



## Performance of Peppermint (*Mentha piperita* L.) in Different Water Deficit and Salinity Management

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### ABSTRACT

Two separate experiments were conducted to investigate the performance of peppermint (*Mentha piperita* L.) in different water deficit and salinity management in 2017 and 2018, a randomized complete block design factorial experiment with three replications was designed in a lysimeter station in the Department of Water Engineering, Campus Agriculture, and Natural Resources of Razi University, Iran. The experiment included three levels of irrigation: 100%, 80% and 60%, and four salinity levels: (control), 2, 3 and 4 dS/m. The results indicated that the water deficit stress effect on aerial characteristics (leaf fresh weight, shoot fresh weight, leaf area, shoot height) and underground organs (root dry weight, root volume, root area, and root length), was significant. The irrigation water salinity effect in two years of the experiment was significant on leaf wet and dry weight, shoot wet and dry weight, leaf area and root dry weight, root volume, root area and root length. The mean comparison test revealed that the effect of deficit irrigation on shoot wet and dry weight was not significant between the 80% and 60% water requirement treatments. For 100% and 60% of irrigation requirements, the maximum and minimum leaf dry weight was observed at 2.47 and 1.54 g/plant in 2017. The mean comparison test result for salinity on underground organs showed that the highest root length in two years was observed in the control treatment and the lowest value of this parameter was observed in the 4 dS/m treatment. In general, water deficit and salinity had a negative effect on peppermint yield during two years of experiments. Therefore, it is not recommended to apply water stress and use water with a salinity of more than 2 ds/m to achieve the maximum yield.

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### 1. Introduction

The growth and development of crops and food production would be limited due to drought (Akhtar *et al.*, 2021). Water plays an important role in crop growth and production, and also it is a determinant of species distribution and evolution (Bhandari *et al.*, 2019; Lalarukh *et al.*, 2022). Many medicinal plants' growth and development are also impacted by the supply of moisture. However, despite the limitation in water resources, it is necessary to adopt strategies to increase the efficiency of water consumption and also to improve the effective substances of medicinal plants (Jamali *et al.*, 2020). Water stress in medicinal plants impacts plant growth and led to a lack of productivity

(Esmailpour *et al.*, 2013). The numerous biotic environmental stresses limited the development and yield of plants worldwide (Hassanisaadi *et al.*, 2022). Salt stress is one of the abiotic stresses that cause much damage to plants. (Aghighi Shahverdi *et al.*, 2018). In addition to reducing crop yield, salinity stress affects the plant's metabolic processes through disruption of cellular water potential and ionic toxicity and disrupts membrane function and absorption of mineral nutrients. (Taghizadeh-Mehrjardi *et al.*, 2021).

Peppermint (*Mentha piperita* L.) is an herbaceous, perennial, rhizomatous, and hybrid plant belonging to Lamiaceae. This plant is a cross between *M. aquatic* and *M. spicata* species. This herb is a well-known herb

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that is commonly used throughout the world in teas, flavorings, and confections (Malekmohammad *et al.*, 2021).

In various researches which have been carried out, the application of drought and salinity stresses have been mentioned as limiting factors for aromatic and medicinal plants from the mint family. The salinity and water stress effects on peppermint medicinal plants and other medicinal plants have been discussed in detail below. Investigating drought stress effects on the morphological traits of peppermint indicated that deficit irrigation led to a decrease in plant traits such as plant height, fresh weight and dry weight (Kheiry *et al.*, 2017).

The research was conducted under the greenhouse peppermint cultivation conditions in Brazil and applying treatments consisting of electric conductivity combined with irrigation water ( $EC_w$ ) of 1.0, 2.0, 3.0, 4.0, and 5.0  $dS\ m^{-1}$  in the presence and absence of bovine biofertilizer, it was reported that the increase in electrical conductivity of the irrigation water levels from 1  $dS\ m^{-1}$  reduced the growth, development, and production of peppermint biomass. Peppermint plants that received bovine biofertilizer had superior results in growth and biomass production, and the application of bovine biofertilizer attenuated the effects of salty peppermint (Veras *et al.*, 2017).

In research conducted on the peppermint plant, reported that the effect of water deficit stress led to a decrease in shoot fresh and dry weight, leaf fresh and dry weight, leaf area, and shoot height; also, the application of water irrigation salinity caused the reduction of the above-mentioned traits. The effects of deficit irrigation and salinity also affected the underground organs of the plant so that it decreased root dry weight, root volume, root length, root area and root density (Basiri *et al.*, 2020).

Research has shown that water stress reduces peppermint growth, but also increases factors such as antioxidant capacity and the amount of phenols and plant pigments (Chrysargyris *et al.*, 2021). The research's result aimed at investigating the effect of urea fertilizer and vermicompost under three irrigation levels (no stress, moderate, and severe drought stress) on the phytochemical properties of peppermint oil showed that the highest leaf area index and dry matter weight were observed in control irrigation, while the maximum amount of essential oil occurred under mild

water deficit stress and the response of the plant in the parameters of dry matter weight and essential oil content was positive in the increase of vermicompost fertilizer (Keshavarz-Mirzamohammadi *et al.*, 2021). The result of deficit irrigation effects on rosemary indicated that applying water stress to the extent of 50% of the water requirement caused a decrease in all plant parameters, essential oil percentage and dry matter. But providing 75% of the rosemary plant's water requirement caused an acceptable performance in dry matter production (Ghamarnia *et al.*, 2022).

