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Research Article

Physiological and morphological responses of peppermint (*Mentha* × *piperita* L.) to the application of seaweed under salinity stress

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Abstract

Biofertilizers, e.g., seaweed extract, have been effective in environmental and agricultural ecosystems. The effects of seaweed extract were studied on the physiological characteristics of peppermints irrigated with water at different salinity levels in a factorial experiment based on a randomized complete block design with four replications in the greenhouse of Yazd University in 2020. The experimental factors included salinity stress at four levels of 0, 2, 4, or 8 dS/m provided from a sodium chloride source and the foliar application of seaweed at four levels of 0, 1/1000, 2/1000, or 4/1000 provided from an Ascoclepe source. In examining morphological traits, results demonstrated that the fresh and dry weight of leaves, aerial parts, total fresh and dry biomass, and harvest index were significant at the P≤0.01 level. The use of seaweed at 4 ppt under stress-free conditions resulted in the highest weight of leaves, aerial, and total biomass. Based on the results, the salinity stress and the foliar application of the seaweed extract were significantly influential on chlorophyll *a* and *b*, total chlorophyll, and proline amino acid at the P≤0.01 level and on dissolved sugars at the P≤0.05 level. The comparison of means revealed that the highest relative water content was 65.8% related to the no-salinity conditions. The highest proline content was obtained from the salinity levels of 4 and 8 dS/m, exhibiting a 37.5% increase versus the control. Total chlorophyll exhibited a positive and significant correlation with other traits, including chlorophyll *a*, chlorophyll *b*, and carotenoids, with correlation coefficients of 0.61, 0.96, and 0.98, respectively. A negative and significant correlation was observed between proline and total chlorophyll, chlorophyll *b*, and relative humidity with correlation coefficients of 0.56, 0.52, and 0.62, respectively. The results generally showed that the application of seaweed extract at the rates of 2/1000 and 4/1000 to the plants exposed to high levels of salinity (4 and 8 dS/m) alleviated the adverse impacts of salinity by improving physiological traits, e.g., proline content.

Keywords: Medicinal plant, Photosynthesizing Pigments, Proline Amino acid, Seaweed extract

Introduction

Peppermint (*Mentha* × *piperita* L.) is an herbaceous rhizomatous perennial hybrid plant species (2n = 48) from the family of Lamiaceae produced by crossing *M. aquatic* and *M. spicataspercia*. In addition to being a medicinal plant, it is a popular plant usually used in tea, as a flavor, and in sweets (Malekmohammad *et al.*, 2021). Peppermint is one of the most widely consumed medicinal plants. The global production rate of peppermint essential oil amounts to 400 tons, about 80% of which is produced in the US (Motiee and Abdoli, 2021).

Peppermint leaves contain 1.2-3.9% (v/w) essential oil with over 300 identified compounds (52% terpene and 9% sesquiterpenes) (Motiee and Abdoli, 2021). Menthol, menthofuran, and 1 8-cineole are the most important compounds in peppermint essential oil (Moetamedipoor *et al.*, 2021). The components of essential oil and the morphological traits vary with environmental and nutritional factors (Mavandi *et al.*, 2021).

Plant growth and yields are limited by numerous biotic and abiotic stresses throughout the world (Hassanisaadi *et al.*, 2022; Esfandiari and Hakimzadeh,

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2022). Salinity is abiotic stress that imposes extensive damage on plants in the world (Aghighi Shahverdi *et al*., 2019; Ardakani and Vahdati, 2018). The most important response of plants to salinity stress is growth reduction. So, salinity stress limits plant growth. Growth reduction is an adaptation strategy for plant survival in stressful conditions (Bernstein, 2019). Salinity stress affects the vegetative and reproductive growth of plants, thereby reducing their dry weight and yields (Fathizad *et al*., 2020; Esfandiari and Hakimzadeh, 2011).

Biofertilizers are a set of preservatives that contain many beneficial microorganisms or metabolic products that are mostly used to supply the nutrient requirement of plants and create proper physical and chemical conditions for their growth in the soil (Kumar *et al*., 2022).

The extract of seaweed is a major source of plant bio-stimulators that can increase agriculture to meet the current global demand. The extract of seaweed based on *Phaeophyceaean Ascophyllum nodosum* and *Durvillaea potatorum* has recently been shown to increase plant growth or tolerance of biotic and abiotic stresses (Islam *et al.*, 2020). The application of seaweed as a bio-stimulator has extensively expanded in the world. It accounts for about 33% of the global market of bio-stimulators and is expected to reach 894 million euros in 2022, algae were estimated to be 10,000 species, which are divided into brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta) based on their pigments. The main group of seaweeds is brown and it includes Ascophyllum, Focus, and Laminaria (Ghatas *et al.*, 2021).

The extract of seaweed contains different amounts of biologically active secondary metabolites, vitamins, thymol compounds, and precursors of vitamins and amino acids. In addition, it contains several plant hormones, such as auxins, cytokinins, gibberellins, abscisic acid, and brassinosteroids (Strik, 2020).

