

Effects of Drought and Salinity Stress on Water Productivity, Photosynthetic Pigments, and Elemental Concentrations of Peppermint (*Mentha piperita* L.) under Melatonin Application

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ABSTRACT

To investigate the effects of drought and salinity stress on water productivity, photosynthetic pigments, and elemental concentrations in peppermint (*Mentha piperita* L.) under melatonin application, a factorial experiment with three replications was conducted in Torbat-e Jam in 2024. The treatments included three levels of salinity (0, 50, and 100 mM NaCl), three levels of drought stress (100, 75, and 50% of water requirement), and two levels of foliar-applied melatonin (0 and 100 μ M). The results indicated that increasing drought stress significantly increased water productivity by 15% and 40%, iron concentration by 6.04% and 68.45%, zinc concentration by 15.38% and 192.3%, sodium concentration by 26.41% and 63%, potassium concentration by 12% and 14.08%, and the Na⁺/K⁺ ratio by 30.55% and 41.66%, respectively. In contrast, chlorophyll a decreased by 9.64% and 25.35%, chlorophyll b by 9.74% and 22.07%, total chlorophyll by 9.67% and 24.19%, and carotenoids by 8.98% and 26.96%, respectively. Increasing drought stress initially increased the essential oil percentage by 20.25%, followed by a reduction of 27.85% at higher stress levels. Salinity stress reduced water productivity by 34.70% and 52.61%, iron concentration by 21.27% and 28%, zinc concentration by 87.11% and 83.65%, chlorophyll a by 7.71% and 32.63%, chlorophyll b by 11.32% and 28.93%, total chlorophyll by 9.21% and 31.23%, carotenoids by 14.89% and 35.10%, and the essential oil percentage by 25.49% and 47.06%, respectively. In contrast, sodium concentration increased from 10.40 to 26.90 and 152.11 mg/g, while potassium concentration increased by 3.3% and then decreased by 3.48%, and the Na⁺/K⁺ ratio increased from 0.053 to 0.15 and 1.13, respectively. Foliar application of melatonin improved water productivity, zinc concentration, potassium concentration, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids by 17.81%, 31.57%, 33.06%, 5.83%, 6%, 5.88%, and 8%, respectively, while reducing iron concentration, sodium concentration, and the Na⁺/K⁺ ratio by 14.71%, 15.44%, and 28.84%, respectively. Overall, the findings demonstrate the effectiveness of melatonin in mitigating the adverse effects of salinity and drought stress on peppermint growth, physiological traits, and mineral nutrition.

Keywords: Chlorophyll, Iron, Potassium, Sodium, Zinc

INTRODUCTION

Drought and salinity are among the most important environmental constraints limiting agricultural productivity by inducing a wide range of physiological, biochemical, and molecular alterations in plants [1]. These stresses impair plant growth and development, ultimately leading to significant yield reduction [2]. The findings of most studies indicate that both salinity and drought stresses significantly reduce the yield of agricultural crops [3]. In addition, both salinity and drought are known to induce oxidative stress and cellular damage in plants [4]. Water productivity and deficit irrigation strategies have therefore emerged as key approaches for improving water management in agriculture, particularly in water-limited regions such as Iran. Water productivity is defined as the ratio of crop yield or economic return to the amount of water consumed, and improving this index is essential for sustainable agricultural production [5].

Peppermint (*Mentha piperita* L.) is a perennial medicinal plant widely used in pharmaceutical, food, cosmetic, and hygienic industries due to its essential oil [6-8]. Recent research on *Mentha* species has demonstrated variation in morphophysiological traits and essential oil composition [9], while studies on *Mentha aquatica* have also characterized its essential oil components, total phenolic content, and antioxidant properties [10]. However, its growth and secondary metabolite production are highly sensitive to environmental stresses. Previous studies have shown that drought and salinity stress can significantly alter nutrient uptake and physiological processes in peppermint and other medicinal plants [11]. Numerous studies indicate that salinity and drought stresses impact the physiological and biochemical characteristics of many medicinal and aromatic plants, including peppermint [12]. For example, salinity stress significantly affected growth and physiological and biochemical

parameters of *Dracocephalum moldavica*, while the application of amino-acid-based biostimulants alleviated some of the adverse effects of salinity stress [13]. One study demonstrated that increasing salinity stress in peppermint from 0 to 6 dS/m reduced photosynthetic pigment content [14]. Another study found that, in different soybean genotypes, drought stress at various growth stages decreased total chlorophyll, chlorophyll a, and chlorophyll b [15]. Similarly, drought stress has been reported to induce biochemical changes in medicinal and aromatic plants, while foliar application of stress-mitigating compounds can improve physiological responses under water deficit conditions [16]. The results of another study showed that increasing salinity (0, 50, and 100 mmol NaCl) and drought stress (100, 75, and 50% of water requirement) in peppermint plants led to reductions in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents. Furthermore, drought stress increased the concentrations of iron (Fe), zinc (Zn), sodium (Na), and potassium (K), while salinity stress reduced iron and zinc concentrations and increased sodium accumulation [5]. The results of another study demonstrated that drought stress (50% field capacity) significantly decreased chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in peppermint [17].

Recently, biostimulants have been recognized as an effective strategy to mitigate environmental stresses and improve crop quality and stability. Among them, melatonin plays a key role in regulating plant metabolism by enhancing phytohormone biosynthesis, nutrient uptake, defense responses, and root growth, ultimately improving yield quality and quantity. It is considered a practical and economical approach for improving plant growth [18]. In addition to its antioxidant properties, melatonin enhances germination, growth, biomass production, and leaf area development [19]. However, its effects can vary depending on concentration, showing both stimulatory and inhibitory responses [20]. Considering the importance of salinity and drought stresses in arid regions, it is necessary to investigate their effects on water productivity, photosynthetic pigments, and elemental concentrations in peppermint under melatonin application.

MATERIALS AND METHODS

To investigate the effects of salinity and drought stress on photosynthetic pigments and the concentrations of certain elements in peppermint under melatonin application, the study was conducted in a greenhouse with an ambient temperature of 25/22 °C (day/night), relative humidity of $52 \pm 5\%$, a light period of 13 hours/day, and geographical coordinates of 60.64° degrees E and 35.23° degrees N, at an altitude of 982 m above sea level in Khorasan Razavi province, Torbat-Jam, Iran. In this study, the experimental factors included three levels of salinity stress (0, 50, and 100 mM NaCl), three levels of drought stress (100, 75, and 50% of water requirement), and two levels of foliar-applied melatonin (0 and 100 μ M), arranged in a factorial experiment with three replications. Figure 1 shows peppermint plants grown under different treatments in the greenhouse.