Iran is one of the best regions in the world for the cultivation of medicinal plants in terms of climate and geographical location (Hakimzadeh *et al.*, 2023). But, a large part of the country is affected by salinity and water stress (Hakimzadeh and Vahdati, 2018). Consequently, the present research was conducted to investigate the effect of irrigation water salinity and water stress on peppermint plant and their aerial and underground characteristics, in order to improve irrigation management for this product.

## 2. Materials and methods

These studies were conducted in two separate experiments, one to study the effect of deficit irrigation and the other to study the salinity of irrigation water on peppermint by lysimeter (45 cm diameter and 100 cm height) at the lysimeter research station (longitude of  $47^{\circ}\ 9'$  east and latitude of  $34^{\circ}\ 21'$  north and altitude 1319 meters above sea level) of the agriculture faculty of Razi University, Iran in 2017 and 2018 (Fig. 1). Each experiment was carried out in a randomized complete block design with three replications. During the deficit irrigation experiment, the investigated treatments included three control levels (full irrigation or 100% water requirement), 20% deficit irrigation (80% water requirement) and 40% deficit irrigation (60% water requirement). The investigated treatments in the irrigation water salinity test included four levels: 0.9 (control), 2, 3 and 4  $dS/m$ . Furthermore, different salinity levels were created using pure NaCl and  $CaCl_2$  salt each with a 50% of combination.

A fully automatic meteorological station was located fifty meters from the test site to receive daily meteorological data. Furthermore, to calculate the water requirement, the single plant coefficients of peppermint were used, which were reported as 0.69, 1.03, and 1.27 in the initial, development, and middle

growth stages, respectively (Ghamarnia and Mousabeygi, 2014). Which is presented in Fig. 2. Table

1 provides the meteorological parameters during two years of research.



Figure 1. The lysimetric research station in the Faculty of Agriculture and Natural Resources, Razi University, Kermanshah, Iran.

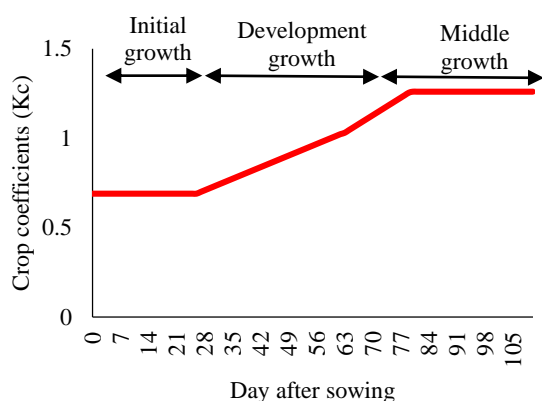


Figure 2. Single plant coefficients of peppermint in different growth stages were used in both growth seasons (Ghamarnia and Mousabeygi, 2014).

Table 1. Meteorological parameters for 2017 and 2018.

Year	Month	Mean temperature (°C)	Mean relative humidity (%)	Mean wind speed (m/s)	Total precipitation (mm)	Radiation (W/m <sup>2</sup> )
2017	June	24.31	30.00	1.25	0	316.82
	July	29.61	18.61	1.02	0	325.69
	August	29.76	21.04	1.10	0	282.68
	September	27.29	19.82	0.99	0	268.15
2018	June	26.33	25.51	1.06	0.02	339.22
	July	28.86	19.63	1.04	0	340.84
	August	29.47	22.3	0.91	0	295.34
	September	25.82	23.66	1.14	0	267.96

In this study using the Penman-Monteith equation (Eq. 1), reference plant evapotranspiration (ET<sub>o</sub>) and using (Eq. 2), actual plant evapotranspiration (ET<sub>c</sub>) were obtained.

$$ET_o = \frac{0.408(R_n - G) + \gamma \frac{900}{T_a + 273} U_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34U_2)} \quad (1)$$

In this equation ET<sub>o</sub> is reference evapotranspiration (mm/day), R<sub>n</sub> is net radiation at the crop surface (MJ m<sup>-2</sup> day<sup>-1</sup>), G is soil heat flux density (MJ/m<sup>2</sup>/day), T is the mean daily air temperature at 2 m height (°C), u<sub>2</sub> is the wind speed at 2 m height (m/s), e<sub>s</sub> is saturation vapor pressure (kPa), e<sub>a</sub> is actual vapor pressure (kPa), e<sub>s</sub> - e<sub>a</sub> is saturation vapor pressure deficit (kPa), Δ is slope vapor pressure curve (kPa/°C), and g<sub>s</sub> is psychrometric constant (kPa/°C) (Allen et al., 1998).

$$ET_c = ET_o \times K_c \quad (2)$$

K<sub>c</sub>: plant coefficient, ET<sub>c</sub>: the actual plant evapotranspiration (mm) (Allen et al., 1998).

This research used surface and direct irrigation in cultivated lysimeters, and according to the controlled conditions of the project and the lack of drainage, the irrigation efficiency was considered 100%. Table 2 represents the cumulative water consumption of the cultivation months.

**Table 2. Water requirement of Peppermint during the growing months.**

Year	Month	Irrigation level (mm)		
		T100	T80	T60
2017	June	63.8	51.04	38.28
	July	100.46	80.37	60.28
	August	225.81	180.65	135.49
	September	195.69	156.55	117.41
2018	June	70.06	56.05	42.04
	July	143.93	115.14	86.36
	August	237.77	190.22	142.66
	September	165.29	132.23	99.17
Total water requirement (mm)		601.4	481.12	360.84

The soil physical and chemical characteristics of the study area are presented in Tables 3 and 4. In 2017 and 2018, planting operations were carried out on June 10 and considering a cultivation distance of 10 cm per lysimeter, 10 rhizomes were cultivated at a depth of 4 cm, and after 110 days were harvested on September 28<sup>th</sup>.