Seaweed extract has recently been used as an alternative to chemical fertilizers in agriculture. The advantages of seaweed fertilizers include better growth and development of roots, better and faster germination of seeds, delayed aging of fruits, the extension of postharvest shelf life, and the increase in plant vigor and resistance to biotic and abiotic stresses. Due to their fibrous and sticky structure, algae increase soil porosity, enhance its water retention capacity, and reduce soil salinity (Mukherjee and Patel, 2020; Stirk *et al.*, 2020). Seaweed extract has a beneficial impact on plant growth and development and increases their resistance to salinity or drought due to its growth hormone contents (e.g., auxins and cytokinins), nutrients (nitrogen, iron, zinc, copper, cobalt, molybdenum, Manganese, Magnesium, Nickels), vitamins, and amino acids (Taghizadeh-Mehrjardi *et al*., 2021; Xu and Leskovar, 2015; Kamali *et al*., 2021; Hakimzadeh and Esfandiari, 2025). Ali *et al*. (2021) reported that seaweed extract increased plant growth and increased tolerance to pests, diseases, and abiotic stresses. Seaweed extract increases antioxidant content, too.

Iran is climatically and geographically located in one of the best regions of the world for the cultivation of medicinal plants. The use of seaweeds as a soil conditioner has increased crop yield and productivity in an environmentally friendly way as they contain many nutrients as well as plant growth hormones which stimulate plant growth (Shojaei *et al*., 2019; Singh *et al.*, 2019; Abdullahi *et al.*, 2021). Given the significance of medicinal plants and the problems of salinity and water scarcity, there are currently no reports on the impact of seaweed liquid on *Mentha* × *piperita* to reduce salt stress. The present study aimed to investigate the changes in the physiological traits and the tolerance of peppermints to different levels of salinity in response to seaweed extract.

Materials and methods

Experimental materials and treatments: The research was conducted in the greenhouse of Yazd University as a factorial experiment based on a completely randomized design (CRD) with four replications to study the quantitative and qualitative responses of peppermint to the application of seaweed and salinity stress. The salinity stress was applied at four levels of 0, 2, 4, and 8 dS/m, and the seaweed was applied at four rates of 0, 1/1000, 2/1000, and 4/1000. The rhizomes of the peppermints were planted in 64 pots with a height of 25 cm and a diameter of 19 cm. Table 1 presents soil characteristics including its nutrients (Zinc, Copper, Iron, and Manganese) and other attributes such as pH, EC, and organic carbon. All micronutrients were at adequate levels for preserving plant growth. After testing the soil, the rhizomes of the same size were planted in the pots containing 2 kg of soil at a rate of one rhizome per pot. After five weeks, salinity was applied at four levels using sodium chloride (NaCl). The saline water with various electrical conductivity (EC) was prepared using NaCl based on Eq. (1). For confidence, the EC of the solutions was checked with an EC-meter.

$$\text{NaCl (mg/L)} = \text{EC (dS/m)} \times 640 \quad (1)$$

The pots were watered based on their weight daily. After 5 weeks, the seaweed was applied by the foliar application at four rates three times every two days. The seaweed extract used in the research was Ascokelp manufactured by the Kimia Cadde Bahar Company, Iran. The control was treated with water instead of seaweed extract. According to the manufacturer, Ascokelp is the extract of *Ascophilium nodosum* mixed with *Laminaria japonica* and macro and micronutrients, making it an effective compound that is rich in natural growth stimulators.

Measurement of physiological traits: Eight weeks after the cultivation and the application of the treatments, the physiological traits were measured in all experimental units. The recorded physiological parameters included proline content, dissolved sugars, and photosynthesizing pigments.

Table 1. Some physical and chemical parameters of soil at depths of 0 to 30 cm

Soil texture	N ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)	ECe ($dS.m^{-1}$)	pH	OC (%)
Sandy loam	1200	7.4	250	2.8	6.93	1.7

Relative water content (RWC): RWC was measured by Yamasaki and Dillenburg's (1999) method. First, fresh leaf weight (FW) was measured with a digital scale. Then, the samples were placed in distilled water and their saturated weight (TW) was measured after 24 hours. They were, then, oven-dried at 70°C for 24 hours to determine their dry weight (DW). Then, RWC was determined by Eq. (2):

$$RWC(\%) = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100 \quad (2)$$

Photosynthesizing pigments: Photosynthesizing pigments, including chlorophylls and carotenoid, were measured by Arnon's (1967) method for which 0.2 g of plant leaves was washed with distilled water, crashed in a mortar, and added with 5 mL of acetone 80%. Then, it was infiltrated through filter paper and centrifuged at 3500 rpm for 15 minutes. Next, a spectrophotometer was calibrated with 80% acetone, and the absorbance of the samples was read at 663, 645, and 470 nm. Finally, Eq. (3)-(6) were used to determine the contents of photosynthesizing pigments:

(3)

Chl a (mg/g FW) = (19.3 × A664 – 0.86 × A645) × 20/100 × g plant

(4)

Chl b (mg/g FW) = (19.3 × A645 – 3.6 × A663) × 20/100 × g plant

(5)

Car (mg/g FW) = (1000 × A470 – 1.82 × Chl a – 85.02 × Chl b)/198

(6)

Total chl = Chl a + Chl b

Total soluble carbohydrates: To determine soluble sugars, 10 mL of 70% ethanol was added to 0.1 g of plant dry matter and was stored in a refrigerator for one week. Then, 1 mL of the supernatant was added with 1 mL of 5% phenol and blended. In the next step, 5 mL of thick sulfuric acid was added to the solution. The color of the yellow solution obtained gradually changed to light brown. After 30 minutes in the laboratory temperature, its absorbance was read at 485 nm with a spectrophotometer calibrated with ethanol. Then, the variations in sugars were determined in mg/g DW by Eq. (7) using the standard glucose curve (Kochert *et al.*, 1978).

$$Y = \frac{M \times T}{W} \times 10 \quad (7)$$

In which *Y* represents the measurement of total soluble carbohydrates, *M* represents the reading by the spectrophotometer, *T* represents the control solution (here, 7 mL), and *W* represents the weight of the leaf sample used (here, 1 g).

