-1}$), whereas apple chips exhibited the lowest levels (5.60 mg $100g^{-1}$) (Table 1). Vitamin C is susceptible to heat and oxidation, leading to significant losses during processing, particularly in high-temperature treatments (Duarte et al., 2009). Future research should investigate alternative preservation techniques, such as freeze-drying or vacuum drying, which have shown potential in minimizing vitamin C loss while maintaining fruit quality (Ratti 2001).

Interestingly, titratable acidity remained unaffected by the processing method (Table 1), suggesting that organic acids in apples are more stable during processing than phenolic compounds. Kschonsek et al. (2018), who observed minimal changes in titratable acidity across different apple processing methods, have reported similar findings. This stability may be due to the resilience of organic acids such as malic acid, a primary component of apple acidity, which remains relatively unchanged under moderate processing conditions. However, in some cases, prolonged drying or extreme heating may lead to minor acid degradation or conversion, which should be explored further in future studies (ElGamal et al., 2023).

Table 1. Quality characteristics of different apple products

Apple Product	Total Phenolic (mg GAE kg^{-1})	Anthocyanin (mg kg^{-1})	Antioxidant (µmol trolox g^{-1})	Vitamin C (mg $100g^{-1}$)	Titratable Acidity (malic %)
Chips	471.7 ± 11.0 C*	5.56 ± 0.03 A	1.12 ± 0.05 C	5.60 ± 0.55 C	0.477 ± 0.01 ns[1]
Dried	598.3 ± 3.3 B	2.43 ± 0.02 B	2.19 ± 0.01 B	7.55 ± 0.30 B	0.481 ± 0.01 ns
Fresh	672.0 ± 1.1 A	1.22 ± 0.01 C	3.47 ± 0.01 A	9.89 ± 0.20 A	0.475 ± 0.01 ns
Significant Effects P values	0.000	0.000	0.000	0.001	0.951

*Capital letters show differences among apple products and [1]ns non-significance at $P \leq 0.05$-error level according to Tukey's test.

Color changes in apple products were also significant (Table 2). Among processed products, apple chips exhibited the highest color change (ΔE = 11.24 units), followed by dried apples (ΔE = 7.45 units).

ΔE is a crucial parameter in evaluating the perceptual difference in color before and after processing. A higher ΔE value in apple chips indicates a significant alteration in color, which can be attributed to more intense thermal processing, likely causing caramelization and Maillard reactions (Atrooz, 2008; Pathare et al., 2013). The lower ΔE in dried apples suggests that drying under controlled conditions may mitigate extreme color changes compared to frying, which is known to induce more profound pigment transformations. Additionally, the increase in ΔE

correlates with anthocyanin degradation, non-enzymatic browning, and pigment oxidation, which are intensified under high-temperature exposure (Maskan, 2001).

As for the browning index, the elevated value was also highest in apple chips (82.19), followed by dried apples (70.68) (Table 2). The elevated browning index in apple chips can be attributed to the Maillard reaction, which is promoted at high temperatures and leads to brown pigments and flavor compounds (Akyıldız and Öcal, 2006). Though significant, the browning in dried apples is likely due to enzymatic browning caused by polyphenol oxidase (PPO) activity rather than thermal-induced Maillard reactions (Toivonen and Brummell, 2008). The combination of PPO activity and non-enzymatic reactions contributes to the observed browning in apple products, with the processing conditions playing a key role in determining the extent of color change.

The color stability of apple products is crucial for consumer acceptance, as discoloration indicates quality deterioration. Several strategies, such as using antioxidants, controlled drying conditions, and modified atmosphere packaging, have been proposed to mitigate browning and maintain color quality in apple products (Amodio et al., 2011). Further research is needed to explore the impact of different processing techniques, including vacuum frying and freeze-drying, on the color retention of apple products while minimizing undesirable browning effects.

The sugar content of apple products showed significant variations, particularly in fructose, glucose, and sucrose levels (Table 2).

The highest fructose content was observed in apple chips, followed by dried and fresh apples, with 24.94, 22.10, and 16.67 g kg^{-1}, respectively. This aligns with previous studies indicating that drying tends to concentrate sugars in fruits due to water loss (Smith et al., 2018). The increased fructose in apple chips could also be attributed to the concentration effect during the dehydration process at higher temperatures (Jones and Taylor, 2020). The finding that apple chips contain the highest level of fructose suggests that consumers may need to be cautious when considering their fructose intake from processed apple products, especially those sensitive to fructose (Harris et al., 2019).

In contrast, the highest glucose content was found in dried apples (15.06 g kg^{-1}), while fresh apples exhibited the lowest glucose levels (12.69 g kg^{-1}). Apple chips exhibited glucose levels statistically similar to those of dried and fresh apples. This may be explained by the fact that glucose, unlike fructose, is more stable in the drying process and thus may not accumulate as much in dehydrated products (Kim et al., 2021). The statistical similarity in glucose levels between apple chips and dried apples suggests that the processing temperature of apple chips might not cause as significant a rise in glucose concentration as it does with other sugars (Lee et al., 2022).

Sucrose content did not differ significantly across the different apple products. All three apple products were grouped in the same statistical category. This could indicate that sucrose levels are more stable across these products, regardless of the drying or dehydration process. Turner et al. (2017), who found that sucrose concentration in apples remains relatively consistent even after processing, have reported similar findings. This stability in sucrose levels suggests that the product characteristics of apple processing might have less impact on sucrose content than fructose and glucose.

Table 2. Quality characteristics of different apple products

Apple Product	Color Difference (ΔE)	Browning Index	Fructose (g kg^{-1})	Glucose (g kg^{-1})	Sucrose (g kg^{-1})
Chips	11.24 ± 0.40 A*	82.19 ± 2.13 A	24.94 ± 0.25 A	13.36 ± 0.40 AB	5.82 ± 0.52 A
Dried	7.45 ± 0.91 B	70.68 ± 3.85 B	22.10 ± 0.14 B	15.06 ± 0.40 A	5.76 ± 0.09 A
Fresh	0.00 ± 0.00 C	0.00 ± 0.00 C	16.67 ± 0.62 C	12.69 ± 0.56 B	6.15 ± 0.18 A
Significant Effects P values	0.000	0.000	0.000	0.027	0.041

*Capital letters show significant differences among apple products at $P \leq 0.05$-error level according to Tukey's test.

The results of the correlation analysis statistically evaluated the relationships between the parameters in the apple samples (Figure 1). The total phenolic content (TP) showed a positive and strong correlation with the total antioxidant capacity (TEAC) ($R= 0.976$, $p \leq 0.01$). This indicates that phenolic compounds contribute significantly to antioxidant capacity. At the same time, TP is negatively correlated with the color difference ΔE ($R= -0.926$, $p \leq 0.01$). When analyzing the relationships between biochemical variables, there was a strong and negative correlation between brown index (BI) and total phenolic content ($R= -0.848$, $p \leq 0.01$). This supports the effect of phenolic compounds in preventing oxidative browning. Similarly, vitamin C content (C) also shows a significant positive correlation with TP ($R= 0.920$, $p \leq 0.01$), indicating that phenolic compounds and vitamin C together play a role in the antioxidant defense mechanism. When sugar components were analyzed, the correlation between fructose (F) and glucose (G) was not significant ($R= 0.336$). However, there was a negative and significant correlation between sucrose (S) and fructose ($R= -0.743$, $p \leq 0.05$), suggesting that fructose levels may change with the hydrolysis of sucrose. In conclusion, correlation analysis shows strong relationships between phenolic

compounds, antioxidant capacity, and color change. Phenolic compounds reduce oxidative discoloration, increase antioxidant capacity, and act in parallel with vitamin C. In addition, relationships between sugar components should be evaluated during processing. These results provide important information for understanding the dynamics between biochemical composition and quality characteristics of apple samples.

	TP	A	TEAC	DeltaE	TA	BI	C	F	G	S	
TP	1										1
A	-0.989**	1									0.8
TEAC	0.976**	-0.954**	1								0.6
DeltaE	-0.926**	0.896**	-0.972**	1							0.4
TA	0.022	0.029	-0.021	0.178	1						0.2
BI	-0.848**	0.796*	-0.937**	0.966**	0.080	1					0
C	0.920**	-0.910**	0.956**	-0.940**	-0.122	-0.916**	1				-0
F	-0.932**	0.899**	-0.977**	0.973**	0.108	0.957**	-0.923**	1			-0
G	-0.135	0.033	-0.273	0.428	0.065	0.529	-0.218	0.336	1		-1
S	0.656	-0.566	0.714*	-0.754*	0.110	-0.775*	0.537	-0.743*	-0.696*	1	-1

Figure 1. Correlation matrix analysis between the data of the studied parameters. The correlation values varied between -1 (shaded in red tones) and 1 (shaded in green tones). A single asterisk "*" denotes significant differences at $p \leq 0.05$, while a double asterisk "**" represents significance at $p \leq 0.01$. TP: Total phenolic, A: Anthocyanin, TEAC: Antioxidant, TA: Titratable acidity, BI: Browning index, C: Vitamin C, F: Fructose, G: Glucose, S: Sucrose.

Principal component analysis (PCA) was utilized to assess the interaction between drying treatments and the variables under investigation, as illustrated in Figure 2. A data set comprising three treatments (fresh, dried, chips) and ten variables was analyzed using the covariance matrix. The PCA results indicated substantial variations in the chemical composition and processing methodologies employed in the samples. The first component (PC1) accounts for 72.5% of the total variance, exhibiting notably high loading values on sugar components (fructose, sucrose) and color change parameters (Delta E, BI). This finding underscores the notion that PC1 is the most dominant component in decomposing factors such as maturity level, processing method, and color change of apple samples. The second component (PC2) describes the factors associated with the dryness of the samples, explaining 13.5% of the total variance. The cumulative variance explanation rate for these two components is 86%, signifying their substantial representation of the influence of the analyzed variables on the samples. As demonstrated in Figure 2, the chips exhibit high loading values in the positive direction of PC1. The chips are distinguished by their notably elevated fructose level, exhibiting a robust correlation with the Delta E and BI parameters that denote color change. The findings of this study suggest that the process of water evaporation during the drying phase leads to a significant concentration of sugar components, which in turn results in a pronounced color change in the fruit. Conversely, fresh apple samples (fresh) exhibited a negative association with PC1. They demonstrated a more pronounced correlation with the TEAC parameter, representing phenolic compounds (total phenolic, vitamin C) and antioxidant capacity. Phenolic compounds and antioxidant capacity were higher in fresh apples, suggesting that the drying process causes a loss of these bioactive components. This phenomenon can be attributed to the sensitivity of phenolic compounds to heat and oxidation, which may lead to their breakdown during the drying process, consequently reducing antioxidant capacity.

When analyzed along PC2, it is evident that the samples are segregated according to their dryness status. Samples categorized as "dry" are positioned in the positive direction of PC2 and demonstrate a strong correlation with glucose content. This finding suggests that dried samples exhibit a pronounced differentiation in sugar composition, particularly in the form of an increase in glucose concentration. Conversely, fresh apple samples exhibited a negative association with PC2, demonstrating a correlation with phenolic compounds and antioxidant capacity parameters. With respect to sugar components, fructose and glucose contents exhibit high loading values on the positive side of PC1, while sucrose is positioned on the negative side of PC1. This finding suggests that sucrose may be broken down into glucose and fructose during the drying process. The hydrolysis of sucrose, due to its exposure to enzymes and water loss in the fruit tissue, may lead to changes in the distribution of these sugar components. Regarding color change, the BI and Delta E parameters exhibited high loading values in the positive direction of PC1, signifying that the drying process substantially influenced the color change in fruit. Processes such as enzymatic darkening and oxidation of phenolic compounds can affect color change in fruits. The elevated values of these parameters in the dried samples imply that the chemical changes occurring during the drying process substantially affect the color stability of fruit tissue.

In conclusion, the PCA analysis demonstrates significant differences between apple samples depending on the processing method. Dried apples exhibited elevated levels of sugars and discoloration, while fresh apples demonstrated higher concentrations of phenolic compounds and antioxidant capacity. These findings are

significant in understanding the effects of fruit processing techniques on chemical composition and reveal how different apple processing methods can affect nutritional quality. Furthermore, PCA results demonstrate that the drying process leads to a loss of phenolic compounds, increased sugar concentration, and changed color, providing significant data for the quality control of apple-based processed products. This analysis is a powerful tool for understanding the relationships between the biochemical components of apple samples. The findings of this study lay the foundation for future research, which can investigate the impact of diverse processing techniques on fruit composition in more depth.

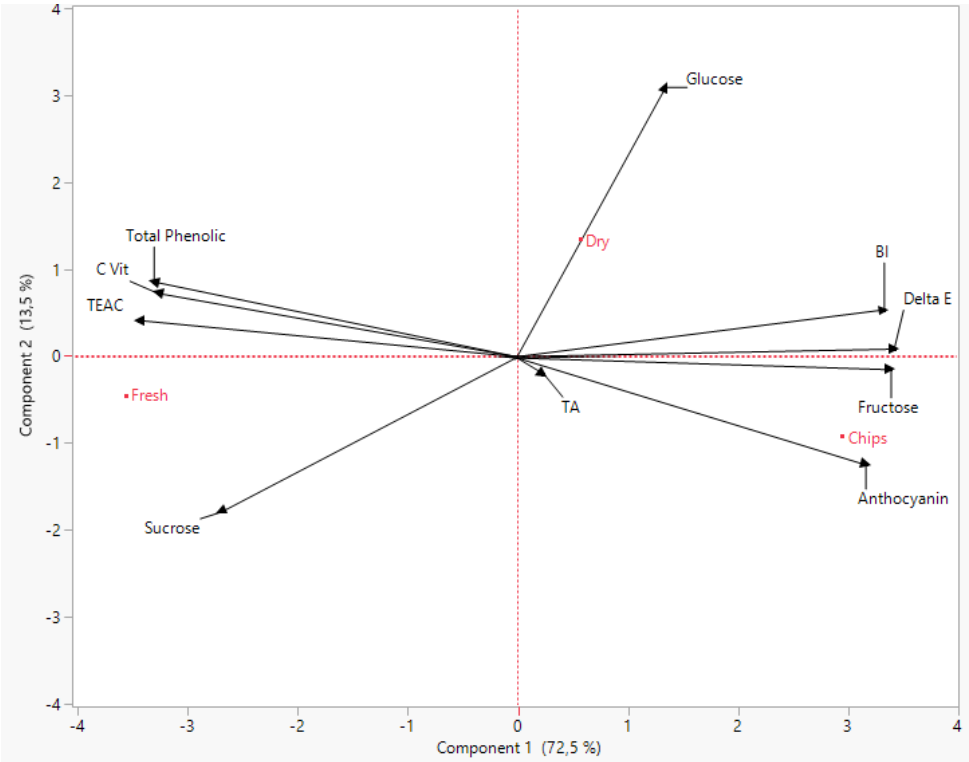


Figure 2. The biplot of scores and loadings for the Principal Component Analysis (PCA) of all data shows the distribution of reaches according to the first two components.