**Table 3. The soil physical characteristics of the study area.**

Year	Soil depth (cm)	Bulk density (g/cm <sup>3</sup> )	Soil Texture	Sand (%)	Silt (%)	Clay (%)
2017	25-0	1.3	Silty Clay	8.7	46.9	44.4
2018	25-0	1.3	Silty Clay	8.1	49.3	42.6

To meet the fertilizer requirement of the peppermint plant based on soil analysis (Table 4), urea fertilizer (147 kg/ha) and vermicompost fertilizer (14.22 kg/ha) were used in this research.

**Table 4. The soil Chemical characteristics of the study area.**

Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	OC (%)	K (ppm)	P (ppm)	EC (μmols/cm)	pH
1.64	1.36	11.9	7.8	1.38	440	26	1.2	7.3

**Table 5. The water chemical and physical characteristics of the study area.**

TDS (mg/lit)	pH	EC (μmols/cm)	SAR (%)	Na <sup>+</sup>	Mg <sup>2+</sup> Ca <sup>2+</sup>	SO <sub>4</sub> (meq/lit)	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub>
640	7.1	1000	0.54	1.08	8.15	1.18	1.9	6.15	0.0

The measurement parameters included two parts as aerial characteristics and underground plant organs. The aerial characteristics included: shoot wet weight, shoot dry weight, leaf wet weight, leaf dry weight, leaf area and shoot height and the underground plant organs included: root dry weight, root volume, root area, root length and root density. The shoot length was measured with a tape measure. At the end of the growth period, the different parts of the aerial characteristics of the peppermint were separated from the plant and their wet weight was measured with a digital scale with an accuracy of 0.001 grams. The root and leaf dry weight was also measured by placing them in an oven at 75 °C for 72 hours. The leaf area was measured using a scanner and Digimizer software, and the root length and area were determined using the Tennant and Atkinson methods, respectively (Alizadeh, 2005). To analyze the variance and the comparison means test, SAS software (version 9.4) was used, and to compare the means of the treatments, the LSD test was used at the 5% level probability. Finally, graphs were drawn using Excel software.

### 3. Results

The analysis variance of deficit irrigation treatment's effect on peppermint aerial characteristics is represented in Table 6.

**Table 6. Variance analysis of water requirement treatment's effect on the peppermint aerial characteristics.**

Year	Source of variation	Degree of freedom	Mean squares					
			Fresh leaf weight	Leaf dry weight	Fresh shoot weight	Shoot dry weight	Leaf area	Shoot height
2017	Replication	2	0.509 <sup>ns</sup>	0.013 <sup>ns</sup>	0.406 <sup>ns</sup>	0.039 <sup>ns</sup>	62.837 <sup>ns</sup>	0.173 <sup>ns</sup>
	Deficit treatment	2	16.104 <sup>**</sup>	0.712 <sup>**</sup>	16.016 <sup>**</sup>	0.233 <sup>*</sup>	19770.11 <sup>**</sup>	148.34 <sup>**</sup>
	Error	4	0.371	0.016	0.297	0.018	110.19	0.602
	CV (%)	-	7.45	6.23	7.11	8.69	2.58	2.81
2018	Replication	2	0.044 <sup>ns</sup>	0.001 <sup>ns</sup>	0.312 <sup>ns</sup>	0.024 <sup>ns</sup>	137.291 <sup>ns</sup>	0.170 <sup>ns</sup>
	Deficit treatment	2	22.640 <sup>**</sup>	0.078 <sup>*</sup>	16.922 <sup>**</sup>	0.175 <sup>*</sup>	55274.21 <sup>**</sup>	88.501 <sup>**</sup>
	Error	4	0.171	0.012	0.369	0.013	603.09	1.048
	CV (%)	-	4.85	5.18	9.41	10.84	7.14	4.02

\*: Significant at 5% level, \*\*: Significant at 1% level and ns: No significant.

Based on the results, deficit irrigation treatment's effect on leaf wet weight, shoot wet weight, leaf area and shoot height was significant at 1% level in 2017 and 2018. In addition, the leaf dry weight was significant at the levels of one and five percent in 2017 and 2018, respectively.

Fig. 3 shows the deficit irrigation average comparison of the peppermint aerial characteristics. Means compared results showed that in both years, the maximum and minimum amount of leaf dry weight,

leaf wet weight, shoot dry weight, shoot wet weight, shoot height and leaf area was observed in the treatment with 100 and 60% water requirement, respectively. The minimum leaf area amount (206.64 cm<sup>2</sup>) was detected in the 60% water requirement treatment for 2017. There was no significant difference between the dry shoot weight between the treatments of 80% and 60% of water requirement in 2017 and 2018 (Fig. 3). It should be noted that the irrigation treatments in 2017 and 2018 have been statistically analyzed separately.