Proline: Proline content was measured by the Bates *et al*. (1973) method, for which 0.5 g of the fresh seedling tissue was homogenized in 10 mL of 3% aquatic sulfosalicylic acid. The aquatic solution was, then, infiltrated through Whatman paper No. 2. Then, 2 mL of the infiltrated solution, 2 mL of ninhydrin acid, and 2 mL of acetic acid were mixed in a test tube and placed in a hot water bath at 100°C for 1 hour. Finally, the reaction mixture was extracted by 4 mL of toluene and was cooled down to room temperature. Then, its absorbance was read with a spectrophotometer at 520 nm. Using the standard curve, the proline content was calculated in terms of mg/g FW with Eq. 8.

$$Y = \frac{M \times T}{W} \quad (8)$$

in which M represents the reading of the spectrophotometer, T represents the volume of toluene used (2 mL), and W represents the weight of the leaf sample (0.5 g).

Morphological traits: Morphological parameters, like fresh and dry root weight and shoots, were weighed with a digital scale with an accuracy of 0.001 g (Sartorius Quintix, Germany) .To determine the dry weights, the harvested samples were dehydrated at 75°C for 48 h.

Statistical analysis: All collected data were analyzed by SAS (Statistical Analysis Software, 9.2). A CRD was conducted to estimate the components of the variance in the simple and interactive effects of salinity stress and seaweed foliar application. Furthermore, the difference between the treatments was evaluated by Duncan's MRT only when the ANOVA F test showed a significance level of 0.05. Pearson's correlation of the traits was checked by SAS 9.4.

Results

Photosynthesizing pigments: The results of analysis of variance (ANOVA) revealed that the simple and interactive effects of salinity stress and seaweed foliar application were significant (P≤ 0.01) on chlorophyll *a* and *b*, total chlorophyll, and carotenoid. According to Table 2, salinity stress reduced these traits, whereas the foliar application of seaweed (at a high rate) increased the photosynthesizing pigments. The highest total chlorophyll content was 4.33 mg/g FW, obtained from the foliar application of seaweed at the rate of 4/1000 with no stress, and the lowest was 1.38 and 1.42 mg/g FW, obtained from the salinity stress levels of 4 and 8 dS/m with no seaweed application, respectively (Figure 1A).

The foliar application of seaweed at the rates of 1/1000, 2/1000, and 4/1000 was related to the highest chlorophyll *a* content (3.10, 2.95, and 3.40 mg/g FW), showing 43.5%, 40.6%, and 48.5% increases versus the control, respectively. The lowest chlorophyll *a* content (1.17 mg/g FW) was related to no seaweed application at the salinity level of 4 dS/m (Figure 1B).

As is seen in Figure 2a, the application of seaweed at the rates of 2/1000 and 4/1000 produced the highest chlorophyll *b* content (0.93 and 0.92 mg/g FW,

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Table 2. The analysis of variance for the effects of seaweed foliar on physiological characteristics of *Mentha × piperita* L. under salinity stress (mean squares).

S.O.V	df	Mean Squres						
		Total chlorophyll	Chlorophyll a	Chlorophyll b	Carotenoids	RWC	Total soluble carbohydrate	Proline
Salinity (S)	3	8.56**	5.26**	0.40**	27.05**	730.17**	0.079^{ns}	0.0000189**
Seaweed (SW)	3	1.87**	1.09**	0.15**	7.34**	112.67^{ns}	0.29*	0.0000064**
S × SW	9	0.48**	0.30**	0.59**	2.82**	243.99^{ns}	0.16*	0.0009641^{ns}
Error	32	0.104	0.084	0.012	0.86	118.47	0.075	0.0006323
CV (%)		14.41	15.66	22.72	19.77	19.02	2.52	3.50

ns, * and **: Not-significant and significant at 5% and 1% probability levels, respectively

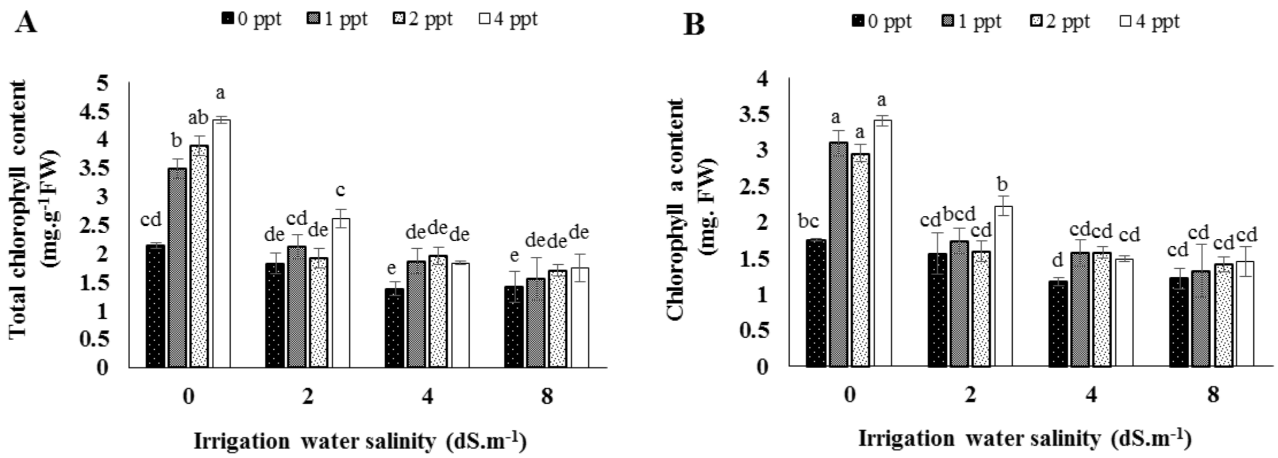

Figure 1. Total chlorophyll (A) and chlorophyll a (B) content of peppermint under salinity stress and seaweed concentrations. Different letters in each factor indicate significant differences at P < 0.05 (Duncan's MRT).