CONCLUSION

The present study demonstrates that drying processing significantly influences the physicochemical and functional properties of 'Fuji' apples, particularly regarding bioactive compound retention, color stability, and sugar composition. Fresh apples exhibited the highest levels of total phenolic content and antioxidant activity, reinforcing the adverse effects of thermal processing on bioactive compounds. While anthocyanin extraction improved in apple chips, vitamin C degradation and increased browning were observed in dried and chip products. The correlation analysis and PCA results highlighted strong relationships between phenolic content, antioxidant capacity, and color stability, further emphasizing the impact of processing methods on apple quality. Given the rising consumer demand for nutritionally rich dried apple products, optimizing drying conditions is essential to minimizing nutrient loss while preserving desirable sensory characteristics. Future research should explore advanced drying techniques to enhance the retention of phenolic compounds and other antioxidants. These insights contribute to developing high-quality dried apple products that meet nutritional and market expectations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Ö. Horzum, H. Tahmaz Karaman, H. Dumanoğlu declare that they have no competing interests.

Author contribution

All authors contributed to the study conception and design. Material preparation by Ö.H, H.T.K., H.D. The first draft of the manuscript was written by Ö.H and H.T.K. and all authors read and approved of the final manuscript.

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Salt stress response of cauliflower (*Brassica oleracea* var. *botrytis*) seedlings under different LED light wavelengths

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Abstract

Cauliflower is one of the major brassica vegetables that receive attention due to the presence of glucosinolates that are known to have health benefits. These metabolites are a part of plant defence system and influenced under stress conditions. Light Emitting Diodes (LED) are gaining interest as to provide artificial light at different wavelengths that modulate morphological, physiological and biochemical responses of plants. The objective of the present study was to explore salt stress response of cauliflower (*Brassica oleracea* var. *botrytis*) seedlings that are grown under different LED light wavelengths in terms of morphological parameters, antioxidant enzyme activities and glucosinolate contents. The seedlings were treated with 200 mM NaCl to generate salt stress or without NaCl as the control and were grown under different LED light wavelengths including red, blue, red+blue, red+far red, blue+far red and white (control) lights. Shoot and root growth parameters, antioxidant enzymes activity (CAT, SOD, APX), H_2O_2 content, aliphatic and indole glucosinolates were determined. The findings revealed that salt and light treatments independently influenced shoot and root growth. The antioxidant enzymes CAT and APX activities were highest in the control plants of blue and blue+far red lights whereas SOD activity was highest in salt treated plants under blue light. Aliphatic and indole glucosinolate content varied among LED light treatments. Salt treatment decreased the aliphatics while indoles particularly the glucobrassisin was increased with the influence of salinity and light wavelengths. The results demonstrated the salt stress response of cauliflower seedlings by elucidating changes in the antioxidant enzymes, glucosinolate contents, major shoot and root growth parameters under a range of different light wavelengths. These findings may provide a comprehensive understanding of the influence of different light wavelengths in mediating plant stress response.

Keywords: Cauliflower, Antioxidant enzymes, Glucosinolates, LED lights, Salinity

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INTRODUCTION

Salinity has negative impact on plant growth and development. Accumulation of salts limit the water uptake of plants that reduces the osmotic potential of the cells (Ahmad et al., 2010; Van Zelm et al., 2020), interrupt water transport across plant cell membranes and disturbs water use efficiency that leads to several physiological changes (Kim et al., 2018; Zhou et al., 2024). Additionally, the excessive accumulation of Na^+ and Cl^- ions may cause toxic effect in plant cells (Davenport et al., 2005).

Plants have multiple adaptive mechanisms at morphological, physiological, biochemical and molecular levels in order to tolerate salt stress. Among these, reactive oxygen species (ROS) signalling has been shown to be an adaptation response to sustain plant growth (Huang et al. 2019). ROS was shown to function as signal molecules

to regulate Na^+/K^+ homeostasis and improving the salt tolerance of Arabidopsis (Ma et al., 2012). The generation of ROS is reported to increase during salinity stress (Kesawat et al., 2023). The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and non-enzymatic antioxidant molecules such as ascorbic acid, tocopherol, glutathione and phenolic compounds are often synthesized to avoid cellular damage by ROS under stress conditions (Gill and Tuteja, 2010).

Synthesis of plant secondary metabolites are often associated with plant stress response. Glucosinolates in Brassicaceae are a part of plant defence system usually linked to several biological properties when hydrolyzed (Sarıkamış et al., 2006). Studies have been conducted to explore the relationship between salt stress and glucosinolates in plants and variable effects of salt stress on individual glucosinolates at various developmental stages were reported (Yuan et al., 2010; Martinez-Ballesta et al., 2013; 2015; Sarıkamış and Çakır, 2017). NaCl treatment caused a significant variation in terms of aliphatic, aromatic, indole and total glucosinolates in *Thellungiella salsuginea*, which is a relative of Arabidopsis known as tolerant to high salinity (Pang et al., 2012). These researchers suggested that aliphatic glucosinolates decreased at higher salt concentrations. Furthermore, the myrosinase activity and the relative electrolyte leakage was shown to increase with salt stress (Pang et al., 2012).

Light is one of the most important environmental factors mainly influencing photosynthesis, stomatal movement and has a major impact on plant growth and development. Light intensity and quality affect plants at physiological, biochemical, morphological and molecular level (Tang et al., 2022). Light spectrum is an important determinant of light absorption and CO_2 fixation efficiency (Hogewoning et al., 2012). Light Emitting Diodes (LED) lights are receiving attention by providing artificial source of light at different wavelengths and combinations that has been shown to modulate physiological and biochemical responses and to optimise plant growth and nutritional properties (Demir et al., 2023).

Research on tomato seedlings reported that lower red to far-red light ratios improved tomato tolerance to salt stress demonstrating that light quality could improve salt stress tolerance (Cao et al., 2018). The influence of pretreatment with four LED light sources (red, blue, red + blue, and white) on biomass and phytochemical accumulation under both control and salinity stress conditions was demonstrated in basil (Rafeie et al., 2022). Far-red light was reported to interact with salinity by changing the plant's stress tolerance in *Cucumis sativus* (Shibuya et al., 2024)

Brassica species are among the prominent vegetable species due to the health benefits of the sulfur containing glucosinolates. Research is ongoing in several brassica species, particularly in terms of these beneficiary secondary metabolites at different growth stages including microgreens (Demir et al., 2023). In this study, the objective was to explore the morphological and biochemical responses of cauliflower seedlings assessed with root and shoot growth, antioxidant enzymes (CAT, SOD, APX) and glucosinolate content under salt stress and different light wavelengths.

MATERIALS AND METHODS

Plant Material and Led Light Treatments

Cauliflower (*Brassica oleracea* var. *botrytis*) F1 seeds were sown in plastic plugs each cell 6x7x7 cm containing peat moss and perlite (3:1). They were germinated and grown in a climate controlled growth cabinet that was divided into 8 chambers equipped with LED lamps producing different light wavelengths. The seedlings were watered with 200 ml dH_2O each time and given an equal amount of nutrient solution containing 20 + 20 + 20 NPK and microelements with an EC of 1.2 dS m^{-1} once a week. The temperature was set to 22±2°C and relative humidity was adjusted to 70 % in the growth cabinet. They were grown under red (R), blue (B), red+blue (R+B) (1:1), red+far red (R+FR) (1:1), blue+far red (B+FR) (1:1) and white (W) (control) LED lights at 16 h light/8 h photoperiod as described by Demir et al. (2023).

Salt Treatment

Salt (NaCl) application was initiated when the seedlings reached to 4-5 leaf stage, one month after sowing. Salt was applied at 4 times by adding 50 mM NaCl each time reaching to the final concentration of 200 mM NaCl (Giuffrida et al. 2017; Kuşvuran et al. 2021). Control plants were watered with equal amounts of tap water. Morphological measurements and collection of leaf samples for biochemical analysis were performed one week after salt treatment both from NaCl treated samples and the control plants.

Growth Parameters

The seedlings were carefully removed from the growth medium. The roots were washed with tap water and dried on a filter paper. The shoot length (cm) and shoot diameter (mm) were determined. The root length (cm) was measured as the longest root. Subsequently, root and shoot tissue was separated and weighed (g) to assess the fresh weights. They were then dried in an oven set at 70°C until reached to a constant weight in order to determine the dry weights (Toscana et al. 2021).

Antioxidant Enzyme Activity Assay

Fresh leaf tissue (0.5 g) collected from each treatment was homogenized with 5 mL extraction solution containing 0.1 M Na-phosphate, 0.5 mM Na-EDTA and 1 mM ascorbic acid. The pH was adjusted to 7.5. The

antioxidant enzymes CAT, SOD and APX activity of the homogenate were determined by Shimadzu UV/VIS 1201 spectrophotometer.

Catalase (CAT, EC 1.11.1.6) activity was determined by using a reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and the enzyme extract. The CAT activity was determined by monitoring the disappearance of H_2O_2 in 1 minutes at 240 nm according to the method of Jebara et al. (2005). Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to Rahnama and Ebrahimzadeh (2005), based on the reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by the decrease at 290 nm for 1 minute following decomposition of H_2O_2 due to the content of ascorbic acid (Nakano and Asada, 1981).

H_2O_2 Analysis

The content of H_2O_2 determined calorimetrically at 415 nm (Mukherjee and Choudhuri, 1983).

Glucosinolate Extraction and Analysis

Glucosinolates were extracted from freeze dried and ground leaf tissue for each treatment and the control. The extraction was performed using 70 % (v/w) methanol in a hot water bath at 70°C and the extracts were passed through an ion exchange column, desulfated with the enzyme sulphatase. Desulfoglucosinolates were eluted in dH_2O and analyzed by HPLC-UV (Shimadzu®) with a gradient of water and acetonitrile as described by Sarıkamış et al., 2006. The quantification was performed using glucotrapeolin as the internal standard and using correction factors reported by Brown et al. (2003) for each individual glucosinolate.

Experimental Design and Statistical Analysis

The experiment was designed according to the randomized plot design with 3 replications, 12 seedlings in each replicate. In the study, the data analyzed according to the F test ($P<0.05$) with the Minitab 17 Package Program (MINITAB Inc.) using the analysis of variance method. Significant differences were determined at 0.05 level with the Duncan test.

RESULTS

Shoot and Root Growth Parameters

Morphological parameters including shoot and root growth of cauliflower seedlings were determined. In terms of shoot growth parameters, shoot length, shoot diameter, shoot fresh and dry weights were measured and given as mean of the replicates. The influence of LED light wavelengths on shoot length, shoot diameter, shoot fresh weight, root length and root fresh weight was statistically significant ($P<0.05$). In terms of shoot length, the highest was recorded in red+far red light as 13.91±0.42 cm. This was followed by blue+far red (13.13±0.70), red (12.18±0.52), blue (10.68±0.41) and white (10.10±0.29). The lowest value was determined in red+blue light as 7.66±0.24 cm ($P<0.001$). However, the difference in terms of salt treatment and the interaction between light and salt treatments were not significant ($P>0.05$). Shoot diameter, was highest in red and blue lights both measured as 4.14±0.21 and 4.14±0.15 mm followed by red+blue (3.80±0.10) and white (3.73±0.18) lights. Shoot diameter was lower in red+far red and blue+far red lights measured as 2.80±0.13 and 2.75±0.11 mm respectively ($P<0.001$). The statistical significance between salt and control treatments was significant in terms of shoot diameter ($P<0.05$). Shoot fresh weight was highest in red light determined as 4.46±0.47 g, followed by blue (4.27±0.36) white (3.93±0.29), red+blue (3.84±0.16) lights and lowest in blue+far red as 1.84±0.15 g ($P<0.001$). The fresh weight of salt treated seedlings were higher (3.90±0.26 g) compared to control (3.08±0.21 g) ($P<0.001$). The interaction between salt treatment and light wavelengths was not significant. Salt and light wavelengths together influenced shoot dry weight, the interaction in between was statistically significant ($P<0.05$). The highest was determined in salt treated plants under blue light as 0.55±0.05 g, followed by red (0.52±0.03) and white (0.43±0.06) lights. The lowest values were found with red+far red and blue+far red wavelengths both in control and salt treatment (Table 1).

Root length was highest in blue light as 16.27±1.29 cm followed by red+blue (14.75±0.86), white (14.21±1.29), red (12.61±1.41) lights. Lower values were determined with red+far red (6.62±0.74) and blue+far red (4.79±0.35 cm) ($P<0.001$). Salt treated plants had longer roots (12.43±1.32 cm) compared to control (10.65±1.11 cm) ($P<0.05$). The interaction between salt treatments and light wavelengths was not significant. Root fresh weight was highest in blue+red light weighed as 0.29±0.03 g followed by red (0.25±0.04), blue (0.22±0.03) and white (0.21±0.04) lights. Lower values were determined with red+far red (0.05±0.01) and blue+far red wavelengths (0.04±0.01) ($P<0.001$). However the difference in terms of salt and control treatments and the interaction between light wavelengths and salt treatment was not significant ($P>0.05$). Root dry weight was highest in blue+red light (0.05±0.01 g) followed by blue (0.04±0.01), red (0.04±0.01), white (0.02±0.01) lights. Red+far red and blue+far red lights revealed lower values (0.01±0.00 and 0.01±0.00 g) ($P<0.001$). The difference between salt and control and the interaction between light wavelengths and salt treatment was not significant ($P>0.05$) (Table 1).