The pots used in this study had a diameter of 25 cm and a height of 40 cm and were filled with soil. The physical and chemical properties of the soil are presented in Table 1.



Fig. 1 Examples of peppermint plants treated in the research greenhouse

Table 1 Some physical and chemical properties of the tested soil

Organic matter	pH	Electrical conductivity (dS m ⁻¹)	Permanent wilting point (%)	Field capacity (%)	Bulk density (g cm ⁻³)	Clay (%)	Silt	Sand	Soil texture
0.6	7.75	1.28	14.2	28.5	1.31	13	41	46	Loamy

Approximately 20 days after rhizome transplantation, and immediately after the emergence of new leaves, melatonin treatments (0 and 100 μ M) were applied as foliar sprays. Melatonin (98% purity, powder, Sigma-Aldrich, USA) was applied every seven days throughout the salinity and drought stress treatments. For solution preparation, the required amount of melatonin was first dissolved in ethanol and then diluted to the desired volume with distilled water. The 100 μ M concentration was selected based on prior reports of optimal stress mitigation and our

preliminary trials [21, 22]. The aerial parts of the peppermint plants were harvested at the early flowering stage, and then growth indices were measured. To minimize the negative effects of saline irrigation water, salinity treatments were applied gradually, starting from the lowest concentration and increasing stepwise. Weed control was performed manually during a single operation. In this study, irrigation water with the physicochemical characteristics presented in Table 2 was used, and additional NaCl concentrations were added to achieve the desired salinity treatments.

Table 2 Qualitative characteristics of irrigation water

SAR	TDS (meq/l)	EC (ds/m)	PH
0.96	526	1.41	7.94

Water requirement was estimated based on cumulative evaporation from the evaporation pan after applying the pan coefficient according to the method recommended in FAO-56 and considering the installation conditions at the site, with an average pan coefficient of 0.7. Evapotranspiration was calculated using Equation (1):

$$ET_a = K_c \times K_p \times E_{pan} \quad (1)$$

Where ET_a is the actual evapotranspiration (mm/day), K_c is the crop coefficient, K_p is the pan coefficient, and E_{pan} is the evaporation from the pan surface (mm/day). To calculate the water requirement of the plant and apply the crop coefficients for peppermint, the results of [23] were used. Accordingly, the crop coefficients for peppermint at the initial, development, and mid-growth stages were considered to be 0.3, 0.8, and 0.5, respectively.

Water productivity was calculated using Equation (2), where Y is the yield per plant (kg plant^{-1}) and W is the total water consumption (m^3). $WP=Y/W$ (2)

The nutritional requirements of the plants were estimated based on established scientific sources and supplied accordingly. Hoagland's nutrient solution was used in this study. After harvest, photosynthetic pigments and the concentrations of iron (Fe), zinc (Zn), sodium (Na), potassium (K), and the Na^+/K^+ ratio were determined. For the determination of photosynthetic pigments, 0.5 g of fresh leaf tissue was ground in a porcelain mortar using liquid nitrogen. Subsequently, 20 mL of 80% acetone was added, and the mixture was centrifuged at 6000 rpm for 10 min. The supernatant was then transferred to Falcon tubes, and absorbance was measured using a spectrophotometer at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids [24]. For elemental analysis, 0.5 g of dried and ground leaf samples were weighed and ashed in a furnace at 550 °C for 3 h. Then, 5 mL of 2 N HCl was added to each sample, and the final volume was adjusted to 50 mL with distilled water. The concentrations of Fe, Zn, Na, and K in the leaf samples were determined using an atomic absorption spectrophotometer (GBC Savantaa, Australia). Data were analyzed using SAS software (version 9.4), and graphs were prepared using Microsoft Excel. Mean comparisons were performed using the LSD test at the 5% and 1% probability levels.

RESULTS AND DISCUSSION

Photosynthetic Pigments

According to the analysis of variance (ANOVA) for photosynthetic pigments in peppermint (Table 3), in addition to the significant main effects of salinity, drought, and melatonin, the two-way interaction between salinity and drought stresses

was also significant at the 1% probability level. The results of the mean comparison for the main effects of salinity stress, drought stress, and melatonin on water productivity and photosynthetic pigments of peppermint (Table 4) showed that increasing drought stress from 100% to 75% and 50% of the water requirement increased water productivity by 15% and 40%, respectively. In contrast, chlorophyll a decreased by 9.64% and 25.35%, chlorophyll b by 9.74% and 22.07%, total chlorophyll by 9.67% and 24.19%, and carotenoid content by 8.98% and 26.96%, respectively.

Similarly, increasing salinity from 0 to 50 and 100 mM NaCl decreased water productivity by 34.70% and 52.61%, respectively. Chlorophyll a decreased by 7.71% and 32.63%, chlorophyll b by 11.32% and 28.93%, total chlorophyll by 9.21% and 31.23%, and carotenoid content by 14.89% and 35.10%, respectively.

Foliar application of melatonin increased water productivity, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content by 17.81%, 5.83%, 6.00%, 5.88%, and 8.00%, respectively.

Table 3 Results of variance analysis of water productivity and photosynthetic pigments under different salinity, drought and melatonin treatments

Mean squares						
Water productivity	Carotenoids	Total chlorophyll	Chlorophyll b	Chlorophyll a	Degrees of freedom	Sources of variation
0.30 **	0.46 **	9.15 **	0.96 **	4.20 **	2	Salinity
9.35 **	0.26 **	5.01 **	0.52 **	2.29 **	2	Irrigation
1.89 **	0.047 **	0.67 **	0.11 **	0.249 **	1	Melatonin
1.28 ns	0.016 **	0.13 **	0.011 **	0.069 **	4	Salinity × Irrigation
0.0324 ns	0.0006 ns	0.027 ns	0.0009 ns	0.030 ns	2	Salinity × Melatonin
0.022 ns	0.0006 ns	0.034 ns	0.0003 ns	0.030 ns	2	Melatonin × Irrigation
0.045 ns	0.004 ns	0.038 ns	0.004 ns	0.025 ns	4	Salinity × Irrigation × Melatonin
0.050	0.0009	0.013	0.0018	0.0073	34	Error
11.79	3.97	3.025	3.13	3.46		Coefficient of variation (percent)

In each column, the means sharing at least one common letter are not significantly different.

Table 4 Comparison of the simple effect of drought stress, salt stress, and melatonin on water productivity and photosynthetic pigments of peppermint plant.