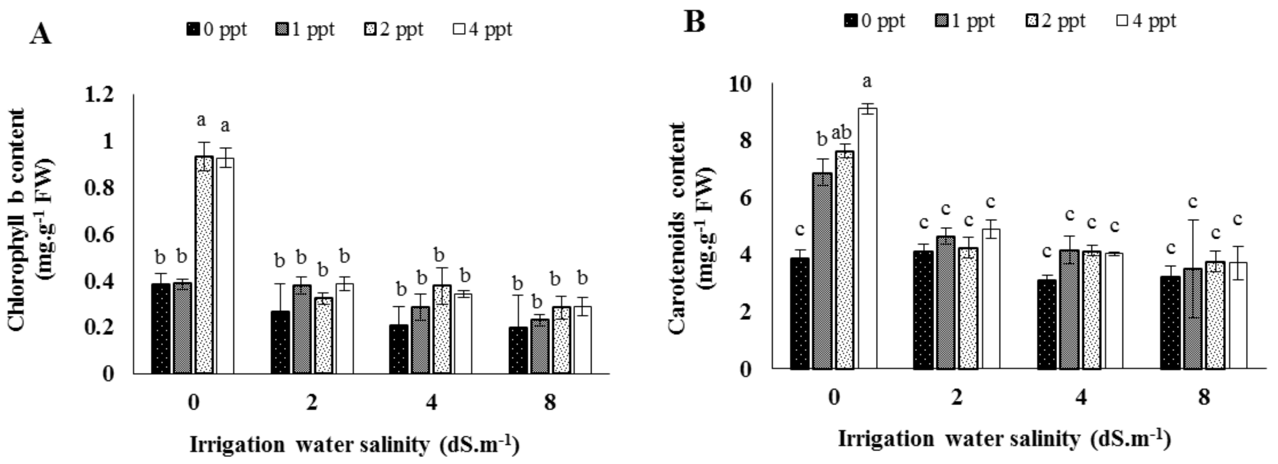

Figure 2. Chlorophyll b (A) and carotenoid (B) content of peppermint under salinity stress and seaweed concentrations. Different letters in each factor indicate significant differences at P < 0.05 (Duncan's MRT).

respectively) when the plants were not exposed to salinity stress. They were 59.1% and 58.6% higher than that of the control, respectively. The lowest chlorophyll *b* content was observed in the application and non-application of seaweed and the saline and non-saline conditions.

The carotenoid variations were similar to the chlorophyll *b* variations. Indeed, the interaction of 4/1000 seaweed and no salinity was related to the highest carotenoid content (9.10 mg/g FW), which was 57.4% higher than that of the control. The lowest carotenoid content was exhibited by the salinity treatments of 2, 4, and 8 dS/m irrespective of the application of seaweed (Figure 2B).

Relative water content (RWC): Based on ANOVA (Table 2), the effect of salinity was significant (P≤0.01) on RWC. However, seaweed and salinity × seaweed had no significant effect on RWC. The highest and lowest RWC were 48.8% and 65.8% related to the salinity levels of 0 and 8 dS/m (Table 2), respectively. An impact of salinity stress is the inhibition of water uptake and drought stress. The decrease in RWC can be

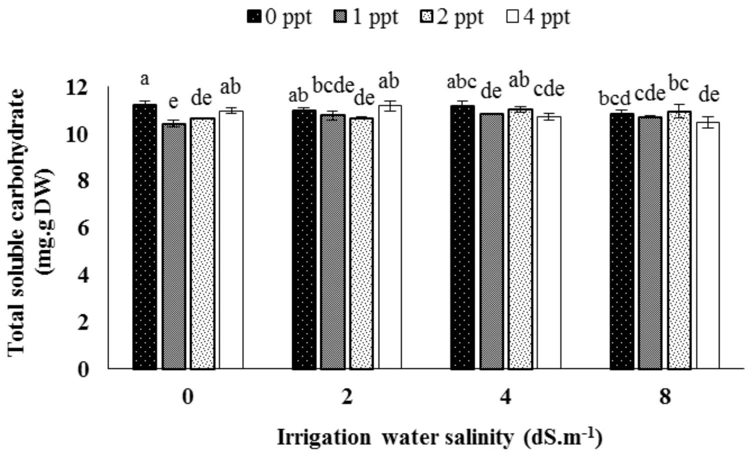

Figure 3. Total soluble carbohydrate content of peppermint under salinity stress and seaweed concentrations. Different letters in each factor indicate significant differences at P < 0.05 (Duncan's MRT).

ascribed to the decrease in water potential and the decrease in water uptake by the roots in drought conditions (Colom and Vazzana, 2003).

