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Evaluation of the impact of drought stress and mycorrhizal fungus on the morphological characteristics of mint species

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Drought stress is among the most important stresses limiting the growth and production of crops and medicinal plants. The co-existence of plants with mycorrhizal fungus reduces drought stress impacts. The present experiments were conducted seeking to investigate the influence of drought stress and mycorrhizal fungus on the morphological characteristics of mint (Mentha pulegium L) species in Namin County over 2020-2021 as a split-factorial study in the form of randomized complete blocks with three iterations. The evaluated treatments varied in the three factors of drought (a) (a1: no stress (normal) and a2: 50% field capacity), factor b (b1: no inoculation and b2: mycorrhizal fungus inoculation), and factor c (c1: Marivan, c2: Salmas, and C3: Miandoab). Results of variance analysis indicated significant differences between the levels of individual impacts of a (drought stress level), b (mycorrhizal fungus levels), and c (cultivars) in terms of all the studied traits. Moreover, significant differences at 1% and 5% levels were observed between the levels of the ABC interaction effect (drought stress with mycorrhizal fungus inoculation in various cultivars) in terms of the traits of dry and wet weight, the number of branches, essential oil content, essential oil performance, and root colonization. The 50% field capacity treatment resulted in a 31.1%, 19.1%, 6.4%, 4.9%, and 36.9% decline in the traits of wet and dry plant weight, number of branches, number of flowers, and root colonization, while causing a 7%, 21.6%, 31.9%, and 53.1% increase in the traits of plant height, root length, essential oil performance, and essential oil content, respectively. Inoculation with mycorrhizal fungus increased all the traits investigated in the present study. The growth traits revealed to increase in the plant through proper water resource management and the use of mycorrhizal fungus seeking optimal use of water resources and nutrients. Results revealed that Inoculation with mycorrhizal fungus increased the resistance of mint against the stress through the improvement of the studied traits.