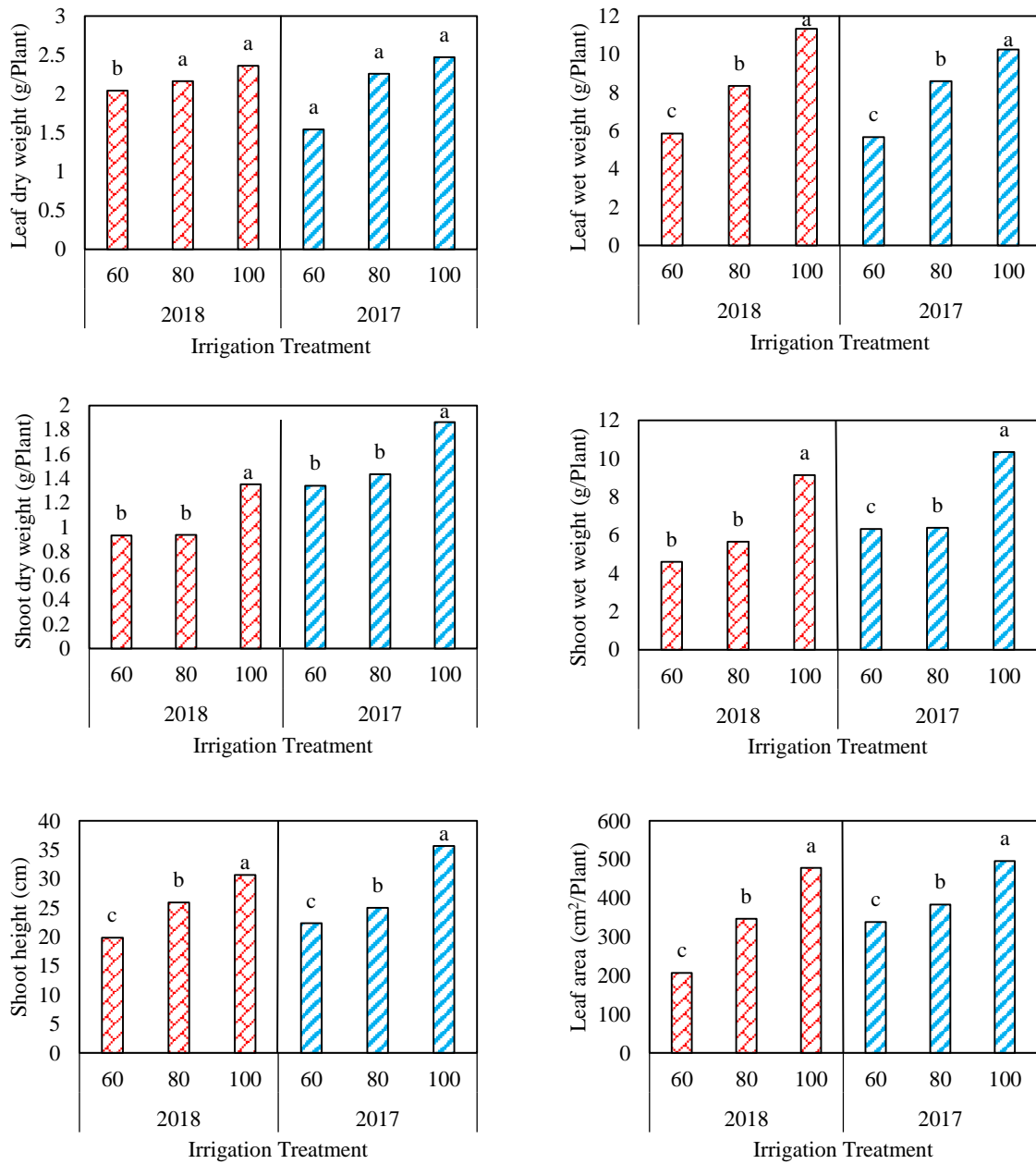


Figure 3. Deficit irrigation average comparison on the peppermint aerial characteristics.

### 3.1. The effect of salinity treatments on the peppermint aerial characteristics

Table 7 represents the variance analysis of the salinity treatment's effect on the peppermint aerial characteristics during two years of the experiment. The

mentioned table indicated that the salinity treatment effect on wet and dry leaf weight, wet and dry shoot weight, and leaf area was significant at a 1% level. Over 2017 and 2018, the shoot height was also significant, at the level of 1 and 5%, respectively.

**Table 7. Variance analysis of the salinity treatments on the peppermint aerial characteristics.**

Year	Source of variation	Degree of freedom	Mean squares					
			Fresh leaf weight	Leaf dry weight	Fresh shoot weight	Shoot dry weight	Leaf area	Shoot height
2017	Replication	2	0.096 <sup>ns</sup>	0.121 <sup>*</sup>	0.279 <sup>ns</sup>	0.031 <sup>ns</sup>	1135.51 <sup>ns</sup>	1.85 <sup>ns</sup>
	Salinity treatment	3	6.737 <sup>**</sup>	0.348 <sup>**</sup>	17.193 <sup>**</sup>	0.377 <sup>**</sup>	28442.21 <sup>**</sup>	24.82 <sup>**</sup>
	Error	6	0.479	0.018	0.389	0.035	131.99	0.883
	CV (%)	-	7.81	6.29	8.45	13	2.93	2.99
2018	Replication	2	0.277 <sup>ns</sup>	0.128 <sup>ns</sup>	0.6332 <sup>ns</sup>	0.0225 <sup>ns</sup>	390.91 <sup>ns</sup>	3.251 <sup>ns</sup>
	Salinity treatment	3	20.874 <sup>**</sup>	0.881 <sup>**</sup>	22.202 <sup>**</sup>	0.334 <sup>**</sup>	38912.82 <sup>**</sup>	16.055 <sup>*</sup>
	Error	6	0.338	0.055	0.477	0.012	260.21	3.209
	CV (%)	-	6.65	12.47	12.61	12.58	4.47	6.44

\*: Significant at 5% level, \*\*: Significant at 1% level and ns: No significant.