Total soluble carbohydrates: As is evident in Table 2, the simple effect of seaweed and the interactive effect of salinity and seaweed were significant (P≤0.05) on total soluble carbohydrates. The highest total soluble carbohydrate content (11.25 mg/g DW) was related to no seaweed application under no salinity stress. The lowest (10.43 mg/g DW) was obtained from the application of seaweed at the rate of 1/1000 and no salinity stress (Figure 3).

Proline: Based on the results, the effect of salinity stress and seaweed foliar application was significant (P ≤ 0.01) on proline content, but their interaction could not influence this trait significantly (Table 2). As the comparison of means revealed (Table 2), the salinity of the irrigation water increased proline content. The highest proline content (0.008 mg/g FW) was obtained from the salinity levels of 4 and 8 dS/m, and the lowest (0.005 mg/g FW) from no salinity level. In addition, the application of 4/1000 seaweed resulted in the highest proline content (0.008 mg/g FW), and the other concentrations of seaweed were all related to the lowest proline content (Table 2).

Morphological traits: The results of analysis of variance showed that seaweed had significant effects on growth and performance traits under salinity stress conditions. Also, the interaction effect of salinity stress and seaweed on the fresh and dry weight of leaves, aerial parts, total fresh and dry biomass, and harvest index was significant at the level and had no significant effect on the fresh and dry weight of roots. (Table 3).

Fresh and dry root weight: As summarized in Table 3, salinity stress and application of seaweed to the leaves were significant for fresh and dry root weight (P≤0.01). However, interaction effects of salinity and seaweed were not significant for these traits. The salinity was caused by decreasing fresh and dry weight. The highest root fresh weight was related to 0 and 2 $dS.m^{-1}$ (63.08 and 58.58 $g.plant^{-1}$), whereas the highest root dry weight was achieved without salinity conditions (21.09 $g.plant^{-1}$). Severe salinity stress (8 $dS.m^{-1}$) reduced the mean fresh and dry weight of roots by 37.25 and 48.74%, respectively, compared to the control treatment (Table 3). Applying seaweed extract to the leaves resulted in fresh and dry root weight. The most effective concentration of the seaweed extract was 2 ppt, resulting in the highest fresh and dry root weight (81.04 and 22.8 $g.plant^{-1}$). The lowest mean fresh and dry root weight was associated with not using the extract (Table 3).

Fresh and dry leaf weight: As shown in Table 3, the effects of salt stress, seaweed application, and salinity-seaweed interaction effects were significant on fresh and dry leaf weight (P≤0.01). Salinity stress was reduced; however, application of seaweed extract to the leaves increased the fresh and dry weight of the leaves.
As shown in Figure 4A and Figure 4B, the highest leaf fresh (23.6 and 24.7 $g.plant^{-1}$) and dry weight (10.3 and 11.5 $g.plant^{-1}$) were related to 2 and 4 ppt concentrations of seaweed application under non-stress conditions. Under high salinity, application of 2 ppt seaweed kelp increased leaf fresh and dry weight by 80.8% and 94.4%, respectively. The non-application of seaweed under the high salinity (8 $dS.m^{-1}$) was caused by the lowest fresh and dry weight of the leaves (3.05 and 0.44 $g.plant^{-1}$).

Fresh and dry aerial weight: The results represented the significant effect of salinity stress, foliar application of seaweed, and salinity stress × seaweed application on aerial fresh and dry weight (P≤0.01). The results showed that a low salinity level did not significantly reduce the mean aerial fresh and dry weight (Table 3). Results showed that seaweed application increased fresh and dry aerial weight under stress and non-stress conditions. Seaweed application (2 and 4 ppt values) under non-stress conditions showed the highest aerial fresh and dry weight. Application of 2 ppt seaweed under high salinity conditions (8 $dS.m^{-1}$) showed 66.2% and 75.8% increasing aerial fresh and dry weight, respectively, compared to no seaweed with

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Table 3. **The analysis of variance for the effects of seaweed foliar on growth and yield attributes of *Mentha x piperita* L. under salinity stress (mean squares)**

S.O.V	df	Mean Squres							
		Leaf fresh (g)	Leaf dry (g)	Aerial fresh (g)	Aerial dry (g)	Root fresh (g)	Root dry (g)	Biomass fresh (g)	Biomass dry (g)
Salinity (S)	3	217.4**	38.16**	302.92**	60.0*	1467.84**	228.42**	4927.24**	824.32**
Seaweed (SW)	3	217.6**	58.37**	461.37**	108.15**	7299.53**	400.00**	14311.26**	1332.44**
S × SW	9	62.63**	14.29**	112.51**	30.29**	83.99ns	6.78ns	537.39**	94.98**
Error	32	10.94	2.35	22.92	4.64	73.96	14.87	155.66	28.37
CV (%)		24.48	26.25	21.32	22.62	16.66	25.22	14.24	17.36

ns, * and **: Not-significant and significant at 5% and 1% probability levels, respectively