Table 1. Shoot and Root Growth Parameters of Cauliflower Seedlings Under Salt Stress and Different LED Light Wavelengths

Growth		Light Wavelengths						Mean
		White	Red	Blue	Red+Blue	Red+Far red	Blue+Far red	
Shoot Length (cm)	Control	10.05 ±0.19	12.13±0.84	10.75±0.64	7.78±0.17	13.73±0.56	12.68±0.62	11.18±0.45
	Salinity	10.15±0.60	12.22±0.73	10.60±0.62	7.55±0.47	14.10±0.67	13.57±1.33	11.37±0.55
	Mean	10.10±0.29 C	12.18±0.52 B	10.68±0.41 C	7.66±0.24 D	13.91±0.42 A	13.13±0.70 AB	
Shoot Diameter (mm)	Control	3.53±0.17	3.80±0.33	4.10±0.31	3.93±0.14	2.58±0.17	2.65±0.12	3.43±0.15 b
	Salinity	3.93±0.31	4.48±0.10	4.18±0.08	3.68±0.13	3.03±0.14	2.85±0.19	3.69±0.14 a
	Mean	3.73±0.18 A	4.14±0.21 A	4.14±0.15 A	3.80±0.10 A	2.80±0.13 B	2.75±0.11 B	
Shoot Fresh Weight (g)	Control	3.29±0.10	3.94±0.78	3.55±0.19	3.64±0.19	2.52±0.17	1.55±0.04	3.08±0.21 b
	Salinity	4.58±0.33	4.98±0.49	4.98±0.47	4.05±0.23	2.71±0.17	2.14±0.20	3.90±0.26 a
	Mean	3.93±0.29 A	4.46±0.47 A	4.27±0.36 A	3.84±0.16 A	2.61±0.12 B	1.84±0.15 C	
Shoot Dry Weight (g)	Control	0.26±0.02 CDb	0.43±0.12 ABa	0.34±0.05 BCb	0.49±0.00 Aa	0.18±0.02 DEa	0.10±0.00 Ea	0.30±0.04
	Salinity	0.43±0.06 ABa	0.52±0.03 ABa	0.55±0.05 Aa	0.39±0.03 Ba	0.19±0.02 Ca	0.16±0.02 Ca	0.37±0.03
	Mean	0.34±0.04	0.47±0.06	0.44±0.05	0.44±0.02	0.19±0.01	0.13±0.01	
Root Length (cm)	Control	12.35±0.83	9.82±1.44	15.15±2.55	14.80±1.16	7.30±1.33	4.50±0.32	10.65±1.11 b
	Salinity	16.07±2.19	15.40±1.42	17.40±0.63	14.70±1.46	5.95±0.68	5.08±0.63	12.43±1.32 a
	Mean	14.21±1.29 AB	12.61±1.41 B	16.27±1.29 A	14.75±0.86 AB	6.62±0.74 C	4.79±0.35 C	
Root Fresh Weight (g)	Control	0.19±0.05	0.21±0.08	0.16±0.05	0.34±0.02	0.06±0.02	0.03±0.00	0.17±0.03
	Salinity	0.22±0.07	0.28±0.04	0.28±0.02	0.24±0.05	0.03±0.00	0.05±0.01	0.18±0.03
	Mean	0.21±0.04 A	0.25±0.04 A	0.22±0.03 A	0.29±0.03 A	0.05±0.01 B	0.04±0.01 B	
Root Dry Weight (g)	Control	0.02±0.01	0.03±0.01	0.03±0.01	0.06±0.00	0.01±0.00	0.00±0.00	0.02±0.00
	Salinity	0.03±0.01	0.04±0.01	0.05±0.00	0.04±0.01	0.01±0.00	0.01±0.00	0.03±0.00
	Mean	0.02±0.01 B	0.04±0.01 A	0.04±0.01 A	0.05±0.01 A	0.01±0.00 BC	0.01±0.00 C	

*The upper case letters indicate differences among LED light wavelengths and lower case letters indicate differences between salt treatment and the control

Antioxidant Enzymes Activity and H_2O_2 Content

The antioxidant enzyme (CAT, APX, SOD) activities were influenced by the interaction of light wavelengths and salt treatment ($P<0.05$). The activity of CAT was highest in blue light determined as 0.43±0.05 mmol g FW min^{-1} in control and 0.21±0.05 mmol g FW min^{-1} in 200 mM NaCl treatment. The decrease was significant in salt treated seedlings under blue light. APX activity was highest in the control plants of blue+far red (7.87±1.83 mmol g FW min^{-1}) and blue lights (7.37±0.42 mmol g FW min^{-1}). Significant decrease was observed in the salt treated seedlings of both light wavelengths. SOD activity was highest 200 mM NaCl treated blue+far red and blue lights. A significant increase in SOD activity was determined in blue+far red light under salt stress. In terms of H_2O_2 content, although a slight increase was observed in B+FR light, neither the difference among light wavelengths nor the NaCl treatment with control was significant and there was no interaction between light and salinity treatmens ($P>0.05$) (Figure 1).

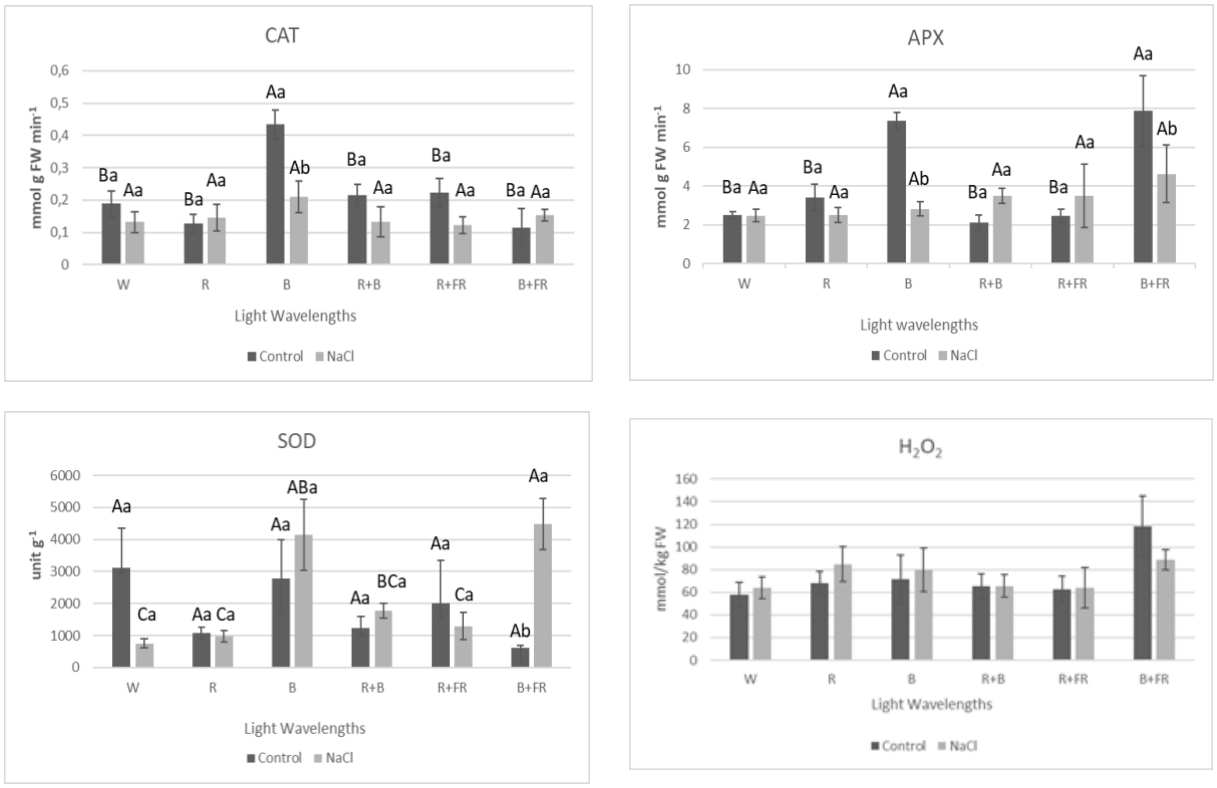


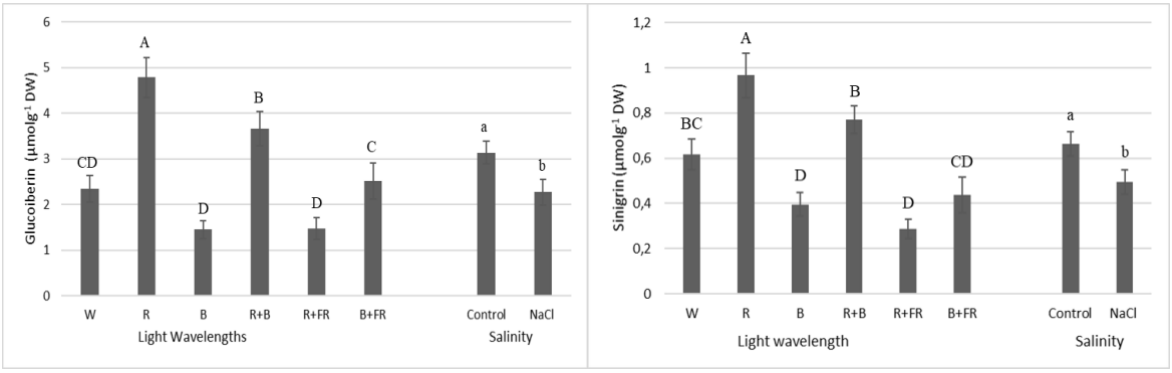
Figure 1. Antioxidant Enzymes Activity and H_2O_2 Content of Cauliflower Seedlings under 200 Mm NaCl and Different LED Light Wavelengths

*The upper case letters indicate differences among LED light wavelengths and lower case letters indicate differences between salt treatment and the control

Aliphatic and Indole Glucosinolate Content

The predominant glucosinolates determined in cauliflower seedlings were sinigrin and glucoiberin of the aliphatic group and glucobrassicin, 1-methoxyglucobrassicin, 4-methoxyglucobrassicin of the indole glucosinolates. The aliphatic and indole glucosinolate contents varied according to salt and light wavelengths. Glucoiberin was quantified as 5.37±0.62 µmolg^{-1} DW under red light, followed by red+blue (3.34±0.39), blue+far red (2.47±0.38) lights. Lower values were found in white (2.26±0.29), red+far red (1.44±0.23) and blue light (1.29±0.19) ($P<0.001$). Glucoiberin content decreased with salt treatment calculated as 3.07±0.26 in control while determined as 2.32±0.35 µmolg^{-1} DW in salt treated seedlings ($P<0.05$). There was no statistically significant interaction between light wavelengths and salt treatment in terms of glucoiberin content ($P>0.05$). Sinigrin content was generally lower compared to glucoiberin. It was determined as 0.967±0.100 µmolg-1 DW in red light. This was followed by red+blue (0.77±0.06), white (0.62±0.07). Lower sinigrin content was found in blue+far red (0.44±0.09), blue (0.40±0.05), red+far red (0.29±0.04) ($P<0.001$). Sinigrin content was decreased with salt (0.49±0.05) compared to control (0.66±0.05) and there was no interaction between light and salinity ($P<0.05$) (Figure 2).

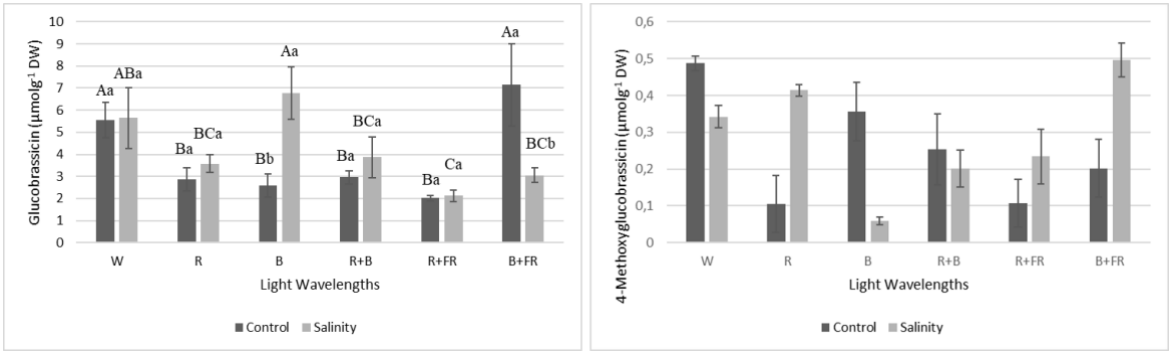
In terms of indole glucosinolates, the predominant indole glucosinolate was glucobrassicin. Light wavelength and salt treatment together significantly influenced glucobrassicin content ($P<0.001$). The highest glucobrassicin content was determined in the control group under blue+far red light (7.14±1.85) followed by salt treated seedlings under blue light (6.78±1.18) and salt treated (5.64±1.38) and control (5.56±0.81) under white light. The variation in terms of 4-methoxyglucobrassicin was not significant among treatments. 1-Methoxyglucobrassicin content was generally low. The interaction between light wavelengths and salt treatment was significant ($P<0.05$). The highest was determined in salt treated plants under red light and control plants under white light (Figure 3).



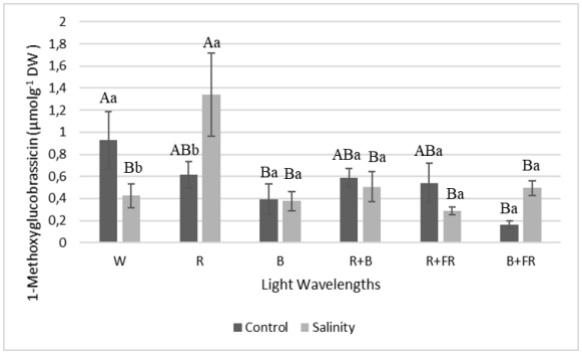
(a) (b)

Figure 2. The Aliphatic Glucosinolate Contents (a) Glucoiberin (b) Sinigrin in Cauliflower Seedlings under LED Lights and Salinity Stress

*The upper case letters indicate differences among LED light wavelengths and lower case letters indicate differences between salt treatment and the control



(a) (b)



(c)

Figure 3. Indole Glucosinolate Content of Cauliflower Seedlings (a) Glucobrassicin (b) 4-Methoxyglucobrassicin (c) 1-Methoxyglucobrassicin

*The upper case letters indicate differences among LED light wavelengths and lower case letters indicate differences between salt treatment and the control

DISCUSSION

Recent research are focused on the influence of salinity and light wavelengths on plant growth and development in order to assess whether different light wavelengths influence plant stress tolerance. In the present study, the effect of light wavelengths and salinity on morphological parameters including shoot and root growth varied significantly. Shoot length was influenced by light wavelengths. Far red light combined with red and blue lights revealed the highest shoot length among others. Pattaro et al. (2024), demonstrated a strong degree of etiolation with R+FR (R:FR ratio of 0.33) in lettuce. In the present study, shoot diameter and shoot fresh weight were highest in red, blue, white and red+blue lights compared to far red light combinations. The red and blue light wavelengths and their combination to various percentages are shown to influence many plant physiological processes and have a major impact particularly on photosynthesis (Hogewoning et al., 2010; Li et al., 2020). Increasing the proportion

of blue light when used in combination with red exhibited a reduced growth in lettuce (Izzio et al., 2021). In our work, the longest root was determined in blue light, followed by red+blue, white, red and it was lower at far red light combinations. Root fresh and dry weights were high in red+blue, red, blue, white lights and lower in far red light combinations. Izzo et al. (2021) stated that when used as single wavelength, both blue and red light reduced root dry weight in lettuce.

In the present study, the activity of antioxidant enzymes generally increased under blue and blue+far red lights. An alleviation in the antioxidant enzyme activity is expected in order to protect plants against damage caused by reactive oxygen species. APX and CAT are the major H_2O_2 scavenging antioxidant enzymes in plants. CAT is important on the H_2O_2 detoxification (Kim et al., 2005). SOD activity was increased in salt treated seedlings under blue and blue+far red light. Accumulation of SOD enzyme is usually thought to protect plants against salt stress damage. In Arabidopsis, salt treatment increased the expression of several SOD genes together with light (Attia et al., 2009). Red light was reported to trigger an antioxidant response in the fourth day of stress in lettuce plants (Arriaga, 2020). In Arabidopsis seedlings, a visible accumulation of ROS was found as a result of cryptochrome activation by blue light (El-Esawi et al., 2017). In basil, seedlings pretreated with LED lights revealed lower H_2O_2 than those in control condition (Rafeie et al., 2022). Inclusion of far red light was shown to reduce the accumulation of H_2O_2 and $O2^-$ of tomato under salt stress (Cao et al., 2018). Pre treatment with red+ blue light was shown to activate antioxidant enzymes and genes associated with stress tolerance, to improve growth and qualtity parameters and to provide better drought stress tolerance in lemon balm (Ahmadi et al., 2019). Utilization of LED lights was reported beneficial for increasing biomass and phytochemical accumulation in basil under both control and salinity stress conditions (Rafeie et al., 2022).