Keywords: mint, drought stress, mycorrhizal fungus, morphological traits

INTRODUCTION

Medicinal herbs have long been used in Iran, and various references concerned with ham have remained from the Iranian and non-Iranian scholars of the Islamic era. Medicinal plants were gradually forgotten with the spread of the chemical drugs but gained new-found attention from the scientific and medical communities given the adverse side effects of the chemicals on human health (Gholamin and Khayatnezhad 2020, Karasakal, Khayatnezhad et al. 2020, Khayatnezhad and Gholamin 2020, Si, Gao et al. 2020, Bi, Chen et al. 2021, Guo, She et al. 2021). Excessive harvest of these plants from nature ended up destroying their resources (Ghahraman & Aukhovat, 2009). Given its vastness and diverse ecological condition, Iran is home to a considerable number of mint genera, some of which are specific to the country. Mentha is the most important genus from the Lamiaceae order, while most other genera have a wide range of applications in the health and cosmetic industries (Akbarzadeh, 2003). Mint species M. pulegium with the Persian name fragrant spearmint is a perennial, herbaceous, dormant, or creeping plant with several stems and a cylindrical appearance with a height of 10-55cm, covered in trichome, leaves with short petioles, and pink to purple flowers that grow wild along water currents and in wet plains (Gholamin and Khayatnezhad 2020, Chen, Khayatnezhad et al. 2021, Hou, Li et al. 2021, Huang, Wang et al. 2021, Liu, Wang et al. 2021). All parts of the plant have a strong fragrance. Its flowers appear in clusters close to the leaves along the stem from July to October (Jamzad, 2012). Improvement of production and yield and stabilizing the production in the face of soil moisture deficiency is the best option to produce products that are resistant to drought (Siddique et al., 2000). Drought is a complex phenomenon and is considered among the factors reducing the yield of crops all around the world (Beltrano and Ronco, 2008). The average rainfall is 240-250mm in Iran, and the average annual precipitation is 413 billion cubic meters, which indicates that Iran has around one-third of the average worldwide rainfall (Gholamin and Khayatnezhad 2020, Karasakal, Khayatnezhad et al. 2020, Cheng, Hong et al. 2021, Ma, Ji et al. 2021, Ren and Khayatnezhad 2021). Meanwhile, the country contains 1.2% of the land of the world, which classifies it into the arid regions of the world (Abdi 2021, Alizadeh 2021, Karasakal 2021, Mohammadzadeh 2021, Radmanesh 2021, Radmanesh 2021). Nowadays, the use of low-input farming systems and the innovative resource utilization management techniques seeking to achieve sustainable agriculture have gained special importance (Soltanian and Tadayyon, 2015). The use of the mycorrhizal fungi which is among the most important microorganisms in the root environment and coexists with the roots of over 90% of plants is among the solutions used in drought-resistant plants (Gholamin and Khayatnezhad 2020, Jia, Khayatnezhad et al. 2020, Khayatnezhad and Gholamin 2021, Sun, Lin et al. 2021, Wang, Ye et al. 2021, Wang, Khayatnezhad et al. 2022). The coexistence of plant roots with mycorrhizal fungi is among the most conventional and oldest ways to increase the nutrient uptake to adapt to environmental stresses (Ortiz et al., 2015). These fungi establish a relationship between the plant roots and soil, and thus increase the roots' absorption speed (Soltanian & Tadayyon, 2015) and improve their growth by increasing nutrient absorption (Mahmoudzadeh et al., 2015). The fungi attenuate the impact of toxic ions by increasing the relative water absorption as well (Gholamin and Khayatnezhad 2021, Khayatnezhad and Nasehi 2021, Li, Mu et al. 2021, Xu, Ouyang et al. 2021, Yin, Khayatnezhad et al. 2021, Zhang, Khayatnezhad et al. 2021, Li, Khayatnezhad et al. 2022). Increased soluble sugar in roots reduces their osmotic potential, while balanced nutrient absorption increases the plants' resistance to environmental stress (Tavasoli & Asgharzadeh ,2009). The present study seeks to investigate the interaction effect of mycorrhizal fungi and drought stress on the morphological traits of mint species in Namin County.

MATERIALS AND METHODS

To investigate the influence of drought stress and mycorrhizal fungus on the morphological characteristics of the examined mint species in Namin County over 2020-2021 as a split-factorial, the present study was conducted in the form of randomized complete blocks with three iterations. The evaluated treatments varied in the three factors of drought (a) (a1: no stress (normal) and a2: 50% field capacity), factor b (b1: no inoculation and b2: mycorrhizal fungus inoculation), and factor c (c1: Marivan, c2: Salmas, and C3: Miandoab). Seeds of the three mint populations (Marivan, Salmas, and Miandoab) were cultivated in seedling trays in a greenhouse in Namin County and were transferred to the main land after the complete establishment of the seedlings. The land was plowed and the seedlings were cultivated in the form of randomized complete blocks with three iterations after land preparation and completion of the irrigation system, with each plot including 15 plants cultivated 40cm away from one another on two 8m rows. Drip irrigation was used to water the plants, and the studied traits were not noted down during the first year given the heterogeneous growth and weakness of the plants. However, the traits of wet and dry plant weight, number of branches, plant height, root length, essential oil yield, essential oil content, number of flowers, and root colonization were noted in the second year. Danesh Pazhouhan Payesh Amir Company identified the components of essential oil using a gas chromatograph connected to a mass spectrometer (GC/MS). The technique proposed by Geneva et al. (2010) was also used to determine root colonization percentage. Variance analysis of the data was conducted using the SAS V.9.4 software and mean comparison was carried out through Duncan's multiple range test at the 1% level.