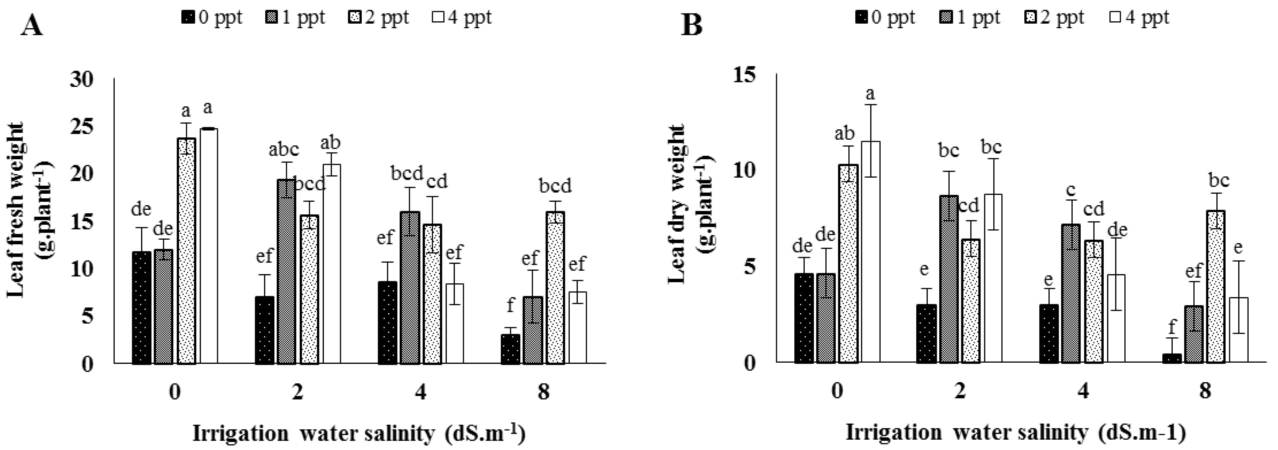

Figure 4. Leaf fresh weight (A) and leaf dry weight (B) of peppermint under salt stress and seaweed concentrations. Different letters in each factor indicate significant differences at P ≤0.05 (Duncan's MRI).

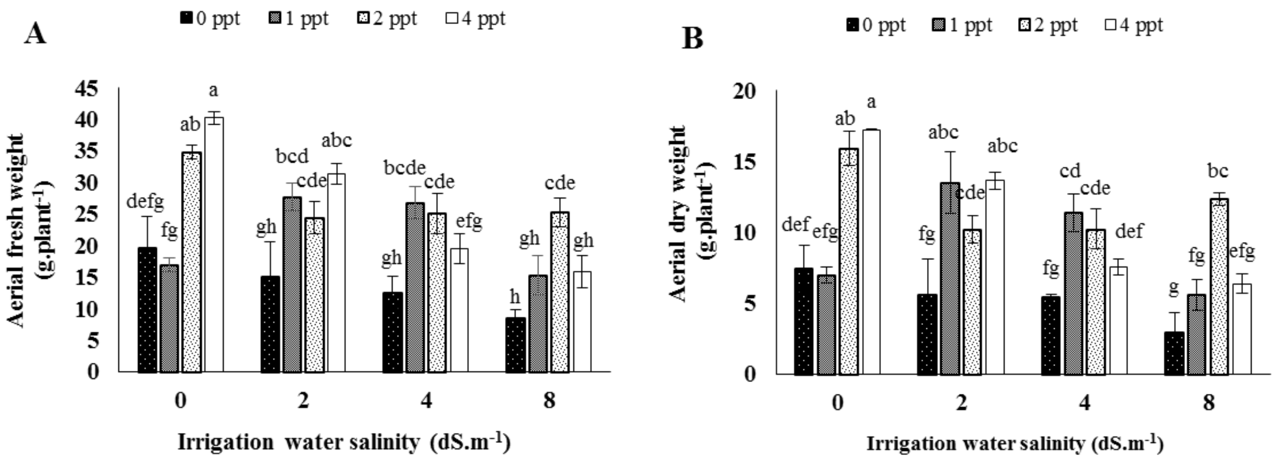

Figure 5. Aerial fresh weight (A) and aerial dry weight (B) of peppermint under salt stress and seaweed concentrations. Different letters in each factor indicate significant differences at P≤0.05 (Duncan's MRI).

no application at the same salinity. The lowest fresh and dry aerial weights (8.52 and 2.98 g.plant^{-1}) were associated with non-application of seaweed under high salinity stress (Figure 5A and Figure 5B).

Fresh and dry biomass weight: The results represented the significant effect of salinity stress, foliar application of seaweed, and salinity stress × seaweed application on biomass fresh and dry weight (P≤0.01) (Table 3). Seaweed extract resulted in an increase in fresh and dry biomass weight under salt stress and non-salt stress conditions. As shown in Figure 6, the highest biomass fresh and dry weights were associated with the application of 2 ppt of seaweed under non-stress conditions (124.9 and 41.97 g.plant^{-1}, respectively). Application of 2 ppt seaweed extract under high salinity conditions (8 dS.m^{-1}) resulted in 79.6 and 80.2% increases in fresh and dry biomass weight compared to no application of seaweed. The lowest means of these traits (20.02 and 6.4 g.plant^{-1}) were related to the non-application of seaweed at high salinity (8 dS.m^{-1}).