Glucosinolate contents in brassica species are of particular interest due to their several biological activities including health benefits and stress response. Studies presented that LED lights significantly modulate glucosinolate contents in brassicas (Demir et al., 2023). In this study, light wavelength and salinity independently influenced aliphatic glucosinolates. Total aliphatics was highest in red light and red+blue light. The lowest was in blue light, red+far red light and blue+far red light. Glucobrassicin was the major indole in cauliflower and its concentration varied with light and salt treatments. A significant increase was observed in salt treated seedlings grown under blue light.

CONCLUSION

The findings of the present study demonstrated the stress response of cauliflower seedlings grown under a range of different light wavelengths. LED lights have a major impact on plant growth and development mainly by influencing changes at the physiological, biochemical and morphological level. They are receiving attention as supplementary lighting to modulate the growth and development as well as nutritional properties of plants. In our study, we have demonstrated that LED light wavelengths influenced the growth, antioxidant enzymes activity and glucosinolate content of cauliflower seedling either independently or interactively with salt stress treatment.

The results may provide a comprehensive understanding of the influence of different light wavelengths in mediating plant stress response.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no known competing interests to declare.

Author contribution

Ayça Kodal: Investigation, Formal Analysis. Gölge Sarıkamış: Conceptualization, Methodology, Supervision, Data curation, Writing original draft, Visualization. Özge Şahin: Methodology, Investigation. Köksal Demir: Review&Editing of the manuscript. This work is part of Ayça Kodal's PhD thesis.

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Investigation of Agronomic and Physiological Performances of Different Peanuts (*Arachis hypogaea* L.) Cultivars in Denizli Ecology

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Abstract

In this study, agronomic, morphological, and physiological characteristics of 10 different peanut (*A. hypogaea* L.) cultivars were comparatively examined. Significant differences were detected among cultivars in terms of yield, pod and seed characteristics, shelling ratio, chlorophyll content, leaf area index, and oil content. The 'Florispan' cultivar had the highest yield (485.24 kg da^{-1}), while the 'Halisbey' cultivar stood out with both high yield (476.91 kg da^{-1}) and the highest oil content (57.28%). Correlation analysis showed a positive relationship between yield and leaf area index during flowering (r=0.21), and a negative relationship between yield and first-quality pod ratio (r=-0.31). Additionally, very strong positive correlations were found between 100-pod weight and 100-seed weight (r=0.91), and between oil content and chlorophyll b content during pod formation (r=0.59). Cluster analysis divided the cultivars into two main groups: Group X (Florispan, Sultan, Çom, Gazipaşa, Halisbey) included yield-focused cultivars, while Group Y (Masal, Georgia Green, Osmaniye-2005, Rigel, NC-7) contained quality-focused cultivars. Similarly, the examined characteristics were divided into two main clusters; Cluster A included yield and morphological characteristics, while Cluster B grouped physiological characteristics and oil content. Our findings emphasize that peanut cultivar selection should be made according to production purpose and highlight the importance of considering physiological characteristics' effects on yield and quality in breeding programs.

Keywords: Peanut (*A. hypogaea* L.), Yield, Correlation

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INTRODUCTION

The origin, distribution, and genetic diversity center of peanut has been determined as South America through comprehensive research. It has been cultivated in South America since 500-750 BC and utilized as a food source (Arıoğlu, 2014). Peanut (*A. hypogea* L.), classified within the Fabaceae family, contains 20-30% protein, 18-20% carbohydrates, and 45-55% oil in its seeds (Anonymous, 2018). Peanut is a rich source of edible oil (47-54%), high-quality protein (22-30%), starch (6-24%), cellulose (1-2%), minerals (2-3%), and calories (5-6%) (Patel et al., 2018). It has a distinct importance among oilseeds due to its various consumption and utilization methods. It can be used for both edible oil and confectionery purposes. Peanut kernels are consumed raw, boiled, roasted, or fried, and are also used in various culinary preparations such as peanut butter, peanut milk, and chocolates (Patel et al., 2018).

Many researchers have examined in detail the basic characteristics that form yield and quality in various species and cultivars, and how the complex interactions between these characteristics determine the quantity and quality of the final product (Aydoğan and Soylu, 2017). Therefore, understanding these characteristics is crucial for the selection and improvement of cultivars for effective yield enhancement. Hence, it is important to understand the relationships between yield and its associated component characters. Correlation analysis measures the mutual relationship between a pair of variables independent of other variables considered (Owen & Jones 1977). Peanut is an unpredictable crop due to its underground pod development. Direct selection for improving yield in peanut

may be inadequate. Knowledge of the degree of variability and relationship among characters contributing to yield and their relative contributions to yield is necessary for developing high-yielding genotypes in peanut (Thakur et al., 2013).

Determining the interactions of physiological processes occurring in plants with environmental factors in their natural development environments and the scope of these interactions provides significant advantages in terms of agricultural production management. Remedial interventions applied in the ecological conditions where plants are grown cause significant changes in physiological activities, especially in terms of quantitative parameters. When these physiological functions develop in the desired direction, both yield increase and positive progress in quality parameters can be observed in plant production (Tunalı et al., 2012). In this context, integrated evaluation of agronomic characteristics such as yield, hundred-seed weight, hundred-pod weight, shelling ratio, and physiological parameters such as total chlorophyll, chlorophyll a, chlorophyll b, and leaf area index in peanut is of great importance for both optimizing yield potential and increasing adaptation capability to changing environmental conditions.

The objectives of this study were to (1) comprehensively evaluate the agronomic, morphological, and physiological characteristics of 10 different peanut (*A. hypogaea* L.) cultivars as selection criteria for breeding programs, (2) determine the variation among cultivars, and (3) reveal the relationships between these characteristics through multivariate analyses.

MATERIALS AND METHODS

Plant Material and Experimental Area

The experiment was established in the main crop conditions of Çal district, Denizli province, during the 2024 peanut planting season. The 10 different peanut cultivars used in the research were obtained from the Osmaniye Oilseeds Research Institute Directorate. The peanut cultivars used as material in the study, their market types, origins, and growth forms are given in Table 1.

Table 1. Market type, origin and growth forms of cultivars

Cultivars	Market type	Origin	Growth Forms
Çom	Virginia	Türkiye	Semi-spreading
Florispan	Spanish	Türkiye	Erect
Gazipaşa	Virginia	Türkiye	Semi-spreading
Georgia Green	Runner	USA	Erect
Halisbey	Virginia	Türkiye	Semi-spreading
Masal	Virginia	Türkiye	Semi-spreading
NC-7	Virginia	USA	Semi-spreading
Osmaniye-2005	Virginia	Türkiye	Semi-spreading
Rigel	Virginia	Türkiye	Semi-spreading
Sultan	Virginia	Türkiye	Semi-spreading

The soil analysis results of the Denizli-Çal region showed an electrical conductivity (EC) value of 450 µS/cm, indicating low-medium soil salinity. The soil pH value was determined as 8.3, showing that the soil has an alkaline character. The lime content was quite high at 25.77%, indicating a calcareous soil structure. Among macronutrients, potassium (K) level was 365 mg kg^{-1}, and phosphorus (P) level was 26.37 mg kg^{-1}. These values indicate that potassium and phosphorus are present at sufficient levels in the soil. However, it should be considered that the high pH value may limit phosphorus uptake by plants. The organic matter (O.M) content of the soil was measured as 1.17%, indicating a low level. Among microelements, copper (Cu) level was 1.7 mg kg^{-1}, and manganese (Mn) level was 11.4 mg kg^{-1}, which are considered sufficient levels (Lindsay and Norvell, 1978). In contrast, iron (Fe) level was measured as 5.4 mg kg^{-1} and zinc (Zn) level as 0.4 mg kg^{-1}, showing that these elements are at low levels (Table 2).

Table 2. Results of soil analysis (0-30 cm depth) of the research area

Location	EC us (µS/cm)	pH	Lime (%)	K (mg kg^{-1})	P (mg kg^{-1})	O.M (%)	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})
Denizli-Çal	450	8.3	25.77	365	26.37	1.17%	1.7	5.4	0.4	11.4

The experiment was conducted in 2024 at the Research and Application Center of the Faculty of Agriculture, Pamukkale University, in Çal district, Denizli. Climate data for the main crop growing season for Denizli-Çal conditions are given in Table 3.

Table 3. 2024 years precipitation, average temperature and relative humidity data for the experimental area (April-October average during the main crop growing season).

2024 Year	A.Precipitation (mm/kg)	A.Temperature (°C)	A.Relative Humidity (%)
April	32.1	16,0	51.1
May	42.5	16.6	58.7
June	10.2	26.8	36.1
July	51.3	26.4	44.6
August	30.3	26.2	37.6
September	0.2	20.9	50.5
October	5.6	15.0	48.8
Total	172.2	-	-

The average rainfall in the growing season of 2024 varied between 0.2-51.3 mm, with the highest rainfall occurring in July. In addition, while the average temperature value was 15-26.8 ºC, the highest temperature data was obtained during the vegetative period. The highest average relative humidity was recorded in May (58.7%) (Table 3) (Anonymous, 2025).

Method

The Research and Application Center of the Faculty of Agriculture, Pamukkale University, in Çal District, Denizli, is located at coordinates 38°05'57" north latitude and 29°25'04" east longitude in the Aegean Region. The study was carried out according to the randomized block design with 3 replications. The total experimental area was 588 m², with plot dimensions of 2.8 m × 5.0 m (14 m²), with 10 plots in each block and each plot consisting of 4 rows of plants. Before planting, the row spacing was 70 cm, and 132.0 seeds were planted per plot manually in furrows opened with a marker at a depth of 6-7 cm. The distance between blocks in the experiment was arranged as 3 m. Just before planting, 30 kg/da of Diammonium phosphate (18% N, 46% P_2O_5) fertilizer was applied and mixed into the soil. After planting, 15 kg da^{-1} of Urea (46% N) fertilizer was applied as top dressing. Additionally, all maintenance operations (weed, disease and pest control, and irrigation) were carried out throughout the growing season. Harvesting was done manually by pulling the plants in the plots and leaving them upside down to dry. Threshing was performed manually when the plant leaves turned brown and the above-ground parts dried.

In this study, a total of 16 characters including yield, one hundred pod weight, one hundred seed weight, shelling ratio, number of pods per plant, first quality pod rate, second quality pod rate, leaf area index during flowering period, total chlorophyll amount during flowering period, chlorophyll a during flowering ,chlorophyll b during flowering, leaf area index during pod period ,total chlorophyll in pod stage, chlorophyll a in pod period, chlorophyll b in pod period and oil content were analyzed (Beycioglu, 2022).

Total chlorophyll and leaf area indices were measured before harvest during the 50% flowering and pod formation stages. Chlorophyll measurements were made with a Falker brand CFL260 model chlorophyll meter during the 50% flowering period and pod formation stages. Leaf Area Index was provided by measurements made on one leaf of each plant during the same periods. Measurements were performed with a BLAM-2 Portable Leaf Area Meter.

Statistical Analysis

The experiment was established on April 28, 2024, according to the Randomized Complete Block Design with three replications. All statistical analyses of the data obtained in the study were performed using the R programming language and statistical computing environment (R Core Team, 2024). Descriptive statistics (mean, standard deviation, minimum and maximum values) were calculated for all agronomic, morphological, and physiological characteristics of peanut cultivars. One-way analysis of variance (ANOVA) was applied to determine whether there were statistically significant differences among cultivars in terms of measured characteristics. For characteristics where ANOVA results were found to be significant ($p<0.05$), Tukey's Multiple Comparison Test (Tukey's HSD) was used as a post-hoc analysis to determine which cultivar means differed from each other. Basic statistical functions of R or packages for agricultural research such as agricolae were used for these analyses (Mendiburu, 2023). Pearson correlation analysis was performed to evaluate the direction and strength of linear relationships between different characteristics examined. Correlation coefficients (r) were calculated and their statistical significance was tested ($p<0.05$). The corrplot package was used for visualization of correlation matrices (Wei & Simko, 2021). Hierarchical cluster analysis was performed using common methods such as Euclidean distance and Ward's method to visualize and group the similarities of cultivars according to measured characteristics. The analysis results were visualized with a heat map containing dendrograms for cultivars and characteristics using ggplot2 (Wickham, 2016) packages.

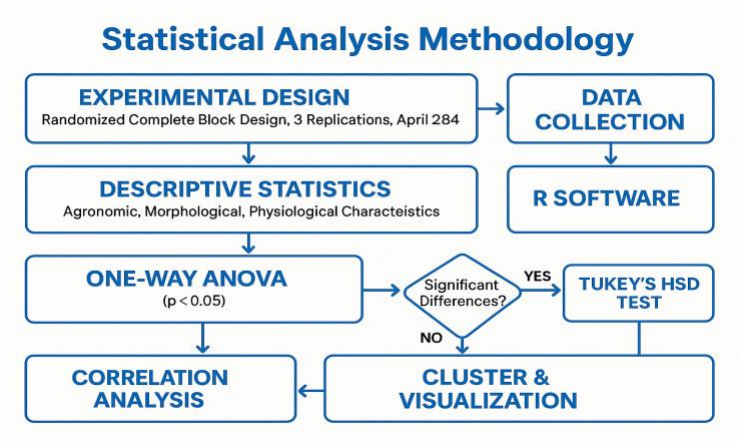


Figure 1. Statistical Analysis Methodology Flowchart

RESULTS AND DISCUSSION

Descriptive statistics of various agronomic and physiological characteristics of peanut (*Arachis hypogaea* L.) are given in Table 4. When the table is examined, yield per decare varies between 341.20-485.24 kg da^{-1}, with an average of 407.56 kg da^{-1}. The coefficient of variation of 12.15% for yield indicates a moderate yield variability among peanut genotypes. The average 100-pod weight is 197.50 g, with a high coefficient of variation of 22.81%, indicating significant differences in pod size among different peanut cultivars/lines. The hundred-seed weight has an average of 88.94 g with one of the highest coefficients of variation at 25.98%, suggesting that seed size could be a suitable criterion for selection. The shelling ratio is an important quality parameter for peanut with an average of 62.15% and shows a relatively low coefficient of variation of 9.62%. Arıoğlu et al. (2018) reported that shelling ratio is one of the most important quality parameters determining economic value in peanut. The number of pods per plant averages 26.58, with a coefficient of variation of 18.69%, making it an important variable among yield components. Canavar and Kaynak (2013) stated that the number of pods per plant is one of the most important components directly affecting yield in peanut. First and second quality pod ratios, with averages of 66.76% and 24.60% respectively, are important parameters in peanut quality classification.