Fig. 4 shows the average comparison of salinity on peppermint aerial characteristics in two years of the experiment. It should be noted that the salinity treatments in 2017 and 2018 have been statistically analyzed separately. During 2017, the maximum leaf dry weight, leaf wet weight, shoot dry weight, shoot wet weight, shoot height and leaf area, were, 2.47, 10.25, 1.86, 10.34 (g/plant), 35.64 (cm), and 495.79 (cm<sup>2</sup>/plant), and was related to the control treatment respectively. Furthermore, the minimum values were 1.67, 6.76, 1.0, 4.62 (g/plant), 29.23 (cm), and 261.48 (cm<sup>2</sup>/plant) were related to the 4 dS/m treatment. This trend was repeated during 2018, with the maximum leaf dry weight, leaf wet weight, shoot dry weight, shoot wet weight, shoot height and leaf area, values were respectively, 2.36, 11.34, 1.35, 9.13 (g/plant), 30.66 (cm), and 478.09 (cm<sup>2</sup>/plant), being related to the control treatments, and the minimum values were respectively, 1.40, 6.21, 0.61, 3.22 (g/plant), 25.19 (cm), and 230.0 (cm<sup>2</sup>/plant), being related to the 4 dS/m treatment.

### 3.2. The deficit irrigation treatment's effect on peppermint underground organs

The analysis variance of the deficit irrigation treatment's effect on peppermint underground organs characteristics is represented in Table 8. According to the mentioned table, deficit irrigation had a significant effect on root dry weight, root volume, root length, and root area in 2017 and 2018. However, root density was

not affected by water stress over 2017, and had no significant effect.

Table 9 illustrates the average comparison effect of deficit irrigation treatments on peppermint underground organs. Considering both years' results, there was no significant difference in the plant root length between the 80% and 60% water requirement treatments. The peppermint underground characteristics of 2017 in the control irrigation treatment had higher performance than other irrigation treatments and had a significant difference at the 1% level. Moreover, except for the root density, other traits with better performance were placed in a different statistical group in 2018.

### 3.3. The effect of salinity treatments on peppermint underground organs

Table 10 represents the variance analysis of the salinity treatment's effect on the peppermint underground organs characteristics in two years of the experiment. According to the results, the salinity stress effect in two years on all underground characteristics except for the root density was significant at the level of 1%. The root density in 2017 had no significant difference and in 2018 there was a significant at 1% level. According to the mean comparison result, the highest root length in two years was observed for the control treatment, and the lowest value was observed for the 4 dS/m treatment.