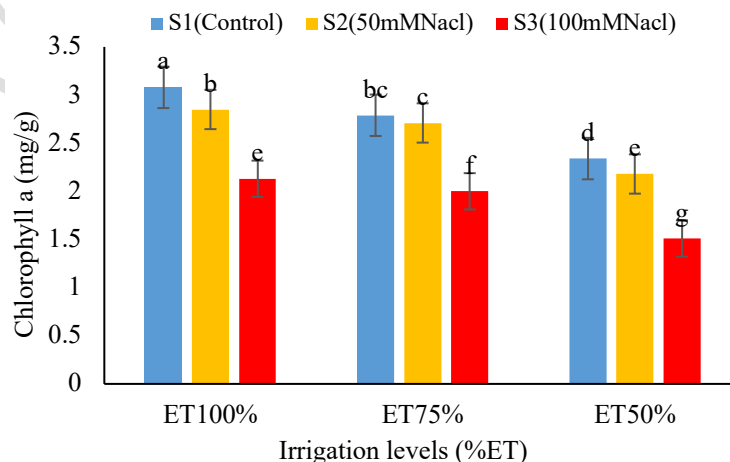
Water productivity (gr/l)	Carotenoids (mg/g)	Total chlorophyll (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a (mg/g)		Treatments		
1.60 c	0.89 a	4.34 a	1.54 a	2.80 a	100	Different irrigation levels (Water requirement %)		
1.84 b	0.81 b	3.92 b	1.39 b	2.53 b	75			
2.24 a	0.65 c	3.29 c	1.20 c	2.09 c	50			
2.68 a	0.94 a	4.45 a	1.59 a	2.85 a	0	Salinity levels (Millimolar sodium chloride)		
1.75 b	0.80 b	4.04 b	1.41 b	2.63 b	50			
1.27 c	0.61 c	3.06 c	1.13 c	1.92 c	100			
1.74 b	0.75 b	3.74 b	1.34 b	2.40 b	0	Melatonin levels (Micromolar)		
2.05 a	0.81 a	3.96 a	1.42 a	2.54 a	100			

ns, *, and **: non-significant, significant at the 5% and 1% probability levels, respectively

The interaction between salinity and drought stresses on photosynthetic pigments (Fig. 2) indicated that the combined effects of salinity and drought further reduced the photosynthetic pigment contents in peppermint. The highest chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were recorded under full irrigation without salinity, with values of 3.08, 1.75, 4.83, and 1.02 mg/g fresh weight, respectively. In contrast, the lowest values were observed under 50% of the water requirement combined with 100 mM NaCl, reaching 1.51, 0.93, 2.44, and 0.47 mg/g fresh weight, respectively.

These findings are consistent with those reported by [5], who demonstrated that increasing the combined levels of drought and salinity stress reduced photosynthetic pigment contents of peppermint. Likewise, [25] reported that increasing salinity from 0 to 50 and 100 mM NaCl significantly reduced the photosynthetic pigment contents in peppermint.

The reduction in photosynthetic pigments under drought and salinity stress may be attributed to the increased production of reactive oxygen species (ROS), which induce lipid peroxidation and subsequently accelerate the degradation of photosynthetic pigments [26].



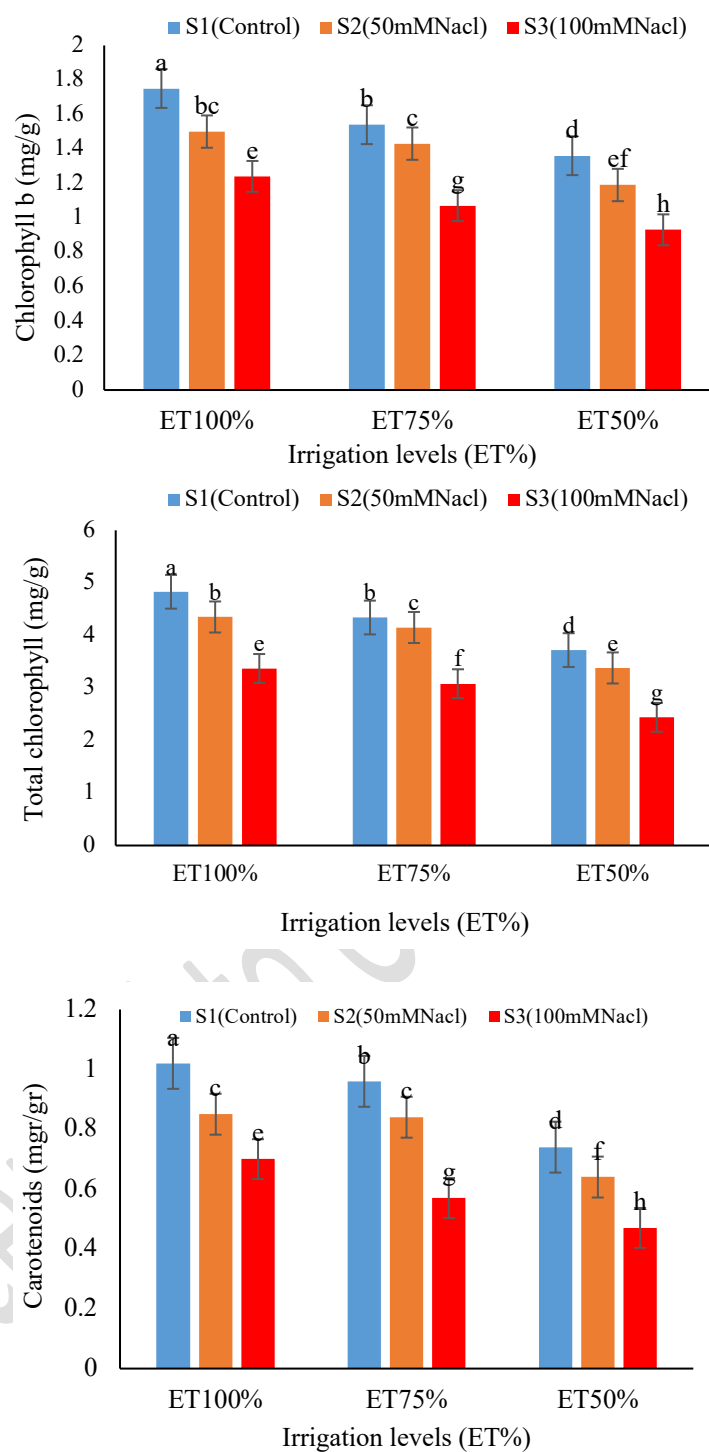


Fig. 2 Interactive effects of salinity and drought stresses on photosynthetic pigments in peppermint. Data are presented as mean $\pm$ SE (n = 3).