In terms of physiological characteristics, leaf area index and chlorophyll values were measured during the 50% flowering and pod formation periods of peanut, with total chlorophyll amount in the pod formation period (41.13) showing an increase compared to the flowering period (38.91), reflecting the change in photosynthetic activity of peanut according to developmental stages. Awal and Ikeda (2002) reported that chlorophyll content in peanut varies according to developmental stages and this variation is related to photosynthetic activity. Chlorophyll b values show high variation in both flowering (23.68%) and pod formation periods (19.78%). Oil content in peanut seeds varies between 46.64-57.28%, with an average of 50.82%, showing that peanut is an important oilseed crop, and the low coefficient of variation (6.85%) indicates that this characteristic is relatively stable. These data can be used in peanut breeding programs to improve yield, quality, and physiological characteristics. Characteristics showing high variation, such as 100-seed weight, 100-pod weight, and chlorophyll content, can be evaluated in selection studies.

The peanut cultivars used in the study show significant variations in terms of agronomic, morphological, physiological, and quality characteristics. The ANOVA results in Table 5 show that there are statistically significant differences among cultivars in all parameters except second quality pod ratio and chlorophyll a content during the pod period ($p<0.01$ or $p<0.05$). Looking at Table 5, the 'Florispan' cultivar has the highest yield with 485.24 kg per decare and is statistically superior to all other cultivars. This is followed by 'Halisbey' (476.91 kg da^{-1}) and 'Osmaniye 2005' (447.4 kg da^{-1}). The lowest yield was observed in the 'Masal' cultivar (341.2 kg da^{-1}). Boydak (2020) found that the yield of peanut plants is affected by environmental factors and determined average yield values between 352.01-553.45 kg da^{-1}. These values are in line with our values.

In terms of 100-pod weight, the 'Rigel' cultivar (249.33 g) has the highest value, while 'Florispan' (105.33 g) shows the lowest value. Kadiroğlu (2012) reported that among different peanut cultivars (Georgia Green, NC-7, Florispan, and Halisbey), the highest 100-pod weight (334.60 g) was obtained from the Halisbey cultivar, while Koldanca (2016) reported that among different peanut cultivars (Halisbey, NC-7, and Batem-5025), the highest 100-pod weight was again obtained from the 'Halisbey' cultivar (64.3 g). The findings obtained partially overlap with the results of the researchers. Pod weight is a parameter affected by factors such as cultivar, climate, soil properties, and cultural practice differences (Karabulut and Tunçtürk, 2019).

For hundred-seed weight, 'NC-7' (112.93 g) and 'Masal' (111.93 g) cultivars stand out. The Florispan cultivar, which has the highest yield, has the lowest 100-seed weight (47.78 g). Aşık et al. (2018) determined 100-seed weight between 53.27-132.68 g in their study. Arıoğlu et al. (2016) obtained the highest 100-seed weight from the 'Osmaniye-2005' cultivar with 137.78 g. Different from these results, Eskalen and Yılmaz (1993) determined 100-

seed weight values between 70.89 g and 90.69 g in their research with NC-7, Çom, Gazipaşa, Shulamith, and NC-17 cultivars.

Table 4. Descriptive Statistics Values of the Investigated Characteristics of Peanut Genotypes

Variable	Abbreviation	Unit	Min	Max	Mean	StDev	CV%
Yield	Yield	kg da^{-1}	341.20	485.24	407.56	49.50	12.15
One hundred pod weight	OHuPoWe	g	105.33	249.33	197.50	45.05	22.81
One hundred seed weight	OHuSeWe	g	47.78	112.93	88.94	23.11	25.98
Shelling ratio	SeRa	%	52.56	71.87	62.15	5.98	9.62
Number of pods per plant	NuPoPePl	quantity	17.74	34.72	26.58	4.97	18.69
First quality pod rate	FiQuPoRa	%	61.43	74.40	66.76	3.37	5.04
Second quality pod rate	SeQuPoRa	%	19.35	28.71	24.60	2.60	10.57
Leaf area index during flowering period	LeArInDuFlPe	LAI	12.60	20.70	16.46	1.99	12.12
Total chlorophyll amount during flowering period	TaChAmDuFlPe	FCI (Falker Chlorophyll Index)	31.70	43.65	38.91	4.54	11.66
Chlorophyll a during flowering	ChADuFl	FCI (Falker Chlorophyll Index)	23.78	29.33	26.84	2.03	7.57
Chlorophyll b during flowering	ChBDuFl	FCI (Falker Chlorophyll Index)	7.33	14.11	11.18	2.65	23.68
Leaf area index during pod period	LeArInDuPoPe	LAI	11.41	16.91	14.03	2.07	14.78
Total chlorophyll in pod stage	ToChPoSt	FCI (Falker Chlorophyll Index)	34.43	45.43	41.13	3.67	8.93
Chlorophyll a in pod period	ChAPoPe	FCI (Falker Chlorophyll Index)	24.67	29.33	27.80	1.46	5.25
Chlorophyll b in pod period	ChBPoPe	FCI (Falker Chlorophyll Index)	7.33	15.33	12.47	2.47	19.78
Oil content	OC	%	46.64	57.28	50.82	3.48	6.85

In terms of shelling ratio, the 'Georgia Green' cultivar has the highest value with 71.87%. Çil et al. (2016) determined the average shelling ratios of genotypes in the range of 57.9-70.1%. For number of pods per plant, 'Georgia Green' (34.72) and 'Osmaniye-2005' (34.03) cultivars stand out, while the 'Halisbey cultivar' (17.74) shows the lowest value. Yaşlı et al. (2020) found that the number of pods per plant was between 32.95-48.68. İnan (2016) reported that the number of pods per plant was 17.23-39.92 in his study. In terms of first quality pod ratio, the 'Georgia Green' cultivar (74.40%) showed superior performance compared to other cultivars. Branch and Culbreath (2008) stated in their study that the 'Georgia Green' cultivar is known for its high-quality pod production characteristic.

For leaf area index during the flowering period, the 'Florispan' cultivar (20.70) is statistically superior to other cultivars. This situation can be explained by the fact that Florispan's high yield may be related to a larger leaf area and thus a higher photosynthetic capacity. Looking at the results in Table 6, in terms of total chlorophyll amount during the flowering period, 'Rigel' (43.65), 'Halisbey' (43.23), and 'Osmaniye-2005' (42.98) cultivars have the highest values. In terms of chlorophyll a content, the 'Gazipaşa' cultivar (29.33) stands out, while for chlorophyll b content, 'Halisbey' (14.11), 'Masal' (13.55), 'Osmaniye-2005' (13.89), and 'Rigel' (13.89) cultivars showed superior performance. These findings are consistent with Awal and Ikeda (2002)'s study reporting that chlorophyll content in peanut is related to photosynthetic capacity and yield potential.

In terms of leaf area index during the pod formation period, the Çom cultivar (16.91) has the highest value, followed by Rigel (16.42). 'Osmaniye-2005', 'Georgia Green', and 'Sultan' cultivars showed the lowest values. Arunyanark et al. (2008) reported that leaf area index during the pod formation period is critically important for assimilate production and pod filling. In terms of total chlorophyll amount during the pod period, 'Sultan' (45.43), 'Halisbey' (44.60), and 'Rigel' (44.10) cultivars stand out. For chlorophyll b content during the pod period, 'Sultan' (15.33), 'Halisbey' (14.67), and 'NC-7' (14.33) cultivars showed superior performance.

Table 5. Mean Values and Groupings of Yield and Morphological Characteristics of Different Peanut Varieties.

Variety	Yield	OHuPoWe	OHuSeWe	SeRa	NuPoPePl	FiQuPoRa	SeQuPoRa	LeArInDuFlPe
Çom	384,67±13,35 d	180,33±4,51 c	74,81±9,65 c	64,74±1,82 b	25,78±3,31 b	67,17±3,38 bc	25,15±2,58 a	16,22±1,34 b
Florispan	485,24±3,23 a	105,33±4,16 d	47,78±0,75 d	64,26±0,69 bc	24,75±2,28 bc	66,63±1,30 bc	26,04±3,45 a	20,70±0,76 a
Gazipaşa	366,16±22,04 de	201,33±9,45 bc	76,99±4,36 c	64,41±4,54 bc	25,94±2,41 b	69,53±0,70 ab	22,30±3,72 a	17,21±1,71 b
Georgia Green	382,92±10,09 d	135,33±5,77 d	58,54±3,95 d	71,87±1,94 a	34,72±1,66 a	74,40±2,15 a	19,35±6,65 a	16,46±0,93 b
Halisbey	476,91±13,4 ab	226,33±9,07 ab	109,21±4,71 ab	59,12±1,03 cd	17,74±2,27 c	66,13±2,33 bc	22,69±2,49 a	15,51±0,34 b
Masal	341,2±13,45 e	214,67±4,16 b	111,93±6,68 a	67,26±1,10 ab	22,49±2,66 bc	66,24±1,66 bc	25,88±1,25 a	16,50±0,35 b
NC-7	433,85±9,27 c	218,00±22 b	112,93±8,37 a	65,16±1,74 b	26,17±0,64 b	65,91±3,44 bc	24,18±3,64 a	12,60±0,14 c
Osmaniye 2005	447,4±8,05 bc	230,67±6,43 ab	99,68±1,44 ab	52,56±0,42 e	34,03±3,94a	61,43±1,00 c	28,71±2,16 a	17,22±1,16 b
Rigel	381,04±9,89 d	249,33±22,30 a	102,11±4,95 ab	56,18±1,03 de	27,65±2,10 ab	65,30±3,06 bc	25,74±2,94 a	16,62±1,18 b
Sultan	376,27±3,93 d	213,67±7,23 bc	95,44±4,53 b	55,90±0,68 de	26,56±2,76 b	64,83±2,57 bc	26,01±2,96 a	15,56±0,28 b
Anova								
F Ratio	52,49**	45,60**	51,31**	30,46**	11,41**	6,14**	1,69	12,91**

*OHuPoWe: One hundred pod weight, OHuSeWe: One hundred seed weight, SeRa: Shelling ratio, NuPoPePl: Number of pods per plant, FiQuPoRa: First quality pod rate, SeQuPoRa: Second quality pod rate, LeArInDuFlPe: Leaf area index during flowering period
** Significant P < 0.01, * significant P < 0.05

Table 6. Mean Values and Groupings of Physiological Characteristics and Oil Content of Peanut Varieties

Variety	TaChAmDuFlPe	ChADuFl	ChBDuFl	LeArInDuPoPe	ToChPoSt	ChAPoPe	ChBPoPe	OC
Çom	32,83±0,62 c	24,11±0,51 cd	8,00±0,33 bc	16,91±0,17 a	39,60±0,10 ab	28,67±1,15 a	10,33±1,15 ab	46,64±2,58 d
Florispan	35,09±1,23 bc	24,44±0,51 b-d	9,22±0,51 bc	15,74±0,89 a-c	37,83±2,80 ab	26,67±1,53 a	10,67±1,53 ab	51,54±1,57 b-d
Gazipaşa	41,65±3,24 ab	29,33±2,51 a	11,33±4,58 ab	14,26±0,38 cd	34,43±2,39 b	26,33±2,52 a	7,33±1,53 b	47,43±1,05 d
Georgia Green	39,19±3,06 a-c	26,78±1,02 a-d	11,55±2,04 ab	11,41±1,24 e	42,47±1,01 ab	28,33±1,15 a	13,33±0,58 ab	50,90±2,20 b-d
Halisbey	43,23±3,46 a	28,22±1,50 a-d	14,11±1,83 a	15,22±0,12 bc	44,60±4,08 a	29,00±1,73 a	14,67±2,52 a	57,28±2,55 a
Masal	42,22±4,56 ab	27,78±1,50 a-d	13,553,02 a	14,33±0,04 cd	42,03±3,67 ab	28,00±1,73 a	13,00±2,64 ab	53,35±3,31 a-c
NC-7	31,7±0,61 c	23,78±0,39 d	7,33±0,33 c	12,69±0,23 de	43,57±3,40 a	28,33±1,53 a	14,33±2,52 a	47,10±1,17 d
Osmaniye 2005	42,98±2,15 a	28,33±0,88 a-c	13,89±1,35 a	11,51±0,39 e	37,20±0,78 ab	24,67±4,04 a	11,67±3,78 ab	48,95±1,30 cd
Rigel	43,65±3,64 a	28,67±1,66 ab	13,89±2,01 a	16,42±0,37 ab	44,10±2,79 a	28,67±0,58 a	14,00±2,00 ab	50,43±0,79 b-d
Sultan	36,55±0,20 a-c	27,00±2,90 a-d	8,89±2,27 bc	11,76±0,69 e	45,43±4,29 a	29,33±1,15 a	15,33±3,05 a	54,59±1,05 ab
Anova								
F Ratio	8,48**	5,05**	4,27**	38,72**	4,87**	1,69	3,40*	9,73**

*TaChAmDuFlPe: Total chlorophyll amount during flowering period, ChADuFl: Chlorophyll a during flowering, ChBDuFl: Chlorophyll b during flowering, LeArInDuPoPe: Leaf area index during pod period, ToChPoSt: Total chlorophyll in pod stage, ChAPoPe: Chlorophyll a in pod period, ChBPoPe: Chlorophyll b in pod period, OC: Oil content
** Significant P < 0.01, * significant P < 0.05

Significant differences in oil content were observed among cultivars. The 'Halisbey' cultivar has the highest oil content with 57.28%, followed by 'Sultan' (54.59%) and 'Masal' (53.35%). The lowest oil content was detected in 'Çom' (46.64%), 'Gazipaşa' (47.43%), and 'NC-7' (47.10%) cultivars. The 'Florispan' cultivar, which has the highest yield, showed average values in physiological parameters. This supports the view of Prasad et al. (2010) that yield in peanut depends not on a single physiological parameter but on the complex interaction of many factors. The study conducted by Bakal et al. is in agreement with our findings (Bakal et al. 2022). On the other hand, the fact that the high-yielding Halisbey cultivar has high chlorophyll content in both flowering and pod periods and high oil content shows the potential effect of these physiological characteristics on yield and quality.

The correlation analysis in Figure 2 shows the relationships between agronomic and physiological characteristics of peanut cultivars. According to the correlation analysis results, a negative correlation (-0.31) was observed between yield and first quality pod ratio. This supports the finding of Canavar and Kaynak (2013) that quality parameters can sometimes be adversely affected in high-yielding peanut cultivars. There is a positive correlation (0.21) between yield and leaf area index during the flowering period.

Among morphological characteristics, there is a very strong positive correlation (0.91) between 100-pod weight and 100-seed weight. Strong negative correlations were observed between shelling ratio and 100-pod weight and 100-seed weight (-0.62 and -0.43, respectively). This supports the finding of Arıoğlu et al. (2018) that shell thickness may increase and shelling ratio may decrease in large-podded cultivars. There is a very strong positive correlation (0.83) between first quality pod ratio and shelling ratio. Kurt et al. (2017) reported that quality pods generally have a higher shelling ratio. There is a very strong negative correlation (-0.91) between first and second quality pod ratios, which is an expected result.