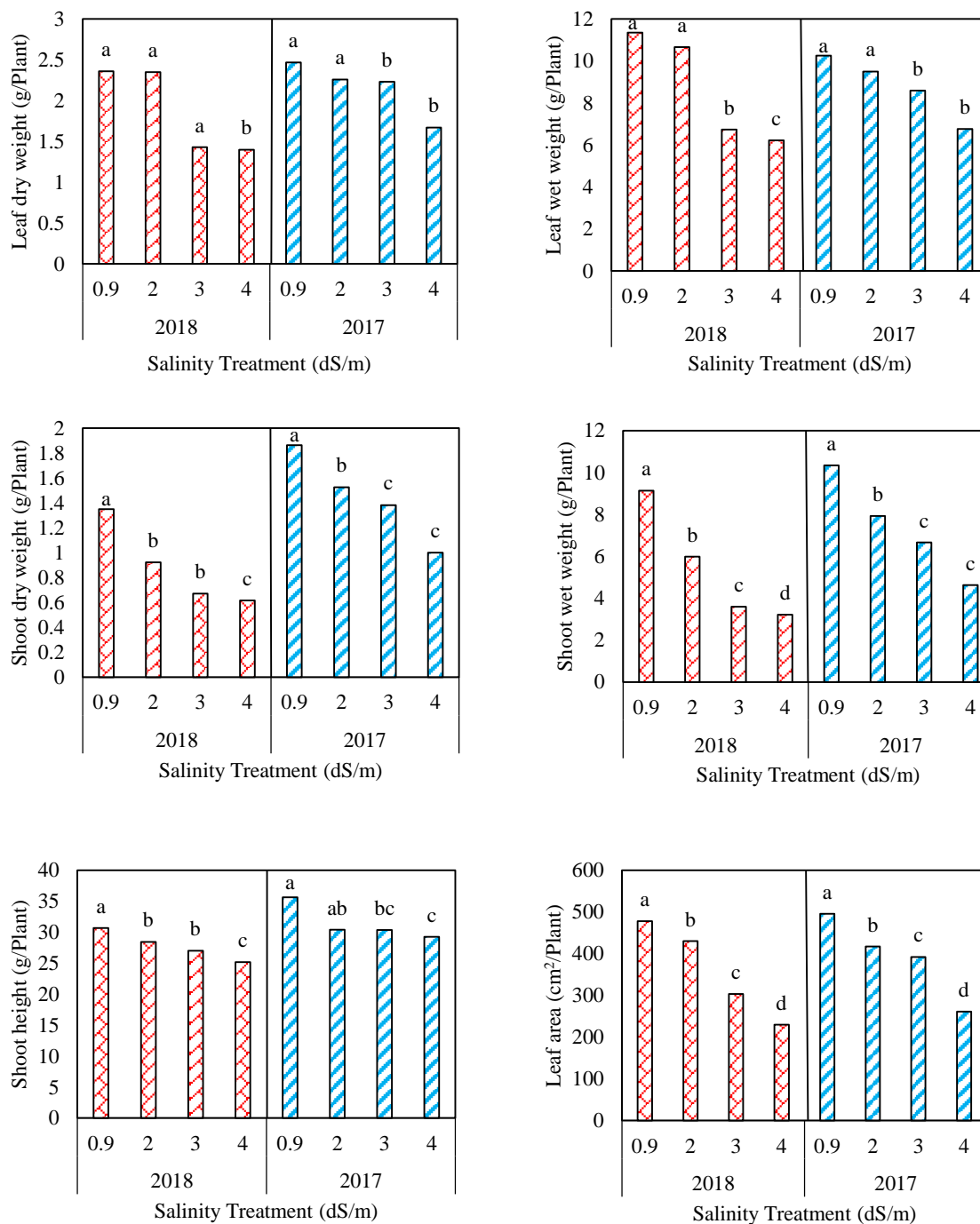


Figure 4. Average comparison of salinity on the peppermint aerial characteristics.

Table 8. Variance analysis of the effect of water requirement treatment on peppermint underground organs.

Year	Source of variation	Degree of freedom	Mean squares				
			Root dry weight	Root volume	Root length	Root area	Root density
2017	Replication	2	0.017 <sup>ns</sup>	2.640 <sup>ns</sup>	246566.83 <sup>ns</sup>	3777.047 <sup>ns</sup>	0.871 <sup>ns</sup>
	Deficit treatment	2	2.332 <sup>**</sup>	186.109 <sup>**</sup>	25889707.54 <sup>**</sup>	807465.99 <sup>**</sup>	0.009 <sup>**</sup>
	Error	4	0.092	1.372	236772.52	4252.64	0.00007
	CV (%)	-	13.16	6.26	8.46	5.65	6.9
2018	Replication	2	0.025 <sup>ns</sup>	0.612 <sup>ns</sup>	24786.80 <sup>ns</sup>	1104.89 <sup>ns</sup>	0.0002 <sup>ns</sup>
	Deficit treatment	2	7.623 <sup>**</sup>	184.555 <sup>**</sup>	43975763.56 <sup>**</sup>	1163380.92 <sup>**</sup>	0.00003 <sup>ns</sup>
	Error	4	0.049	0.837	120054.25	3583.92	0.00001
	CV (%)	-	5.75	5.20	5.73	5.19	5.08

<sup>\*</sup>: Significant at 5% level, <sup>\*\*</sup>: Significant at 1% level and ns: No significant.

**Table 9. The average comparison effect of deficit irrigation treatments on peppermint underground organs.**

Year	Deficit treatment (%)	Root dry weight (g/Plant)	Root volume (cm <sup>3</sup> /Plant)	Root length (cm/Plant)	Root area (cm <sup>2</sup> /Plant)	Root density (g/cc/Plant)
2017	100	3.21 <sup>a</sup>	25.3 <sup>a</sup>	9081.0 <sup>a</sup>	1699.3 <sup>a</sup>	0.150 <sup>a</sup>
	80	2.24 <sup>b</sup>	20.8 <sup>b</sup>	4610.1 <sup>b</sup>	1096.9 <sup>b</sup>	0.126 <sup>b</sup>
	60	1.45 <sup>c</sup>	10.0 <sup>c</sup>	3544.3 <sup>b</sup>	666.5 <sup>c</sup>	0.106 <sup>c</sup>
2018	100	5.69 <sup>a</sup>	26.5 <sup>a</sup>	10442.8 <sup>a</sup>	1866.6 <sup>a</sup>	0.233 <sup>a</sup>
	80	3.15 <sup>b</sup>	14.4 <sup>b</sup>	4238.3 <sup>b</sup>	875.2 <sup>b</sup>	0.220 <sup>a</sup>
	60	2.75 <sup>b</sup>	11.9 <sup>c</sup>	3454.1 <sup>b</sup>	718.1 <sup>c</sup>	0.213 <sup>a</sup>

Different letters indicate a significant difference in the 1% level probability using Duncan's test.

**Table 10. Variance analysis of the salinity treatments on the peppermint underground organs.**

Year	Source of variation	Degree of freedom	Mean squares				
			Root dry weight	Root volume	Root length	Root area	Root density
2017	Replication	2	0.001 <sup>ns</sup>	3.139 <sup>ns</sup>	100468.13 <sup>ns</sup>	7138.738 <sup>ns</sup>	0.00005 <sup>ns</sup>
	Salinity treatment	3	0.952 <sup>**</sup>	51.619 <sup>**</sup>	12171670.91 <sup>**</sup>	322215.255 <sup>**</sup>	0.00007 <sup>ns</sup>
	Error	6	0.035	1.541	567242.04	8158.91	0.00009
	CV (%)	-	6.72	5.76	11.43	6.78	7.41
	Replication	2	0.052 <sup>ns</sup>	1.762 <sup>ns</sup>	211603.84 <sup>ns</sup>	2036.93 <sup>ns</sup>	0.0002 <sup>ns</sup>
2018	Salinity treatment	3	5.353 <sup>**</sup>	79.356 <sup>**</sup>	29932015.35 <sup>**</sup>	630701.45 <sup>**</sup>	0.0018 <sup>**</sup>
	Error	6	0.077	1.932	240616.93	7612.42	0.0001
	CV (%)	-	6.77	6.15	7.93	6.66	7.50

\*: Significant at 5% level, \*\*: Significant at 1% level and ns: No significant.