Measurement of Elements in Leaves

According to the analysis of variance (ANOVA) for element concentrations in peppermint and essential oil percentage (Table 5), the main effects of salinity stress, drought stress, and melatonin were significant ($P < 0.01$) for all measured elements and essential oil percentage. In addition, the interactions between salinity and drought stresses were significant for all measured elements. The interactions between salinity stress and melatonin, as well as between drought stress and melatonin, were also significant ($P < 0.01$) for all measured elements, except for iron. However, the three-way interaction among salinity stress, drought stress, and melatonin was not significant for any of the measured elements or essential oil percentage.

Table 5 Analysis of variance results of essential oil percentage and concentration of elements in peppermint plant under different salinity, drought and melatonin treatments

Mean squares							
Essential oil percentage	Sodium to potassium ratio	Potassium	Sodium	Zinc	Iron	Degrees of freedom	Sources of variation
1.31 **	6.37 **	9720.19 **	1080.30 **	1.19 **	1.82 **	2	Salinity
1.14 **	0.10 **	2152.27 **	4255.96 **	0.35 **	5.74 **	2	Irrigation
0.0426 **	0.33 **	19937.46 **	952.10 **	0.047 **	0.96 **	1	Melatonin
0.0275 ns	0.037 **	3638.45 **	1100.10 **	0.61 **	1.37 **	4	Salinity × Irrigation
0.0247 ns	0.19 **	1454.20 **	424.52 **	0.014 **	0.02 ns	2	Salinity × Melatonin
0.0287 ns	0.20 **	1193.41 **	2.52 **	0.01 **	0.02 ns	2	Melatonin × Irrigation
0.0113 ns	0.03 ns	3240.42 ns	6.06 ns	0.008 ns	0.008 ns	4	Salinity × Irrigation × Melatonin
0.0002	0.0007	19.35	9.57	0.0002	0.023	34	Error
4.79	6.18	2.65	4.90	6.33	8.24		Coefficient of variation (percent)

In each column, the means sharing at least one common letter are not significantly different.

Table 6 Comparison of the simple effect of drought stress, salt stress, and melatonin on the concentration of elements in peppermint plant

Essential oil percentage (%)	Sodium to potassium ratio	Potassium (mg/g)	Sodium (mg/g)	Zinc (mg/g)	Iron (mg/g)	Treatments
0.79 b	0.36 c	153.07 c	48.64 c	0.13 c	1.49 c	100
0.95 a	0.47 b	171.32 b	61.49 b	0.15 b	1.58 b	75
0.57 c	0.51 a	174.63 a	79.27 a	0.38 a	2.51 a	50
1.02 a	0.05 c	177.12 b	10.40 c	0.52 a	2.22 a	0
0.76 b	0.15 b	182.97 a	26.90 b	0.067 c	1.77 b	50
0.54 c	1.13 a	170.94 c	152.11 a	0.085 b	1.60 c	100
0.79 b	0.52 a	146.46 b	67.33 a	0.19 b	2.005 a	0
0.92 a	0.37 b	194.89 a	56.93 b	0.25 a	1.73 b	100

ns, *, and **: non-significant, significant at the 5% and 1% probability levels, respectively

Iron

The comparison of the simple effects of salinity stress, drought stress, and melatonin application on leaf iron (Fe) concentration in peppermint (Table 6) revealed that reducing irrigation from 100% to 75% and 50% of the crop water requirement increased Fe concentration by 6.04% and 68.45%, respectively. In contrast, increasing salinity from 0 to 50 and 100 mM NaCl decreased leaf Fe concentration by 21.27% and 28.00%, respectively. Furthermore, foliar application of melatonin decreased leaf Fe concentration by 14.71% compared with the untreated control. The interaction between salinity and drought stresses significantly affected leaf Fe concentration (Fig. 3). The highest Fe concentration (2.29 mg/g DW) was recorded under the combined application of the highest salinity level (100 mM NaCl) and the greatest deficit irrigation (50% of the crop water requirement). The results of this study were consistent with the research of [5,27].

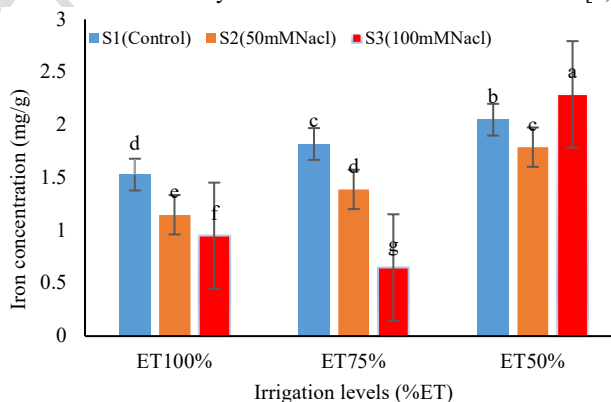


Fig. 3 Interactive effects of salinity and drought stresses on iron concentration in peppermint plant .Data are presented as mean ± SE (n = 3).

Zinc

The results presented in Table 6 showed that increasing drought stress from 100% to 75% and 50% of the crop water requirement increased

Zn concentration by 15.38% and 192.3%, respectively. In contrast, increasing salinity stress from 0 to 50 and 100 mM NaCl decreased Zn concentration by 87.11% and 83.65%, respectively. Moreover, foliar application of melatonin significantly increased Zn concentration by 31.57%. The interaction effect of drought and salinity stresses on Zn concentration (Fig. 4) revealed that the highest Zn concentration (0.165 mg/g) was recorded in the control treatment, whereas the lowest value (0.014 mg/g) was observed under the combined severe drought and salinity stresses. The interaction between salinity stress and melatonin application (Figure 5) showed that the highest Zn concentration (0.191 mg/g) was obtained in the non-saline treatment receiving melatonin, while the lowest value (0.096 mg/g) was recorded under 50 mM NaCl without melatonin application. Furthermore, melatonin foliar application increased Zn concentration under 0, 50, and 100 mM NaCl by 15.75%, 46.87%, and 13.08%, respectively. The interaction effect of drought stress and melatonin on Zn concentration (Figure 4) further demonstrated that melatonin enhanced Zn accumulation under all drought levels. The highest Zn concentration (0.832 mg/g) was recorded in plants receiving melatonin under the 50% crop water requirement treatment. Overall, melatonin foliar application consistently increased Zn concentration at all drought stress levels compared with the corresponding treatments without melatonin. The results of this research were consistent with the results of [27], which showed that by increasing the level of drought stress in the sage plant, the zinc concentration increases.