Correlation coefficients: Table 4 presents the results of Pearson's correlation test between the

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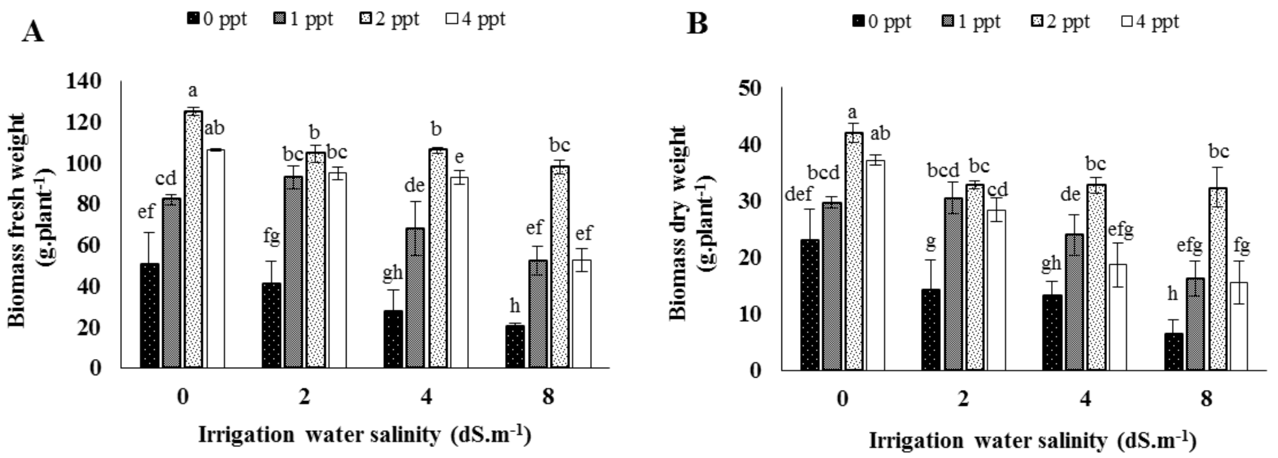

Figure 6. Fresh biomass weight (A) and dry biomass weight (B) of peppermint under salt stress and seaweed concentrations. Different letters in each factor indicate significant differences at P ≤0.05 (Duncan's MRI).

Table 4. Correlation coefficients of physiological traits of the seaweed foliar application under the salt stress

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) total chlorophyll	1						
(2) chlorophyll a	0.98**	1					
(3) chlorophyll b	0.96**	0.89**	1				
(4) carotenoids	0.61**	0.73**	0.38	1			
(5) RWC	0.27	0.36	0.11	0.39	1		
(6) total soluble carbohydrate	-0.15	-0.16	-0.12	-0.19	-0.07	1	
(7) proline	-0.56**	-0.56*	-0.52**	-0.25	-0.62**	-0.07	1

*** P<0.05 and ** P<0.01**

Table 5. Correlation coefficients of morphological traits of the seaweed foliar application under the salt stress

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Fresh leaf weight	1							
(2) Dry leaf weigh	0.99**	1						
(3) Fresh aerial weight	0.97**	0.99**	1					
(4) Dry aerial weight	0.98**	0.99**	0.99**	1				
(5) Fresh root weight	0.76**	0.77**	0.75**	0.75**	1			
(6) Dry root weight	0.74**	0.73**	0.71**	0.7**	0.95**	1		
(7) Fresh biomass weight	0.9**	0.91**	0.89**	0.9**	0.97**	0.92**	1	
(8) Dry biomass weig	0.94**	0.94**	0.93**	0.93**	0.92**	0.92**	0.98**	1

*** P<0.05 and ** P<0.01**

physiological traits. Accordingly, total chlorophyll exhibited strong positive significant (P≤ 0.01) correlations with chlorophyll *a*, chlorophyll *b*, and carotenoids with correlation coefficients of 0.61, 0.96, and 0.98, respectively. Proline was also correlated with the physiological traits of total chlorophyll, chlorophyll *a* and *b*, and RWC negatively and significantly (P≤ 0.01) with correlation coefficients of 0.62, 0.52, and 0.56, respectively. Also, there were positive significant (P≤ 0.01) correlations between chlorophyll *a* and chlorophyll *b* (0.89) and between chlorophyll *a* and carotenoids (0.73). Soluble sugars had no significant relationship with the recorded traits. Similarly, RWC has significant correlations with no trait except for proline content. Under the seaweed foliar application, all the morphological traits were positively correlated, and coefficients correlated were significantly affected (P<0.01) (Table 5).

Discussion

The negative effects of Salinity on the biochemical traits of *Mentha* × *piperita* L. were amended by adding seaweed liquid. Our results revealed that salinity reduced photosynthesizing pigments, including chlorophyll *a* and *b*, total chlorophyll, and carotenoids, significantly. Yasamani Masouleh *et al*. (2020), also,

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reported a decrease in chlorophyll *a*, *b*, and total under salinity stress. With the increase in salt concentration and the decrease in water potential, water uptake decreased in peppermints, so the plants could not preserve the water in their leaves, resulting in their deterioration.

Hegazi *et al*. (2015) reported that the application of seaweed extract under salinity stress reduced the adverse effects of salinity stress on eggplants. Salinity stress increased the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), as well as sodium content, and decreased potassium content in the eggplant buds and fruits. In general, the application of seaweed alone or in combination with different levels of salinity stress slightly increased SOD, APX, and potassium content significantly.

In a study on the effect of seaweed extract on the physiological traits and nutritional quality of spinach in drought conditions, Xu and Leskovar (2015) revealed that seaweed extract did not affect on the growth of spinach in full irrigation conditions. However, seaweed improved the growth of spinach in stressful conditions by increasing water relations and reducing stomatal opening.