Table 11 represents the average comparison effect of salinity treatments on peppermint underground organs. All the investigated parameters had no significant difference between the 2 and 3 dS/m treatments, based on the results obtained in 2017. The results from the control treatment also showed a significant difference in root volume, length, and area compared to the other salinity levels. Another notable point was that the root density trait had almost the same performance at all salinity levels and had no significant differences. In 2018, the results demonstrated that the control

treatment had a higher performance in terms of dry weight, length, area, and root density and had a significant difference compared to other salinity levels. Examining the results revealed that there were no significant differences between the 2 and 3 dS/m treatments on root density and root volume. The average comparison salinity treatments result in the peppermint underground organs in two years indicated that all the traits' performance decreased due to the increase in the water salinity level.

**Table 11. Average comparison effect of salinity treatments on the peppermint underground organs.**

Year	Salinity treatment (dS/m)	Root dry weight (g)	Root volume (cm <sup>3</sup> )	Root length (cm)	Root area (cm <sup>2</sup> )	Root density (g/cc)
2017	0.9 (Control)	3.21 <sup>a</sup>	25.33 <sup>a</sup>	9081.0 <sup>a</sup>	1699.35 <sup>a</sup>	0.1367 <sup>a</sup>
	2	3.11 <sup>ab</sup>	22.95 <sup>ab</sup>	6953.4 <sup>b</sup>	1414.82 <sup>b</sup>	0.1267 <sup>a</sup>
	3	2.83 <sup>b</sup>	22.22 <sup>b</sup>	6091.5 <sup>b</sup>	1304.24 <sup>b</sup>	0.1267 <sup>a</sup>
	4	1.97 <sup>c</sup>	15.64 <sup>c</sup>	4226.5 <sup>c</sup>	908.16 <sup>c</sup>	0.1267 <sup>a</sup>
2018	0.9 (Control)	5.69 <sup>a</sup>	26.55 <sup>a</sup>	10442.8 <sup>a</sup>	1866.6 <sup>a</sup>	0.2333 <sup>a</sup>
	2	4.54 <sup>b</sup>	25.29 <sup>ab</sup>	6639.3 <sup>b</sup>	1451.5 <sup>b</sup>	0.1800 <sup>b</sup>
	3	3.67 <sup>c</sup>	23.45 <sup>b</sup>	4354.8 <sup>c</sup>	1132.4 <sup>c</sup>	0.1667 <sup>b</sup>
	4	2.54 <sup>d</sup>	15.13 <sup>c</sup>	3313.1 <sup>d</sup>	791.1 <sup>d</sup>	0.1567 <sup>b</sup>

Different letters indicate a significant difference in the 1% level probability using Duncan's test.

#### 4. Discussion

Statistical analysis indicated a significant effect of the deficit of irrigation on peppermint aerial characteristics. It should be mentioned that the significant effects of deficit irrigation on various plants have been confirmed by other researchers (Arshi et al., 2005; Forouzandeh et al., 2011; Ardakani et al., 2012;

Akbari Nodehi et al., 2014; Gorgini et al., 2015; Kheiry et al., 2017; Polanski et al., 2018; Parsa et al., 2019; Ghamarnia et al., 2022). The investigation of water deficit treatments over 2017 revealed that the application of 20% water stress compared to the full water requirement, in the leaf dry weight, leaf wet weight, shoot dry weight, shoot wet weight, shoot



height and leaf area, caused reductions of 8.90, 16.19, 23.08, 38.38, 29.82, and 22.62% and applying 40% water deficit in the mentioned traits, caused yield reductions of 37.65, 44.64, 28.07, 38.92, 37.28, and 31.81% respectively. These results were repeated in 2018 as well, so based on the results' application of 20% water stress compared to the full water requirement, in the leaf dry weight, leaf wet weight, shoot dry weight, shoot wet weight, shoot height and leaf area, caused reductions of 8.34, 26.38, 30.88, 38.10, 15.40 and 28.05% and applying 40% water deficit in the mentioned traits, caused yield reductions of 13.55, 48.35, 31.11, 49.72, 35.32 and 56.77% respectively (Fig. 3). In the investigation of the causes for the decrease in plant yield in the examined traits in years following the water stress application, it can be stated that water stress decreases cell division and cell shrinkage, and as a result decreases the pressure of the cell membrane. Finally, it causes a decrease in the shoot height. Furthermore, researchers have reported that with increasing water stress and decreasing the pressure of stomatal protective cells, the stomatal conductance and subsequently the plant height, growth rate and biomass of the plant decreased (Goldani et al., 2021). Therefore, the decrease in the growth rate and plant biomass due to its water stress could be a reason for a decrease in the wet and dry leaf weight, and the wet and dry shoot weight. Stress caused by water deficit has negative effects on plant morphology due to the limited amount of water available to plant roots and the activation of various processes in the plant that involve energy consumption. Dehydration stress causes a decrease in the number of branches as an adaptation mechanism to deal with this condition in the peppermint medicinal plant. The present research results were in line with other researchers such as: (Parsa et al., 2019; Jamali et al., 2020; Goldani et al., 2021; Keshavarz-Mirzamohammadi et al., 2021).