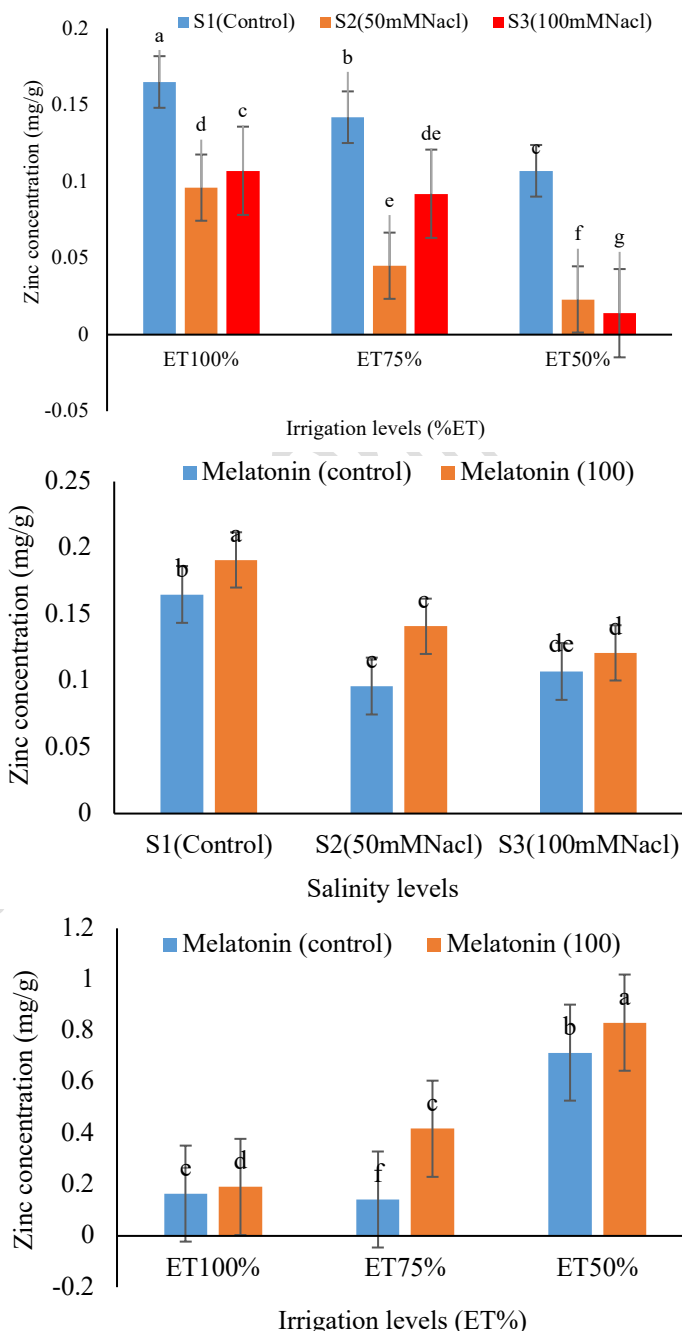


Fig. 4 Interactive effects of salinity and irrigation stress, salinity stress and melatonin, and irrigation stress and melatonin on zinc concentration in peppermint. Data are presented as mean $\pm$ SE (n = 3).

Sodium

The results presented in Table 6 indicated that both drought and salinity stresses significantly increased Na concentration in peppermint leaves. In contrast, foliar application of melatonin reduced Na concentration by 15.44%, demonstrating its effectiveness in mitigating the adverse effects of salinity stress. The interaction effect of drought and salinity stresses on Na concentration (Fig. 5) showed that, at each salinity level, increasing drought severity resulted in a progressive increase in Na concentration in peppermint leaves. The interaction between salinity stress and melatonin application (Fig. 5) further revealed that foliar application of melatonin reduced Na concentration by 24.89%, 18.58%, and 14.24% under 0, 50, and 100 mM NaCl, respectively. Similarly, the interaction effect of drought stress and melatonin application on Na concentration (Figure 5) demonstrated that melatonin significantly reduced Na accumulation under all irrigation regimes. Compared with the corresponding treatments without melatonin, foliar application of melatonin decreased Na concentration by 24.89%, 7.13%, and 5.74% under 100%, 75%, and 50% of the crop water requirement, respectively. The results of this study were consistent with the results of [28,29,5].

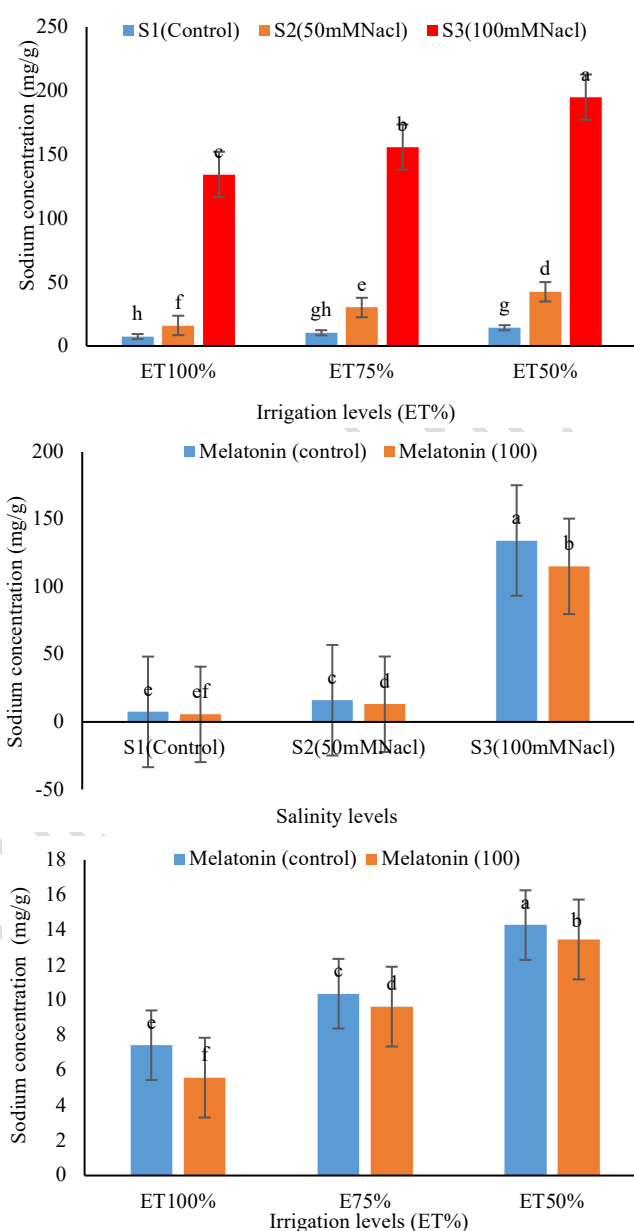


Fig. 5 Interactive effects of salinity and drought stresses, salinity and melatonin stress, and irrigation and melatonin stress on sodium concentration in peppermint plant .Data are presented as mean $\pm$ SE (n = 3).