Very strong positive correlations were observed between total chlorophyll amount during the flowering period and chlorophyll a and chlorophyll b (0.94 and 0.97, respectively). Arunyanark et al. (2008) reported that total chlorophyll amount is directly related to chlorophyll a and b contents and these parameters reflect photosynthetic activity. Similar strong positive correlations (0.83 and 0.95, respectively) were found between total chlorophyll amount during the pod period and chlorophyll a and chlorophyll b. There is a positive correlation (0.30) between leaf area index during the flowering period and leaf area index during the pod period. A strong positive correlation (0.59) was observed between oil content and chlorophyll b content during the pod period. There is a negative correlation (-0.48) between oil content and number of pods per plant. This supports the view of Prasad et al. (2010)

that as the number of pods increases in peanut, the amount of assimilate transferred to each pod may decrease, which may adversely affect oil content.

The heat map and cluster analysis show the hierarchical clustering of peanut cultivars (1-10) and the characteristics examined (Figure 3). Cultivars are divided into two main groups (X and Y). Cluster X consists of subclusters X1 (1, 7, 2) and X2 (3, 4). Cultivars in this cluster generally show similarity in terms of yield and morphological characteristics. Cluster Y consists of subclusters Y1 (5, 6, 9, 10) and Y2 (8). Cultivars in this cluster show similarity in terms of physiological characteristics and oil content.

Characteristics are also divided into two main groups (A and B): Cluster A consists of subclusters A1 (Yield, SeQuPoRa, LeArInDuFlPe) and A2 (LeArInDuPoPe, SeRa, FiQuPoRa, NuPoPePl). This cluster generally includes yield and morphological characteristics. Cluster B consists of subclusters B1 (OHuSeWe, OHuPoWe, TaChAmDuFlPe, ChADuFl, ChBDuFl) and B2 (ToChPoSt, ChBPoPe, ChAPoPe, OC). This cluster covers physiological characteristics and oil content.

Cluster analysis is a method used to group genotypes according to the characteristics examined and determine the relationship between them. Sanches et al. (2006), in their study, examined morphological characteristics such as number of pods, seed weight, pod length, and plant height among 64 peanut cultivars and stated that the relationship between these characteristics stems from genotypes. This also supports the finding of Arunyanark et al. (2008) that physiological characteristics in peanut are related to each other and to quality parameters. Additionally, Holbrook and Stalker (2003) emphasized that agronomic and physiological characteristics in peanut generally cluster in different groups and this situation should be taken into account in breeding studies.

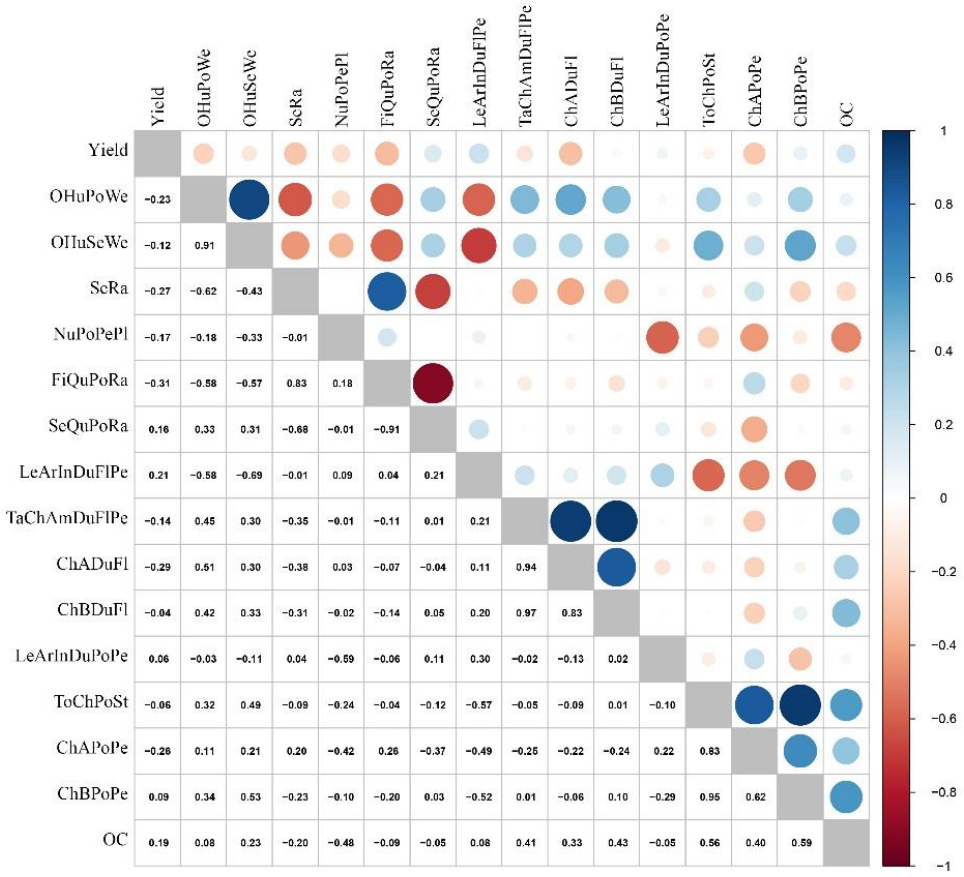


Figure 2. Correlation Analysis of Peanut Varieties.

*OHuPoWe: One hundred pod weight, OHuSeWe: One hundred seed weight, SeRa: Shelling ratio, NuPoPePl: Number of pods per plant, FiQuPoRa: First quality pod rate, SeQuPoRa: Second quality pod rate, LeArInDuFlPe: Leaf area index during flowering period, TaChAmDuFlPe: Total chlorophyll amount during flowering period, ChADuFl: Chlorophyll a during flowering, ChBDuFl: Chlorophyll b during flowering, LeArInDuPoPe: Leaf area index during pod period, ToChPoSt: Total chlorophyll in pod stage, ChAPoPe: Chlorophyll a in pod period, ChBPoPe: Chlorophyll b in pod period, OC: Oil content

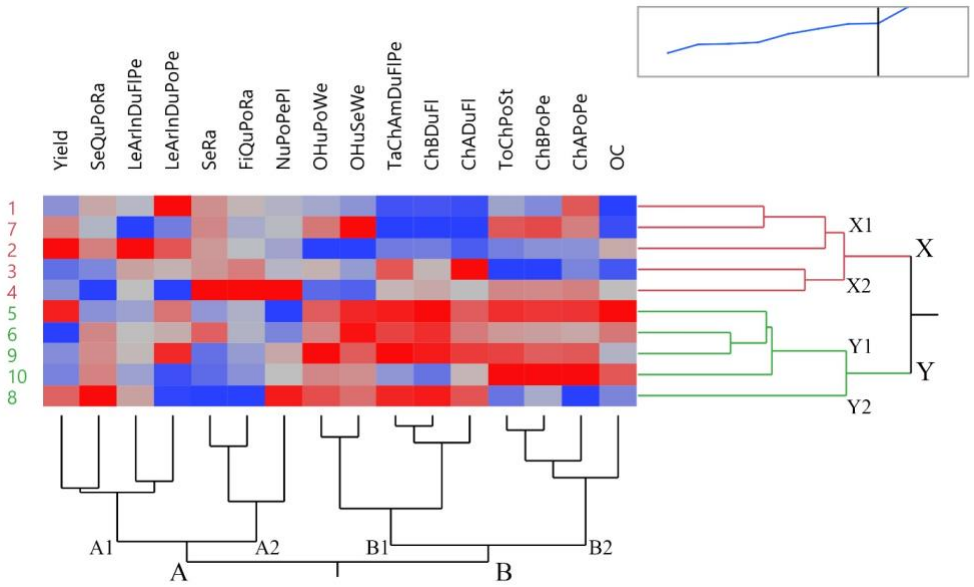


Figure 3. Heat map obtained as an output of the hierarchical cluster analysis of varieties and traits studied. Colors shifting to red indicate an increase and colors shifting to blue indicate a decrease in the temperature scale.
*OHuPoWe: One hundred pod weight, OHuSeWe: One hundred seed weight, SeRa: Shelling ratio, NuPoPePl: Number of pods per plant, FiQuPoRa: First quality pod rate, SeQuPoRa: Second quality pod rate, LeArInDuFlPe: Leaf area index during flowering period, TaChAmDuFlPe: Total chlorophyll amount during flowering period, ChADuFl: Chlorophyll a during flowering, ChBDuFl: Chlorophyll b during flowering, LeArInDuPoPe: Leaf area index during pod period, ToChPoSt: Total chlorophyll in pod stage, ChAPoPe: Chlorophyll a in pod period, ChBPoPe: Chlorophyll b in pod period, OC: Oil content

CONCLUSION

In this study, agronomic, morphological, and physiological characteristics of different peanut genotypes were comprehensively examined. The research results revealed significant variations among peanut cultivars in terms of yield and quality parameters. Yield per decare varied between 341.20-485.24 kg, with the 'Florispan' cultivar achieving the highest yield. In terms of morphological characteristics, the 'Rigel' cultivar (249.33 g) stood out in 100-pod weight, 'NC-7' (112.93 g) in 100-seed weight, and the 'Georgia Green' cultivar (71.87%) in shelling ratio. 'Georgia Green' and 'Osmaniye-2005' cultivars showed superior performance in number of pods per plant, while the 'Georgia Green' cultivar excelled in first quality pod ratio. When physiological parameters were examined, Florispan in leaf area index during the flowering period, and 'Rigel', 'Halisbey', and 'Osmaniye-2005' cultivars reached high values in total chlorophyll amount. During the pod formation period, the 'Çom' cultivar had the highest leaf area index, while 'Sultan', 'Halisbey', and 'Rigel' cultivars stood out in total chlorophyll amount. In terms of oil content, the 'Halisbey' cultivar (57.28%) reached the highest value compared to other cultivars.

According to the correlation analysis, strong positive relationships were determined between 100-pod weight and 100-seed weight (0.91), negative relationship between shelling ratio and pod weight (-0.62), and positive relationship between oil content and chlorophyll b content during the pod period (0.59).

Cluster analysis divided peanut cultivars into two main groups: Cluster X (Çom, NC-7, Florispan, Gazipaşa, Georgia Green) showed similarity in yield and morphological characteristics, while Cluster Y (Halisbey, Masal, Rigel, Sultan, Osmaniye-2005) showed similarity in physiological characteristics and oil content. The examined characteristics were also grouped into two clusters: agronomic-morphological (Cluster A) and physiological-quality (Cluster B) parameters. This grouping facilitates cultivar selection according to target characteristics in breeding studies.

These findings provide valuable information for developing yield, quality, and physiological characteristics in peanut breeding programs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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Effect of encapsulation on aroma and aroma-active components of fresh and black garlic

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Abstract

In this study, aroma profiles and aroma-active compounds of fresh garlic (EFG) and black garlic (EBG) samples encapsulated using the complex coacervation method were investigated. Volatile aroma components were analysed by GC-MS (gas chromatography-mass spectrometry) with the HS-SPME (headspace-solid phase microextraction) method. EFG and EBG samples contained 30 and 37 different aroma compounds, respectively. The chemical changes during the fermentation of black garlic caused a significant decrease in the number of aroma compounds. A total of 13 aroma-active compounds were identified in the encapsulated samples. The odor dilution (FD) factors of these compounds ranged from 4 to 1024. Among the 10 identified compounds, 2 were alcohols, 3 were sulfur-containing compounds, 4 were acids, and 1 was a ketone. The remaining 3 compounds were unidentified aroma-active compounds. This study provides an important contribution in terms of expanding the use of encapsulated garlic products in the food industry and developing products suitable for consumer preferences. These findings may lead to the widespread use of encapsulated garlic products in functional food and nutraceutical fields in the future.

Keywords: *Allium sativum* L, black garlic, encapsulation, GC-MS, aroma-active

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INTRODUCTION

Garlic is a plant of the genus Allium belonging to the Liliaceae family and is widely used throughout the world thanks to its unique flavour. From past to present, garlic has been referred to with many titles such as 'nectar of the gods', 'Russian penicillin', 'natural antibiotic' and 'herbal talisman' (Lidiková et al., 2023). Used in many fields such as agriculture, food, cosmetics, personal care, nutraceuticals and traditional medicine, garlic stands out as a valuable product with a wide range of culinary and medicinal uses. In addition, it is reported that garlic is rich in nutrients and functional components and has important medicinal properties such as antimicrobial, antioxidant, hypoglycaemic, anti-hypertension and anti-tumour effects (Sasmaz et al., 2022, 2024a; Banerjee and Maulik, 2002; Eidi et al., 2006; Wang et al., 2012; Casella et al., 2013; Verma et al., 2023; Renu et al., 2023). Black garlic is a functional product obtained by processing fresh garlic. It is usually produced by a controlled process lasting up to 60 days under conditions of 60-90 °C temperature and 60-80% relative humidity (Zhang et al., 2016; Sasmaz et al., 2022, 2025). Spontaneous fermentation and various chemical reactions are involved in the production of black garlic. This process alters the garlic's color, texture, taste, and nutrient profile, yielding a softer texture and sweeter aroma (Kimura et al., 2017). Compared to fresh garlic, black garlic has a significantly different chemical composition, phenolic compounds, the amount of compounds with high antioxidant capacity increases (Chen et al., 2018; Sasmaz et al., 2022). Black garlic contains bioactive components with antioxidant, anticancer, antimicrobial and anti-inflammatory properties (Tomsik et al., 2017; Rios-Rios et al., 2019). Due to their beneficial properties, these ingredients are increasingly used in functional foods (Dordevic, 2015). However, the stability of

bioactive ingredients is critical for their successful integration into foods, especially for those derived from natural sources. This is because components such as vitamins, probiotics, minerals, polyphenols, omega fatty acids and phytosterols are sensitive to environmental factors such as light, oxygen, heat and water (Augustin and Sanguansri, 2008; Esfanjani and Jafari, 2016). Therefore, the food industry focuses on developing new methods to minimally affect the organoleptic and quality properties of bioactive ingredients. In this context, encapsulation method is recognised as an effective tool to overcome these problems by protecting bioactive components against environmental and chemical effects (Gouin 2004; Zuidam et al., 2019). Encapsulation is the process of coating bioactive components (core-core) with suitable coating agents. This technology is widely used to improve the stability, specificity and bioavailability of food ingredients. Today, coacervation is an effective micro/nano encapsulation method widely used in the food and pharmaceutical industries. Complex coacervation is a process in which electrostatic interactions occur between two biopolymers with different electrical charges (Timilsena et al., 2019). While previous studies have characterised the aroma of fresh and black garlic (Molina-Calle et al., 2017; Sasmaz et al., 2024b), the effect of complex coacervation on aroma and aroma-active compounds in these products has not been investigated.

The encapsulation process offers the possibility of expanding the use of encapsulated garlic products in the food industry due to its potential to partially suppress the pungent aroma of fresh garlic and delay the release of characteristic aroma compounds that occur during fermentation of black garlic.This study aims to characterize the aroma profiles and aroma-active compounds of encapsulated fresh and black garlic using HS-SPME (headspace-solid phase microextraction) with GC-MS (gas chromatography-mass spectrometry), elucidating the impact of encapsulation on sensory properties. To our knowledge, this is the first study in the literature to investigate the aroma characteristics of both encapsulated fresh and black garlic in this context.