A further aim of this study was to evaluate the salinity stress effects on the peppermint aerial characteristics. The results obtained from Table 7 showed that the leaf wet and dry weight, shoot fresh and dry weight, leaf area, and shoot height were affected by different salinity levels. The results of previous research also indicated that salinity stress has an inauspicious effect on the plant (Ghorbani et al., 2018; Rostami et al., 2018) and peppermint (Khorasaninejad et al., 2010; Shahriari et al., 2013;

Roodbari et al., 2013; Veras et al., 2017; Ghamarnia et al., 2022). For two years of the experiment, leaf area was the only trait that was significant among all salinity treatments. There was no significant difference between control (0.9), 2 and 3 (dS/m) salinity treatments in wet and dry leaf weight over 2017 (Fig. 4).

The study of salt stress treatments in 2017 revealed that the 4 dS/m treatment compared to the control treatment (0.9 dS/m) in leaf dry weight, leaf wet weight, shoot dry weight, shoot wet weight, shoot height and leaf area, respectively caused reductions of 32.38, 34.0, 45.96, 55.30, 17.98 and 47.26% and in 2018 in the mentioned traits, , caused yield reductions of 40.67, 45.21, 54.37, 64.73, 17.87, and 51.88% respectively (Fig. 4). One of the adverse effects of salinity stress is the prevention of water absorption and drought stress, and the reason for the decrease in relative water content can be attributed to the reduction of leaf water potential and the reduction of water absorption from roots in dry conditions (Colom and Vazzana, 2003). Salinity stress reduces the power of cell growth by reducing the absorption of nutrients, the lack of usable water in the plant, and the toxicity of elements. It also causes a reduction in the leaf area and photosynthesis (Ghorbani et al., 2018). The experiment conducted in relation to the effect of salinity stress on three different types of mint showed that salinity stress decreased the shoot length of these three species (Aziza et al., 2008). In previous studies, it has been reported that the stem weight of the medicinal plant fenugreek (Archangi et al., 2012), the leaf and stem weight of the Mexican flower (*Agastache foeniculum kuntz*) (Khorsandi et al., 2010), the growth of *Echinacea angustifolia* (Montanari et al., 2008) and basil (Bernstein et al., 2009) decreased under salt stress conditions.

Investigating deficit irrigation treatment's effect on peppermint underground organs, the results revealed that the application of 80 and 60% water requirement treatments compared to the control treatment decreased the amount of root dry weight by 30 and 54%, in 2017 also, during 2018, this decrease was 44 and 51%, respectively (Table 9). The results of the present study were consistent with other similar studies (Ghanbari and Ariafar, 2013; Akbari Nodehi et al., 2014).

The study of water deficit irrigation on other root characteristics, over 2017 revealed that the application

of 20% water stress compared to the full water requirement, in the root density, root area, root length and root volume, respectively caused reductions of 15.53, 35.45, 49.23, and 17.91 %, as well as applying 40% water deficit in the mentioned traits, caused yield reductions of 28.87, 60.78, 60.97, and 60.52 %. These results were repeated in 2018, so based on the results' application of 20% water stress compared to the full water requirement, in the root density, root area, root length and root volume, respectively caused reductions of 5.58, 53.11, 58.46, 38.10, and 45.83% and applying 40% water deficit in the mentioned traits, caused yield reductions of 8.58, 61.53, 66.92, and 55.19% (Table 9).

The study of salt stress treatments in 2017 revealed that the 4 dS/m treatment compared to the control treatment (0.9 dS/m) in the root density, root area, root length, root volume, and root dry weight, respectively caused reductions of 7.32, 46.56, 53.46, 38.26 and 38.63% and in 2018 in the mentioned traits, caused yield reductions of 32.83, 57.62, 68.27, 42.99, and 55.36% (Table 11).

The review of the research conducted on the adverse effect of salt stress on the peppermint plant and the reports presented in this field was also in line with the results of the present study (Ghanbari and Ariafar, 2013; Khorasaninejad et al., 2010; Tabaei-Aghdaei et al., 2004; Roodbari et al., 2013).

## 5. Conclusion

In this present research, the water stress and irrigation water salinity effect on peppermint aerial and underground characteristics were investigated. This study's results revealed that the deficit irrigation effect on leaf wet weight, shoot wet weight, leaf area, and shoot height was significant ( $P \leq 0.01$ ). Also, the effect of salinity on the wet and dry leaf weight, wet and dry shoot weight, and leaf area was significant at the 1% level. The results showed that during two years, the highest and lowest values for all aerial and underground characteristics were related to the 100% and 60% water requirement treatments. Based on the results, the drought, and salinity stress effect on all underground properties except root density was significant at a 1% level. In the study of the effect of irrigation water salinity on aerial and underground characteristics, the results revealed that the highest and lowest values of the mentioned characteristics were observed in the control and four ds/m, respectively. In

general, water deficit and increased water salinity levels had a negative effect on aerial and underground traits and caused a decrease in the peppermint yield during two years of testing. Therefore, it is not recommended to apply water stress and use water with a salinity of more than 2 ds/m to get the maximum yield.

## Conflict of Interests

All authors declare no conflict of interest.

## Ethics approval and consent to participate

No human or animals were used in the present research.

## Consent for publications

All authors read and approved the final manuscript for publication.

## Availability of data and material

All the data are embedded in the manuscript.

## Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

## Informed Consent

The authors declare not to use any patients in this research.

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