Potassium

The results presented in Table 6 showed that increasing drought stress enhanced K concentration in peppermint leaves. Regarding salinity stress, increasing NaCl concentration from 0 to 50 mM resulted in a slight increase (3.3%) in K concentration, whereas a further increase to 100 mM NaCl caused a 3.48% reduction. In addition, foliar application of melatonin significantly increased K concentration by 33.06%. The

interaction effect of drought and salinity stresses on K concentration (Fig. 6) revealed that the highest K concentration (177.68 mg/g) was recorded under the I3S1 treatment, whereas the lowest value (106.43 mg/g) was observed under the I1S3 treatment. Furthermore, the interaction effects of salinity stress $\times$ melatonin and drought stress $\times$ melatonin (Fig. 6) demonstrated that foliar application of melatonin increased K concentration in peppermint leaves under all salinity and drought stress levels compared with the corresponding treatments without melatonin. The results of this study were consistent with the results of [29,30].

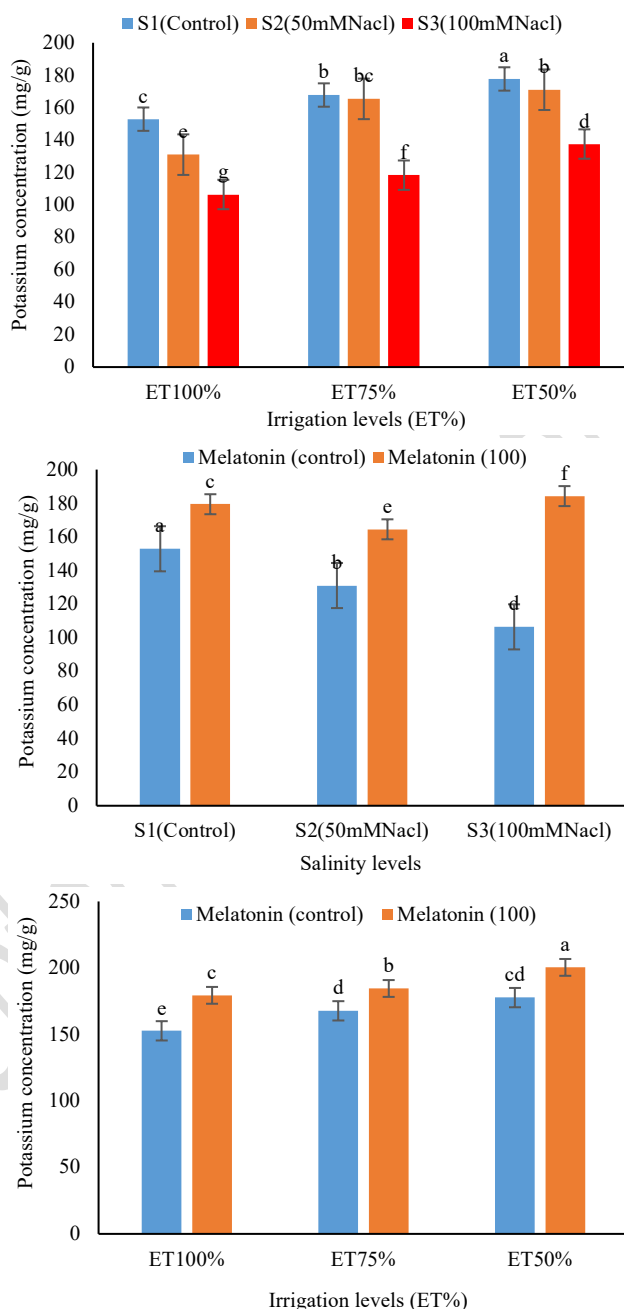


Fig. 6 Interactive effects of salinity and drought stresses, salinity stress and melatonin, and irrigation stress and melatonin on potassium concentration in peppermint plants. Data are presented as mean $\pm$ SE (n = 3).

Sodium to Potassium Absorption Ratio

The results presented in Table 6 showed that increasing drought and salinity stress levels led to a higher Na/K ratio. In contrast, foliar application of melatonin significantly reduced the Na/K ratio by 28.84%. The interaction effect of drought and salinity stresses on the Na/K ratio (Fig. 7) revealed that the highest Na/K ratios were recorded under the most severe salinity treatment (100 mM NaCl), particularly when combined with higher levels of drought stress. Furthermore, the interaction effects of salinity stress $\times$ melatonin and drought stress $\times$ melatonin on the Na/K ratio (Fig. 7) demonstrated that foliar application of melatonin consistently reduced the Na/K ratio across all salinity and drought

stress levels compared with the corresponding treatments without melatonin, indicating its positive role in maintaining ionic homeostasis under stress conditions.