MATERIALS AND METHODS

Material

In our previous study, black garlic encapsulation was optimised by central composite design (CCD) based on response surface methodology on the basis of encapsulation efficiency (EE, %) considering three parameters: core/coating ratio, extract concentration and coacervate/maltodextrin (MD) ratio. As a result of this study, the optimum conditions in terms of EE (%) were determined as 4.0 for coating material/core ratio, 50% for extract concentration and 6.0 for MD/coacervate ratio (Sasmaz et al., 2023). In this study, both black and fresh garlic were encapsulated using these optimum parameters. For the preparation of fresh and black garlic extracts, 30 g of garlic was homogenized with 30 mL of distilled water (1:1 ratio, 50% w/v) using a blender (Waring 8011 EB SET2). The resulting mixture was then subjected to ultrasonic-assisted extraction in a water bath for 10 minutes to enhance extraction efficiency. Subsequently, the samples were centrifuged at 5500 rpm for 20 min at 25°C to separate the solid residue. Finally, the supernatant was filtered through coarse cellulose filter paper to obtain clear extracts (Sasmaz et al., 2023). The encapsulation process was finalized after adjusting the solution pH to 3.2, followed by phase separation achieved by maintaining the solution at 4°C for 30 minutes. Prior to lyophilization, the samples were stored in a freezer at -40°C. The freeze-drying procedure was conducted for approximately 48 hours using a laboratory-scale lyophilizer.

Aroma Compounds Analysis

The method used for aroma analyses was a compact system combining analytical techniques such as solid phase micro-extraction (SPME), gas chromatography (GC, Agilent 7890B) and mass spectrometry (MS, Agilent 7010B), which is a kind of dynamic headspace (HS) method. For aroma analyses, 3 g of encapsulated black and fresh garlic samples were placed in a 20 ml glass vial with 5 µl of internal standard solution (IS:4-nonanol, 40 µg) in an autosampler (Gerstel Inc., Linthicum, MD; USA). The vial was sealed using a screw cap containing a PTFE-silicone septum. The sample vial, which is then taken into the pretreatment chamber by the autosampler, is pre-conditioned (incubated) at 50°C for 15 minutes. After preconditioning, a syringe with a 2 cm fibre (Supelco, Bellefonte, PA, USA) coated with 50/30 µm divinylbenzene/carboxylate/polydimethylsiloxane (DVB/Car/PDMS) absorbent materials was used, 33 mm inserted into the vial and collected the trapped aroma compounds volatilised in the environment for 30 minutes at 50°C and brought to the desorption block at 250°C by the autosampler and injected the trapped aroma compounds into the DB-Wax (60 m x 0. 25 mm i.dx 0.25 µm, J&W Scientific-Folsom, USA) column. In the analysis, the oven temperature was gradually heated from 90°C to 140°C and then to 240°C. Helium was used as carrier gas at a flow rate of 1 mL/min. In the GC-MS system, the interface temperature was set to 250°C and the ionisation source temperature to 180°C. Identification of aroma compounds was carried out by comparing the mass spectra obtained with Wiley 11.0 and NIST 14 mass spectral data libraries and retention times determined using alkane standards in the C8-C32 range. The amount of aroma compounds was calculated quantitatively (microgram/kg) using the internal standard method (Sevindik, 2020). Analyses were conducted in triplicate for each sample.

Aroma Extract Dilution Analysis (AEDA)

The AEDA methodology was employed to characterize the potent aroma compounds of the samples. A purge and trap extraction was employed beforehand and the liquid extract representative of sample's original aroma was

used for AEDA procedure. The purge and trap system comprised a nitrogen source that was regulated by a flow meter and connected to a splitter system that divided the flow into multiple channels to purge three samples concurrently. A standard 20-mL vial, sealed with a crimp cap and a septum, was utilized to contain the solid sample. The needle of the N_2 source and the cartridge were installed through the septum to purge and trap the aroma compounds. The details of this extraction process was explained in detail in our previous work (Sasmaz et al., 2024b). AEDA was performed by gradually diluting the aroma extracts with dichloromethane, with the ratio of extract to solvent initially set at 1:1, then gradually increasing to 1:2, 1:4, 1:8, 1:16, and so forth, until the desired ratio was reached. Three panelists with extensive experience in the field utilization an olfactometric port within a GC-MS-O device to assess the aromatic extracts. The dilution and sniffing procedure was methodically executed until no odor was perceptible. During this procedure, each odor perceived from the olfactometric port was represented as a flavor dilution (FD) factor comprising 4, 8, 12, and so forth. The ratio previously cited indicated that the higher the FD factor of a key aroma compound, the more effective it was on the aroma profile (Sasmaz et al., 2024b).

Statistical Analysis

Statistical analysis was performed using the independent samples t-test in SPSS 22.0 (version 22, SPSS Inc., Chicago, IL, USA). Differences in the content levels were evaluated, and means with p-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Aroma Profiles of Encapsulated Samples

In order to observe the effects of encapsulation process on the aroma components of fresh and black garlic, two samples prepared as encapsulated fresh garlic (EFG) and encapsulated black garlic (EBG) were examined in terms of aroma profiles. For this purpose, analyses were performed on encapsulated garlic samples by HS-SPME method with GC-MS device and the results obtained are given in Table 1. Significant differences were observed in the aroma components between the EFG and EBG samples ($p< 0.05$). 30 aroma compounds in the EFG sample and 37 in the EBG sample were detected. The sulfurous compounds group dominated with 12 compounds in both samples, and apart from these compounds, a total of 45 different aroma compounds were identified and quantified, including four thiols, five aldehydes, five ketones, four alcohols, six volatile acids, one furan, one volatile phenol, two terpenes and five other compounds. When the total concentration of aroma compounds of EFG and EBG samples were analysed, the total concentration of aroma compounds in EBG sample was considerably lower than that of EFG sample (EFG: 41170 µg/kg; EBG: 1353 µg/kg). This finding is thought to be a reflection of the effect of the degradation reactions of flavour precursor amino acids on flavour during black garlic fermentation. There are several studies in the literature on both the minimisation of the release of flavour compounds by the encapsulation process and the loss of important flavour compounds that give garlic its pungent odour by the fermentation process (Lotfy et al., 2015; Molina-Calle et al., 2017). Chromatograms highlighting differences between EFG and EBG samples are shown in Figure 1..

Organosulfur compounds

Black garlic is formed as a result of natural reactions and biochemical transformations of the constituents, without the involvement of microorganisms and without the addition of preservatives, only by the action of enzymes naturally present in the raw material (Molina-Calle et al., 2017; Najman et al., 2022; Sasmaz et al., 2024b). During prolonged controlled heating, garlic is subjected to enzymatic and non-enzymatic browning reactions (such as oxidation, caramelisation and Maillard reactions). These reactions alter the physicochemical properties, bioactive components and nutritional value of garlic, as well as leading to significant transformations in its aromatic structure (Molina-Calle et al., 2017; Najman et al., 2022; Sasmaz et al., 2024b). Aroma compounds such as furfural, furanones and pyrazines formed during Maillard reactions are effective in the formation of sweet, fruity and caramelised tones characteristic of black garlic. In addition, unstable organosulfur compounds such as allicin, which is mainly responsible for the pungent odour in fresh garlic, are degraded by heat treatment into derivatives such as S-alylcysteine and S-alylmercaptocysteine, which are more stable structures. These compounds shape the biological effects of black garlic and form the basis of its characteristic aroma (Najman et al., 2022; Kilic-Buyukkurt et al., 2024; Sasmaz et al., 2024b). Considering the findings obtained, although the total concentrations of aroma compounds were approximately 40 times higher in the EFG sample than in the EBG sample, the number of aroma compounds detected in the EBG sample was higher. When Table 1 is analysed, a total of 14 compounds including seven disulphides, five trisulphides and two other organosulfur compounds were detected in the samples. Organosulfur compounds, which are known to be the most dominant compounds in the flavour profiles of both fresh and black garlic, have also played an important role in the formation of the characteristic odours of encapsulated garlic. Among these compounds, alkenyl sulphates are known to be the characteristic flavour components of garlic. Alkenyl sulfides have been reported to inhibit both the initial and developmental stages of tumourigenesis in an experimental carcinogenesis model for various cancer types (Ariga et al., 2006). In the literature, it has been found that garlic contains more than thirty organosulfur compounds, and it has been observed that the encapsulation process applied in encapsulated fresh and black garlic samples traps or

suppresses many of these compounds. One of the important findings obtained while determining the flavour profiles of encapsulated fresh and encapsulated black garlic is that the total concentration of organosulfur compounds is approximately 180 times more dominant in fresh garlic than in black garlic. The same situation was similarly observed in the flavour profiles of fresh and black garlic whose flavour profiles were examined within the scope of this project and in the flavour table of fresh and black garlic added bread. While the total concentration of disulphides in the EFG sample was 5709 µg/kg, the total disulphide concentration in the encapsulated black garlic samples was only 64.0 µg/kg.

The fact that the S-CH_3 bond is easily broken with heat treatment is accepted as an indicator that organosulfur compounds are not resistant to heat treatment (Plano et al., 2009). In the light of this information, it is expected that the amount of organosulfur compounds that give the garlic its pungent specific odour will decrease considerably with the heat treatment applied during the fermentation of black garlic, such as the result obtained in our study. It has been reported that the diallyl disulphide compound, which was determined in both EFG and EBG samples and is the most dominant compound among the disulphides of EFG, has many protective effects against colon, kidney, skin, breast and oesophageal cancers (Bansal et al., 2018). In previous studies, it has been reported that allinase enzyme degrades cysteine sulfoxides such as alliin in heat treatment applications, releasing characteristic aroma compounds such as thiosulfanes, diallyl sulfide and diallyl disulfide (Puccinelli et al., 2017).

(1E)-1-Propenyl disulphide compound, known in the literature as one of the fastest oxidising compounds of garlic and onion when chopped or crushed, was the second most dominant compound determined in fresh garlic (1440µg/kg). This compound was also found to be greatly reduced by being oxidised by black garlic fermentation and almost disappeared with the encapsulation process (8.3µg/kg). In addition to disulphides, five different trisulphide compounds were determined in the EFG sample and three different trisulphide compounds in the EBG sample, and the total aroma concentrations of these compounds were found to be 13353.3 and 42.1 µg/kg, respectively. As in the case of disulphides, trisulphide compounds were observed to undergo a great loss in quantity by fermentation and encapsulation processes. It has been reported in the literature that allyl trisulfide, the most dominant trisulfide compound detected, and other trisulfide compounds give effective results in the treatment of many diseases, especially cancer (Seki et al., 2008). Many trisulfide compounds were determined in studies on the flavour profiles of garlic and it was determined that most of these compounds were lost during the fermentation and encapsulation processes of black garlic. In the studies conducted in the literature, considering the flavour profiles of fresh and black garlic, in parallel with the results obtained in our study, it was reported that heat treatment degraded the trisulfide compounds predominantly found in garlic and many trisulfide compounds were lost (Abe et al., 2020).

In addition to disulphide and trisulphide compounds, two organosulfur compounds, which are among the most dominant compounds of encapsulated garlic, especially EFG samples, were detected. 3-Vinyl-1,2-dithi-4-ene and 2-vinyl-1,3-dithi-4-ene were the compounds that gave EFG samples their characteristic flavours. It has been emphasised in the literature that these compounds are the compounds that give the pungent odours of fresh garlic (Mangoba et al., 2019).

Thiols

The second important aroma group identified in the aroma profiles of EFG and EBG samples were thiols. 3H-1,2-Dithiol is the most dominant compound among these compounds and was found at a high concentration of 5361 µg/kg in the EFG sample, while this compound was not detected in the EBG sample. In the study conducted by Sasmaz et al. (2024), this compound was detected in black garlic, the fact that it was not detected in encapsulated samples is another proof that the encapsulation process has a direct effect on the flavour profile of black garlic. In addition, it was observed that thiols, which are known as one of the aroma groups that persist in human breath when fresh garlic is consumed, were suppressed by the encapsulation process. This situation shows that the usage areas of encapsulated garlic products have the potential to expand. It has been reported in previous studies in the literature that 3H-1,2-dithiol compound has a negative aroma in garlic (Yang et al., 2019). In the literature, Ikram et al. (2019) determined *Allium fistulosum* in the green onion variety known as winter onion and reported that it contributes to the bitter characteristic odour of green onion.

Aldehydes

Another group found in the aroma profile of encapsulated fresh and black garlic was aldehydes. The compounds furfural, which is known to be formed by Maillard reactions of certain components subjected to heat treatment and is responsible for burnt caramel-like odours, and *(E,E)*-2,4-decadienal, which has a toasted oily odour, were only detected in EBG samples. However, nonanal, *(E)*-2-octenal, known for its pleasant, citrusy and soap-like odours, and benzaldehyde compounds with almondy and caramel odours were detected in both samples. In previous studies, it was reported that aldehydes are the compounds that characterise the specific aroma of black garlic and furfural was the most prominent aldehyde compound in black garlic (Ding et al., 2021). In our study, it was observed that aldehyde compounds, which are predominant in the flavour profile of black garlic and released by the Maillard reaction of fructans under the influence of heat treatment and humidity, were significantly reduced by the effect of encapsulation process.