Compatible solutes, which are known as osmolytes, are a group of diverse chemical organic compounds that are polar and water-soluble and do not interfere with cell metabolism even at high concentrations. These osmoprotectants protect plants against stresses by inhibiting reactive oxygen species (ROS), protecting membrane structure, and stabilizing enzymes and proteins (Hayat *et al*., 2012). These compounds mainly include glycine betaine and sugars. Proline increases in plants exposed to salinity. It acts as an osmoprotectant (Wang, 2017). According to Shahverdi *et al*. (2019), the main factors that contribute to salinity resistance include proline accumulation, catalase activity, and photosystem efficiency. As well, soluble sugars, e.g., sucrose, glucose, and fructose, are closely associated with stress tolerance in plants (Yasamani Masouleh *et al*., 2020). These findings are consistent with Yildiztekin *et al*. (2018) who observed that pepper seedlings exposed to salinity showed an elevated level of proline whereas the use of seaweed extract reduced their proline content significantly.

The concentration of total soluble sugars was increased in salinity stress, which agrees with Manaf (2016) and (Sodaeizadeh *et al*., 2016) who reported that total soluble carbohydrates increased in cowpea plants exposed to salinity significantly and the use of seaweed improved these conditions. Also, Elansary *et al*. (2017) state that the accumulation of carbohydrates is a common mechanism for the stress tolerance of grass, which is related to osmotic adjustment in drought conditions.

Dehnadi *et al*. (2020) found that the increased level of salinity in different treatments increased antioxidant enzymes in tomatoes. According to Taheri *et al*. (2020), salinity stress inhibits plant growth, they can change physiological features and limit crop production.

In the present research, the application of seaweed at the rates of 2/1000 and 4/1000 alleviated salinity effects. Increases were observed in chlorophyll *a*, *b*, and total at salinity levels of 4 and 8 dS/m. According to Kasim *et al*. (2016), seaweeds are a rich potential source of biologically active compounds, and their application can be a good way to reduce salinity stress by inducing several secondary metabolites and the synthesis of new proteins. Rouphael *et al*. (2017) reported that the application of seaweed extract increased photosynthesizing pigments in zucchini squash plants exposed to salinity stress. According to Battacharyya *et al*. (2015), brown seaweed extract is widely used in horticulture for its growth-stimulating effects and its impact on improving crop tolerance of abiotic stresses like salinity, extreme temperatures, nutrient deficiency, and drought. The chemical compounds of seaweed extract include complicated polysaccharides, fatty acids, vitamins, phytohormones, and mineral nutrients. Seaweed extract changes the physical, biochemical, and biological properties of soil. It may also affect root shape and facilitate the effective uptake of nutrients. In general, the use of seaweed extract for improving soil quality may be a potential way for soil recovery or facilitation of more phytoremediation.

Growth and yield attributes of *Mentha × piperita* L. were decreased with the increasing EC. According to Hammam and Awad Alla (2020), on the other side, plant height, plant fresh and dry weights, root length, and root fresh and dry weights of *Vicia pannonica* Crantz. (Mustafa *et al*., 2022) decreased significantly with increasing NaCl.

In a previous study (Ghafarizadeh *et al.*, 2017), exploring the effects of seaweed liquid on the growth of *Triticum aestivum* without salt stress, plant height, root length, dry weights of the plant and roots increased to their highest levels up to 10% seaweed concentration.

It was figured out that seaweed liquid has an alleviating effect in opposition to the harmful effects of salt stress in all explored traits. Likewise, seaweed liquid alleviated the negative effects of salt stress on the growth traits of *Echinacea purpurea* L. (Kara *et al*., 2019), on the growth, photosynthetic and biochemical traits of *Lycopersicon esculentum* L. (Vinoth *et al*., 2017; Awaad, 2021), on the biochemical traits of *Medicago sativa* L. (El-Sharkawy *et al.*, 2017), on the growth traits of *Triticum aestivum* (Hamouda *et al*., 2023), *Solanum lycopersicum* L. (Popescu, 2020) and *Solanum lycopersicum* L. cv. “Rio Fuego” (Hernandez-Herrera *et al*., 2022), on the growth and the amount of essential oil *of Lippia citriodora* (Hakimzadeh Ardakani *et al*., 2023), on the growth and photosynthetic traits of *Pisum sativum* L. (Hamouda *et al*., 2023). Furthermore, plant height, root fresh and dry weights, and total chlorophyll of *L. officinalis* gradually increased under salt stress when the concentration of nano-micronutrients increased (Abdelsadek *et al*., 2022). In contrast to these positive results, Popescu (2020) found

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that seaweed liquid had no alleviating effect on the growth traits of *Cucumis sativus* under salt stress

Conclusion

Soil and water salinity is a grave problem and important environmental stress, especially in arid and semi-arid regions, which reduces crop yields and production. Various solutions have been proposed for increasing salinity tolerance and increasing or stabilizing plant growth and yields in these conditions. One solution is to use biofertilizers. *Mentha* × *piperita* L.was considerably negatively affected by salt stress in terms of biochemical traits. According to the results of this empirical research, the use of seaweed has a positive and increasing effect on plant growth and yields. The results showed that salinity, particularly at high rates (4 and 8 dS/m), decreased chlorophyll *a*, *b*, and total, as well as carotenoids and RWC. Proline was increased with the increase in salt concentration in the growth medium. The foliar application of seaweed increased the yield of the peppermints. Considering all studied traits, the best seaweed concentration was found to be 2/1000. Although 4/1000 was also satisfactory, the rate of 2/1000 is preferred given the cost and other factors. Seaweed improved soluble sugars and amino acid content, thereby increasing plant resistance to salinity stress. In general, the results revealed that the application of seaweed extract to peppermint (*Mentha piperita* L.) exposed to salinity conditions would improve its physiological traits and salt tolerance.

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