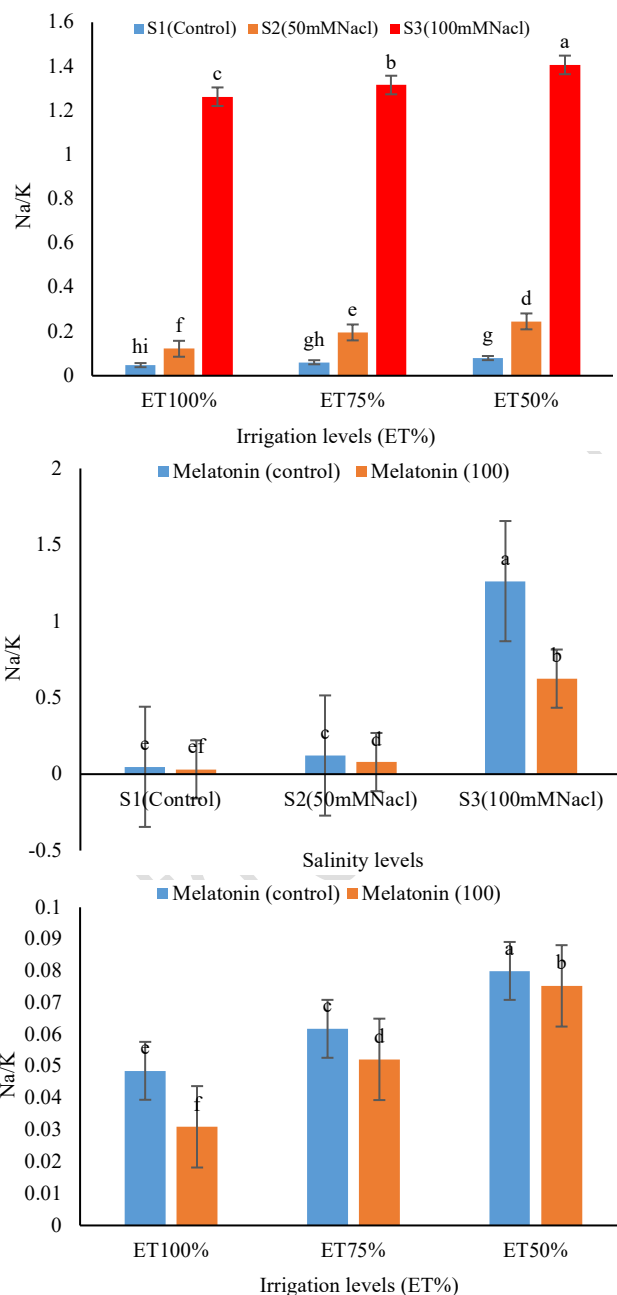


Fig. 7 Interactive effects of salinity and drought stresses, salinity and melatonin stress, and irrigation and melatonin stress on the sodium to potassium absorption ratio in peppermint. Data are presented as mean $\pm$ SE (n = 3).

Essential Oil Percentage

The results presented in Table 6 showed that increasing salinity stress reduced the essential oil percentage of peppermint by 25.49% and 47.06%, respectively. Similarly, increasing drought stress initially increased the essential oil percentage by 20.25%, followed by a reduction of 27.85% at higher stress levels. In addition, foliar application of melatonin significantly improved the essential oil percentage, resulting in a 16.50% increase compared with the control treatment.

CONCLUSION

The results of the present study indicated that reducing irrigation to 75% and 50% of the water requirement increased water productivity by 15% and 40%, respectively. Under the same drought levels, iron concentration increased by 6.04% and 68.45%, zinc concentration by 15.38% and 192.3%, sodium concentration by 26.41% and 63%, potassium concentration by 12% and 14.08%, and the sodium-to-potassium ratio by

30.55% and 41.66%, respectively. The essential oil percentage initially increased by 20.25% under moderate drought stress but declined by 27.85% under severe drought conditions.

In contrast, drought stress negatively affected photosynthetic pigments. Chlorophyll a decreased by 9.64% and 25.35%, chlorophyll b by 9.74% and 22.07%, total chlorophyll by 9.67% and 24.19%, and carotenoids by 8.98% and 26.96% under 75% and 50% of water requirement, respectively.

Also, increasing salinity stress from 0 to 50 and 100 mM NaCl reduced water productivity by 34.70% and 52.61%, iron concentration by 21.27% and 28%, zinc concentration by 87.11% and 83.65%, chlorophyll a by 7.71% and 32.63%, chlorophyll b by 11.32% and 28.93%, total chlorophyll by 9.21% and 31.23%, essential oil percentage by 25.49% and 47.06%, and carotenoids by 14.89% and 35.10%, respectively. In contrast, sodium concentration increased from 10.40 to 26.90 and 152.11 mg/g, while potassium concentration initially increased by 3.3% at 50 mM NaCl but decreased by 3.48% at 100 mM NaCl.

The results also indicated that foliar application of melatonin increased water productivity, zinc concentration, potassium concentration, chlorophyll a, chlorophyll b, total chlorophyll, essential oil percentage, and carotenoids by 17.81%, 31.57%, 33.06%, 5.83%, 6%, 5.88%, 16.50% and 8%, respectively. In contrast, iron concentration, sodium concentration, and the sodium-to-potassium ratio decreased by 14.71%, 15.44%, and 28.84%, respectively.

The interaction effects of salinity and drought stresses on photosynthetic pigments indicated that the simultaneous application of these stresses intensified the reduction of pigment content in peppermint.

Regarding mineral concentrations, the highest iron concentration (2.29 mg/g) was recorded under the combined highest levels of drought and salinity stress. The highest zinc concentration (0.165 mg/g) was observed in the control treatment, whereas the lowest value (0.014 mg/g) was recorded under the highest levels of both stresses. Under constant salinity levels, increasing drought stress led to an increase in sodium concentration in peppermint leaves.

The highest potassium concentration (177.68 mg/g) was recorded in the I3S1 treatment, whereas the lowest value (106.43 mg/g) was observed in the I1S3 treatment. The highest sodium-to-potassium ratio was observed under the most severe salinity level (100 mM NaCl).

The interaction effects of salinity stress and melatonin application showed that foliar spraying of melatonin increased zinc and potassium concentrations at all salinity levels, while reducing sodium concentration and the sodium-to-potassium ratio.

Similarly, the interaction between drought stress and melatonin indicated that zinc concentration was highest under severe drought conditions. Overall, foliar application of melatonin increased zinc and potassium concentrations and decreased sodium concentration and the sodium-to-potassium ratio under all drought stress levels.

It is suggested that, due to the beneficial effects of melatonin foliar application in reducing sodium accumulation and alleviating salinity-induced stress in peppermint, its application may also be recommended for other plant species in the region.