Table 1. Aroma profiles of encapsulated fresh garlic (EFG) and encapsulated black garlic (EBG) (µg/kg)

No	RT	LRI	Compounds	EFG (µg/kg)	EBG (µg/kg)	Sig
			Thiols			
1	30,03	1494	3H-1,2-Dithiol	5360.5 ± 126.1	Nd	*
2	34,86	1628	*(Z)*-5-(Methylthio)-4-penten-2-ol	680.1 ± 55.3	21.6 ± 2.0	*
3	35,79	1636	1,5-Pentanethiol	Nd	7.1 ± 0.2	*
4	38,09	1712	3-methyl 3H-1,2-dithiol	891.1 ± 92.0	10.4 ± 0.8	*
			Total	6931.7 ± 273.4	39.1 ± 3.0	*
			Organosulfur compounds			
			Disulfides			
5	20,61	1283	Allyl methyl disulfide	125.8 ±10.2	Nd	*
6	28,11	1454	*(Z)*-1-Allyl-2-(prop-1-en-1-yl)disulfane	166.8 ±1.5	Nd	*
7	28,62	1463	Diallyl disulfide	3671.0 ±28.7	24.9 ± 1.4	*
8	28,92	1485	Allyl *(E)*-1-propenyl disulfide	Nd	9.6 ± 0.9	*
9	31,29	1516	*(1Z)*-1-propen-1-yl propyl disulfide	Nd	11.4 ± 0.1	*
10	39,20	1780	*(1E)*-1-propenyl disulfide	1439.7 ± 456.3	8.3 ± 0.5	*
11	46,00	2030	Pentyl propyl disulfide	305.7 ± 27.1	9.9 ± 0.6	*
			Total	5708.9 ± 523.7	64.0 ± 3.5	*
			Trisulfides			
12	24,79	1370	Dimethyl trisulfide	86.7 ± 0.2	Nd	*
13	32,48	1592	Allyl methyl trisulfide	2818.9 ± 29.1	18.2 ± 0.6	*
14	40,06	1789	Allyl trisulfide	9680.3 ± 243.5	14.0 ± 1.3	*
15	46,96	2005	Allyl *(E)*-prop-1-enyl trisulfide	601.7 ± 66.0	9.8 ± 0.7	*
16	48,69	2124	Diallyl trisulfide	165.8 ± 41.8	Nd	*
			Total	13353.3 ± 380.6	42.1 ± 2.5	*
			Total sulphides	19062.3 ± 904.3	106.1 ± 6.1	*
			Other organosulfur compounds			
17	38,22	1714	3-Vinyl-1,2-dithi-4-en	6615.7 ± 316.5	Nd	*
18	41,63	1842	2-Vinyl-1,3-dithi-4-en	5393.8 ± 434.6	52.4 ± 1.8	*
			Total	12009.5 ± 751.1	52.4 ± 1.8	*
			Aldehydes			
19	25,53	1390	Nonanal	37.6 ± 1.6	28.2 ± 1.0	*
20	26,65	1429	(*E*)-2-Octenal	62.9 ± 1.0	38.4 ± 0.1	*
21	27,77	1439	Furfural	Nd	33.4 ± 0.7	*
22	29,93	1492	Benzaldehyde	173.7 ± 7.9	105.0 ± 15.4	*
23	40,62	1792	*(E,E)*-2,4-Decadienal	Nd	33.0 ± 3.8	*
			Total	274.2 ± 10.5	237.9 ± 20.9	*
			Ketones			
24	31,57	1570	(*E,E*)-3,5-Octadien-2-one	147.4 ± 1.4	64.8 ± 5.8	*
25	34,75	1624	Acetophenone	87.2 ± 8.0	239.3 ± 5.5	*
26	41,05	1796	3-methyl-1,2-cyclopentanedione	Nd	12.0 ± 0.8	*
27	45,69	2001	Furyl hydroxymethyl ketone	Nd	7.3 ± 0.2	*
28	55,16	2470	Benzophenone	Nd	69.2 ± 7.2	*
			Total	234.6 ± 9.4	392.7 ± 19.4	*
			Carboxylic acids			
29	27,32	1429	Acetic acid	36,2 ± 1,8	107,5 ± 0,6	*
30	35,86	1647	3-Methyl-butanoic acid	288,8 ± 8,5	Nd	*

31	41,81	1852	Hexanoic acid	250,5 ± 3,9	103,1 ± 2,3	*
32	44,53	1914	2-Ethyl-hexanoic acid	Nd	4,5 ± 0,3	*
33	47,22	2070	Octanoic acid	768,0 ± 11,2	33,0 ± 2,1	*
34	49,44	2144	Nonanoic acid	240,2 ± 17,2	Nd	*
			Total	1583,6 ± 42,6	248,1 ± 5,3	*
			Alcohols			
35	18,24	1170	3-Penten-2-ol	85.4 ± 1.5	13.9 ± 1.3	*
36	19,79	1238	2-Hexanol	91.9 ± 1.7	41.1 ± 3.8	*
37	34,26	1621	*(E)*-p-Menta-2,8-dienol	402.1 ± 22.5	20.8 ± 0.3	*
38	41,94	1859	Furaneol	Nd	10.8 ± 0.8	*
			Total	579.4 ± 25.7	86.6 ± 6.1	*
			Terpenes			
39	30,87	1512	Linalool	190.0 ± 16.2	58.7 ± 3.6	*
40	50,31	2228	*α*-Bisabolol	305.0 ± 1.5	32.2 ± 2.9	*
			Total	495.0 ± 17.7	90.9 ± 6.6	*
			Phenols			
41	47,43	2081	3-Methyl-phenol	Nd	26.4 ± 0.2	*
			Total	0.0	26.4 ± 0.2	*
			Furans			
42	29,28	1489	Acetylfuran	Nd	36.1 ± 2.5	*
			Total	Nd	36.1 ± 2.5	*
			Other compounds			
43	27,55	1435	1,3-Dimethylpyrazole	Nd	10.7 ± 1.1	*
44	30,51	1496	Diethyl oxalate	Nd	21.0 ± 2.2	*
45	39,37	1785	3-Acetopyridine	Nd	5.4 ± 0.4	*
			Total	0.0	89.4 ± 5.5	*
General Total				***41170.2 ± 2034.6***	***1353.3 ± 75.5***	*

(*) The symbol in the row indicates statistical differences ($p<0.05$*). Nd: Not detected. EBG:Encapsulated black garlic, EFG: Encapsulated fresh garlic. LRI: Linear retention indexes calculated in DB-Wax column.

Furans

Furans, another aroma group identified with heat treatment, were not found in encapsulated fresh garlic samples as expected, while the only furan compound found in EBG samples was acetylfuran. Acetylfuran compound, which gives black garlic its sweetness and unique flavour, is known to be released by the Maillard reaction of S-alk(en)il-l-cysteine amino acid under high temperature (Molina-Calle et al., 2017).

Alcohols

Another aroma group detected in the encapsulated garlic samples in the study was alcohols (Table 1). 3-Penten-2-ol, 2-hexanol and *(Z)-p*-menta-2,8-dienol were the three volatile alcohol compounds that could be detected in EFG samples, while in EBG samples, especially furfuryl alcohol, alpha kumyl alcohol and furaneol compounds were formed due to the effect of heat treatment. In studies conducted in the literature, Setiyoningrum et al. (2022) reported that the furfuryl alcohol released with the heat treatment applied especially in black garlic makes an important contribution to the aroma of black garlic.

Ketones

When the table of aroma compounds obtained was examined, another important aroma group was ketones. Among the five ketone compounds identified in the samples, *(E,E)*-3,5-octadien-2-one and acetophenone compounds were detected in both EFG and EBG samples, while 3-methyl-1,2-cyclopentanedione, furyl hydroxymethyl ketone, benzophenone compounds were detected only in EBG samples.

Volatile acids

When the results given in Table 1 are considered, it is seen that another aroma group detected in encapsulated garlic samples is volatile acids. Acetic acid, 3-methyl-butanoic acid, hexanoic acid, 2-ethyl-hexanoic acid, octanoic acid and nonanoic acid were the acid compounds detected in encapsulated garlic samples, while 2-ethyl-hexanoic acid was not detected in EFG, 3-methyl-butanoic acid and octanoic acid were not detected in EBG samples. When the results are considered, it is thought that the encapsulated garlic products in the present study do not have a great contribution to the total aroma in terms of acids, since the detection threshold values of volatile acids, which generally give garlic oily odours, are high.

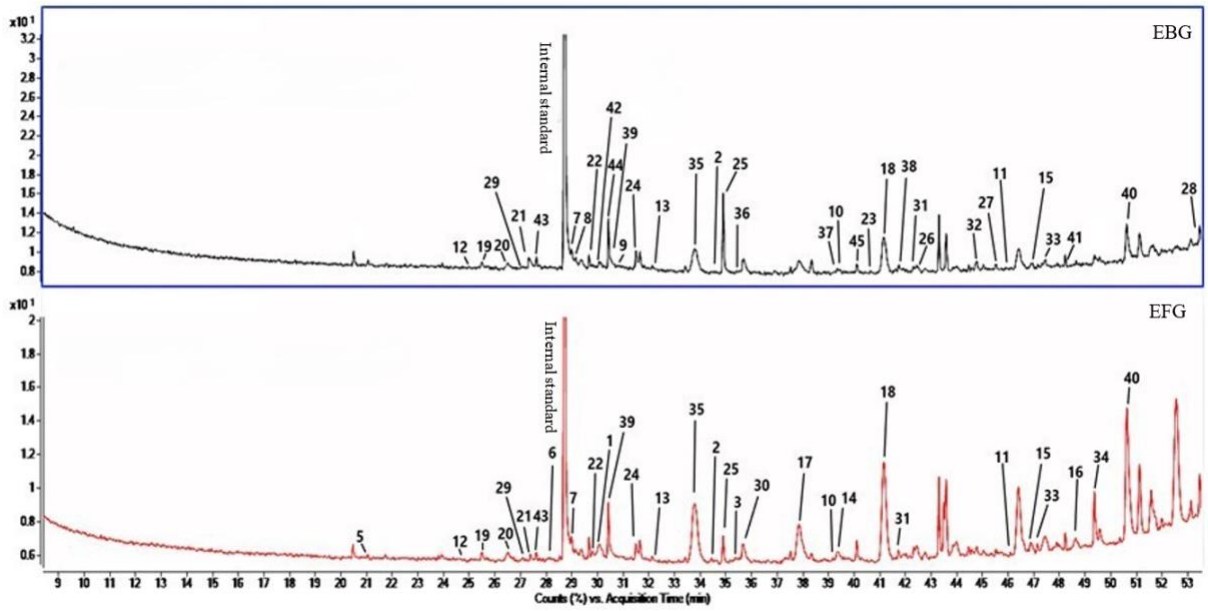


Figure 1. GC chromatograms of encapsulated fresh garlic (EFG) and encapsulated black garlic (EBG). The numbering refers to the order in table 1.

Aroma-active compounds of encapsulated samples

The aroma-active compounds, odours given by these compounds and odour dilution factors (FD) of the products obtained by encapsulating fresh and black garlic samples are given in Table 2. A total of 13 aroma-active compounds were determined in encapsulated samples. Significant differences were observed in the aroma-active compounds between the EFG and EBG samples ($p< 0.05$). The FD factors of the aroma-active compounds varied between 4-1024. Of the 13 aroma-active compounds, 10 were identified and 3 were unidentified (unknown) aroma-active compounds. Unknown odorants (LRI: 1134, 1356, and 1510) were detected by GC-MS-O, but could not be identified by GC-MS due to their low concentration or to limited spectral matches. Of the identified compounds, 2 were alcohols, 3 were sulfur compounds, 4 were acids and 1 was a ketone compound. When the aroma activities of encapsulated garlic samples were compared with unencapsulated fresh and black garlic samples in our previous study by Sasmaz et al. (2023), it was clearly observed that the FD values of encapsulated samples were lower. Osorio et al. (2011) encapsulated guava fruit using maltodextrin, arabic gum and spray dryer and compared the flavour of the encapsules with fresh fruit. It was determined that the encapsules obtained had an aroma very close to the odour of guava fruit when dissolved in water. However, it was also reported that no compounds were detected when the encapsules were analysed for aroma in solid form without dissolving in water. Researchers have explained this situation as the compounds are effectively transferred to the encapsulated structure. In our study, the FD values of encapsulated and non-encapsulated fresh and black garlic samples were analysed.

The highest FD values in encapsulated garlic samples were determined as organosulfur compounds (allyl methyl trisulfide, diallyl disulfide, *(1Z)*-1-propen-1-yl propyl disulfide) as in non-encapsulated samples. It is known that organosulfur compounds formed by the degradation of alliin and its derivatives are very important for both fresh and black garlic flavour and are responsible for the characteristic odours of garlic. In encapsulated fresh and black garlic samples, these two compounds have higher FD values in encapsulated fresh garlic samples. It was determined that these data were also compatible with the aroma compounds of the samples.

A total of 4 aroma-active acid compounds, namely acetic acid (vinegar smell, sour), hexanoic acid (sweat smell), octanoic acid (pungent, mouldy) and nonanoic acid (waxy) were detected in encapsulated garlic samples. The FD factors of these compounds ranged from 4 to 16 and generally had higher values in encapsulated black garlic. 3-Penten-2-ol and 2-hexanol were the aroma-active alcohol compounds identified in the encapsulated garlic and gave the samples oily, green and pungent odours, respectively. It has been reported that these compounds were determined as aroma-active compounds in many products such as passion fruit, mussel meat, olive oil and contributed to the odour in previous studies (Hui et al., 2010; Kesen et al., 2013, 2014).

The only ketone compound identified in encapsulated samples was acetophenone, which is responsible for sweet, pungent odours. In the literature, it has been reported that ketones are generally produced from the peroxidation of lipids and amino acids through the Maillard reaction as a result of heat treatment and have significant effects on food odour (Wang et al., 2019). Acetophenone compound has been reported to be one of the important aroma-active compounds responsible for sweet, pungent, floral odours in various samples such as yeast extract (Wang et al., 2019), dried figs (Yao et al., 2021), yoghurt (Liu et al., 2022) in previous studies.

Table 2. Aroma-active compounds of EFG and EBG samples (FD≥4)

No	LRI	Compounds	Odour Descriptions	FD		
				EFG	EBG	Sig
1	1134	Unknown	Garlic	128	Nd	*
2	1170	3-Penten-2-ol	Oily, green	32	4	*
3	1238	2-Hexanol	Pungent	16	4	*
4	1356	Unknown	Popcorn	256	Nd	*
5	1429	Acetic acid	Vinegar smell, sour	4	128	*
6	1463	Diallyl disulfide	Garlic	1024	64	*
7	1510	Unknown	Green, grass	4	Nd	*
8	1629	Acetophenone	Sweet, fruity	8	256	*
9	1516	*(1Z)*-1-propen-1-yl propyl disulfide	Sulfurous, umami	64	32	*
10	1592	Allyl methyl trisulfide	Umami, garlic	16	128	*
11	1852	Hexanoic acid	Buttery	16	64	*
12	2070	Octanoic acid	Ripe fruit	4	32	*
13	2144	Nonanoic acid	Waxy, buttery	4	32	*

(*) The symbol in the row indicates statistical differences ($p<0.05$*). Nd: Not detected. EFG: Encapsulated fresh garlic, EBG:Encapsulated black garlic,

CONCLUSION

In this study, the aroma profiles and aroma-active compounds of fresh garlic (EFG) and black garlic (EBG) samples encapsulated using the complex coacervation method were investigated, demonstrating for the first time the method's ability to modulate garlic's aroma profile by reducing pungent organosulfur compounds while retaining key aroma-active components.The findings revealed that the encapsulation process had significant effects on the characteristic aroma components of garlic. While 30 different aroma compounds were detected in the EFG sample, this number reached 37 in the EBG sample. However, the concentration of total aroma compounds was about 40 times higher in EFG samples compared to EBG samples. This result reveals that the chemical reactions that take place during the fermentation of black garlic cause a significant reduction in aroma compounds. In particular, organosulfur compounds were found to be much more predominant in fresh garlic than in black garlic. The encapsulation process partially suppressed the pungent flavour of fresh garlic and delayed the release of the characteristic aroma compounds arising during the fermentation process of black garlic. Moreover, encapsulation was observed to reduce the odour dilution factors (FD) of flavour-active compounds. These findings reveal the potential of encapsulation technology to preserve and provide controlled release of aroma components of garlic. This study provides an important contribution in terms of expanding the use of encapsulated garlic products in the food industry and developing products suitable for consumer preferences. Future research should focus on sensory evaluation and encapsulation stability to further optimize garlic-based functional foods.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Statement of the conflict of interest to the manuscript should be specified in this section.

Author contribution

Hatice Kubra Sasmaz: conceptualization, data curation, formal analysis, funding acquisition, writing - first draft, writing - review & editing, Onur Sevindik: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing). Gamze Guclu: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing). Serkan Selli: Conceptualization, data curation, formal analysis, funding procurement, original draft writing, review, and editing. Hasim Kelebek: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing).

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