REFERENCES

1. Husain S., Geissler N., Koyro H.W. Effect of NaCl salinity on *Atriplex nummularia* L. with special emphasis on carbon and nitrogen metabolism. *Acta Physiologiae Plantarum*. 2013;35:1025–1038.
2. Liang G., Liu J., Zhang J., Guo J. Effects of drought stress on photosynthetic and physiological parameters of tomato. *Journal of the American Society for Horticultural Science*. 2020;145(1):12–17. doi:10.21273/JASHS04725-19.
3. Pirasteh-Anosheh H., Emam Y., Sepaskhah A.R. Improving barley performance by proper foliar application of salicylic acid under saline conditions. *International Journal of Plant Production*. 2015;9:467–486.
4. Munns R. Genes and salt tolerance: Bringing them together. *New Phytologist*. 2005;167:645–663.
5. Shamsabadi V., Banejad H., Ansari H., Nemati S.H. Investigating the influence of drought and salinity stresses on photosynthetic pigments and elemental concentrations of *Mentha piperita* L. under selenium application. *Water Management in Agriculture*. 2023;10(1):95–110.
6. Foster S. Peppermint (*Mentha piperita* L.). *American Botanical Council Botanical Series*. 1996;306:3–8.
7. Eccles R. Menthol and related cooling compounds. *Journal of Pharmacy and Pharmacology*. 1994;46:618–630.
8. Fleming T. *PDR for Herbal Medicines*. 1st ed. Montvale, NJ: Medical Economics Company; 1998.
9. Latifi M., Kheiry A., Razavi F., Sanikhani M. Influence of vermicompost and nitrogen treatments on the enhancement of morphophysiological traits and essential oil composition of spearmint (*Mentha spicata* L.) for double harvesting seasons. *Journal of Medicinal Plants and By-products*. 2024;13(4):1–11. doi:10.22034/jmpb.2024.363701.1613.
10. Nakhaei F. Identification of essential oil components, total phenolic content and antioxidant properties of *Mentha aquatica* in Southern Khorasan Province, Iran. *Journal of Medicinal Plants and By-products*. 2024;13(1):43–49. doi:10.22092/jmpb.2022.354236.1354.
11. Jafari T., Iranbakhsh A., Kamali K., Daneshmand F., Seifati S.E. Effect of salinity stress on growth parameters, mineral ion concentration, osmolytes, non-enzymatic antioxidants, and phenylalanine ammonia-lyase activity in three genotypes of *Chenopodium quinoa* Willd. *Novelty in Biomedicine and Cellular and Molecular Biology Journal*. 2021;12(45):63–85.
12. Kumar D., Punetha A., Chauhan A., Suryavanshi P., Padalia R.C., Kholia S., Singh S. Growth, oil, and physiological parameters of three mint species grown under saline stress levels. *Physiology and Molecular Biology of Plants*. 2023;29(7):1061–1072. doi:10.1007/s12298-023-01337-1.
13. Seyedi A., Fathi S., Movlodzadeh R. The effect of biostimulants based on free amino acids on some growth and physiological parameters of *Dracocephalum moldavica* L. under salinity stress. *Journal of Medicinal Plants and By-products*. 2024;13(2):469–477. doi:10.22092/JMPB.2022.356634.1424.
14. Ghorbani M., Movahedi Z., Kheiri A., Rostami M. Effect of salinity stress on some morphophysiological traits and the quantity and quality of essential oil in peppermint (*Mentha piperita* L.). *Environmental Stresses in Crop Sciences*. 2018;11(2):413–420. doi:10.22077/escs.2018.953.1188.
15. Kamrava S., Babaeian Jolodar N., Bagheri N. Evaluation of drought stress on chlorophyll and proline traits in soybean genotypes. *Journal of Crop Breeding*. 2017;9(23):95–104.

16. Afshari M., Pazoki A., Sadeghipour O. Biochemical changes of coriander (*Coriandrum sativum* L.) plants under drought stress and foliar application of salicylic acid and silicon nanoparticles. *Journal of Medicinal Plants and By-products*. 2023;12(3):197–207.
17. Ghiyasi S., Movahedi Z., Rostami M. Effect of drought stress and stress modifier on morphophysiological traits of peppermint (*Mentha piperita* L.). *Crop Science Research in Arid Regions*. 2026;7(4):739–758. doi:10.22034/csrar.2024.470040.1427.
18. Shamsabadi V., Modoodi M., Moradi M. The effect of salinity stress on water productivity and some characteristics of *Dracocephalum moldavica* L. using melatonin. *Iranian Journal of Irrigation and Drainage*. 2025;19(2):175–183.
19. Zhang N., Sun Q., Zhang H., Cao Y., Weeda S., Ren S.H., Guo Y.D. Roles of melatonin in abiotic stress resistance in plants. *Journal of Experimental Botany*. 2015;66:647–656.
20. Sarropoulou V.N., Dimassi-Theriou K., Therios I., Koukourikou-Petridou M. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates, and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). *Plant Physiology and Biochemistry*. 2012;61:162–168.
21. Beyrami H., Rahimian M.H., Dehghani F., Ranjbar G., Hasheminejad Y. Effect of irrigation water salinity on the yield of two *Salicornia* species (*Salicornia bigelovii* and *S. persica*). *Journal of Agricultural Science and Sustainable Production*. 2019;29(3):95–111.
22. Moradi M., Roosta H.R., Nasrabadi H.N., Shamsabadi V. Melatonin improves growth indices, photosynthetic performance, and essential oil content of *Thymus daenensis* L. under salt stress. *BMC Plant Biology*. 2026;26:505. doi:10.1186/s12870-026-08356-6.
23. Shahriari S., Azizi M., Aroiee H., Ansari H. Effect of different irrigation levels and mulch application on growth parameters and essential oil content of peppermint (*Mentha piperita* L.). *Iranian Journal of Medicinal and Aromatic Plants Research*. 2013;29(3):568–582. doi:10.22092/ijmapr.2013.4041.
24. Arnon D.I. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 1949;24(1):1–15.
25. Danace E., Abdossi V. Effects of silicon and nano-silicon on some morphophysiological and phytochemical traits of peppermint (*Mentha piperita* L.) under salinity stress. *Iranian Journal of Medicinal and Aromatic Plants Research*. 2021;37(1):98–112. doi:10.22092/ijmapr.2021.343340.2810.
26. De la Luz L. Chilling-enhanced photooxidation: The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiology*. 2004;83:278–282.
27. Sodaieizadeh H., Mansouri F. Effects of drought stress on dry matter accumulation, nutrient concentration, and soluble carbohydrate content of *Salvia macrosiphon* as a medicinal plant. *Journal of Arid Biome*. 2014;4(1):1–9.
28. Parsa M., Kamaei R., Yousefi B. Effects of different biofertilizers on nutrient elements, essential oil, and yield of peppermint (*Mentha piperita* L.) under drought stress conditions. *Iranian Journal of Medicinal and Aromatic Plants Research*. 2019;35(5):860–875. doi:10.22092/ijmapr.2019.126004.2543.
29. Caparros P.G., Romero M.J., Llanderal A., Ceacero P., Lao M.T., Segura M.L. Effects of drought stress on biomass, essential oil content, nutritional parameters, and production costs in six Lamiaceae species. *Water*. 2019;11(3):573.
30. Mohammadi S., Boroomand N., Moghbeli E. Effect of different mycorrhizal inoculation treatments on nutrient element concentration, yield per plant, and antioxidant activity of peppermint (*Mentha piperita* L.) under salt stress. *Journal of Soil Management and Sustainable Production*. 2019;8(4):127–142. doi:10.22069/ejsms.2019.12936.1734.