RESULTS AND DISCUSSION

Table 1 demonstrates the results of variance analysis of the studied traits. Significant differences were observed between the levels of individual impacts of a (drought stress level), b (mycorrhizal fungus levels), and c (cultivars) in terms of wet and dry plant weight, number of branches, plant height, root length, essential oil yield, essential oil content, number of flowers, and root colonization (Table 1). Significant differences were also observed between the levels of the ac interaction effects (drought stress in cultivars) in terms of all traits except plant height, and between the various levels of bc interaction effect (drought stress with mycorrhizal fungus inoculation) in terms of wet and dry plant weight, root colonization, and number of branches at 1% and 5% significance levels. Moreover, significant differences at 1% and 5% levels were observed between the levels of the ABC interaction effect (drought stress with mycorrhizal fungus inoculation in various cultivars) in terms of the traits of dry and wet weight, the number of branches, essential oil content, essential oil performance, and root colonization (Table 1). Among the studied traits, plant height had the highest, and the number of flowers had the lowest coefficient of change at 3.63% and 1.07%, respectively (Table 1).

Dry and wet plant weight

A comparison of the mean triple effects of a*b*c on the wet weight trait (Table 1) indicated that the combination of normal irrigation and mycorrhizal fungus inoculation in the Marivan cultivar yielded the highest figures at 790gr/plant, while the combinations of 50% FC stress without inoculation in Salmas cultivar and 50% FC with without inoculation in Miandoab cultivar yielded the lowest wet weight and were classified into the class j (Diagrams 2-4). A comparison of the mean triple effects of a*b*c on the dry weight trait (Table 1) indicated that the combination of normal irrigation and mycorrhizal fungus inoculation in the Salmas cultivar yielded the highest figures at 284.6gr/plant, while the combinations of 50% FC stress without inoculation in Marivan cultivar resulted in the lowest dry weight. Badavi et al. (2015) and Ruiz Lozano et al. (2001) also reported reduced vegetative traits in plants placed under drought stress. The reduced absorption, transfer, and consumption of nutrients due to drought is among the reasons for the reduced dry plant weight (Khayatnezhad and Gholamin 2020, Peng, Khayatnezhad et al. 2021, Shi, Khayatnezhad et al. 2021, Wang, Shang et al. 2021, Zhao, Wang et al. 2021). Mycorrhizal fungus inoculation increases the root absorption surface and nutrient and water absorption (Esmaielpour et al., 2013) by expanding the roots (Ghorbanian et al., 2015). Moghadasan et al. (2015) investigated the role of Mycorrhizae in drought tolerance and found that the mycorrhizal fungus increases the photosynthetic pigments and growth parameters in plants.

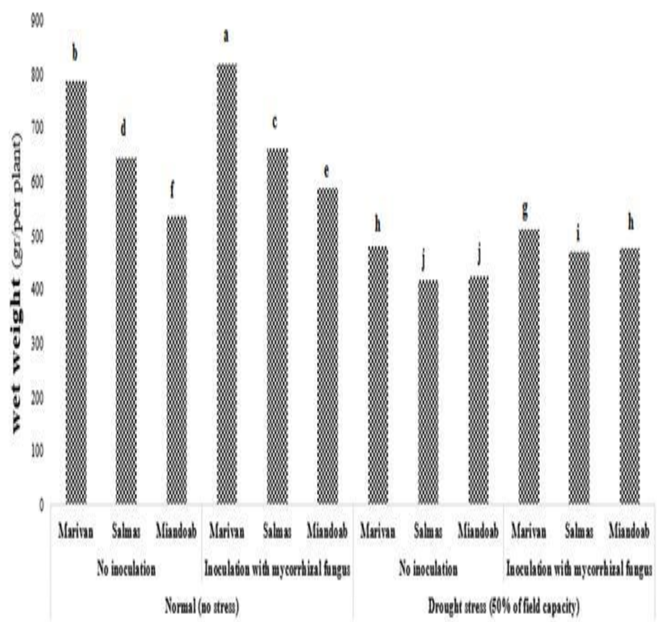

Figure 1: comparison of the mean triple effect (drought stress and mycorrhizal fungus across cultivars) for the wet weight trait (gr/plant)

Identical letters indicate no significant difference at the level of P≤ 0.05

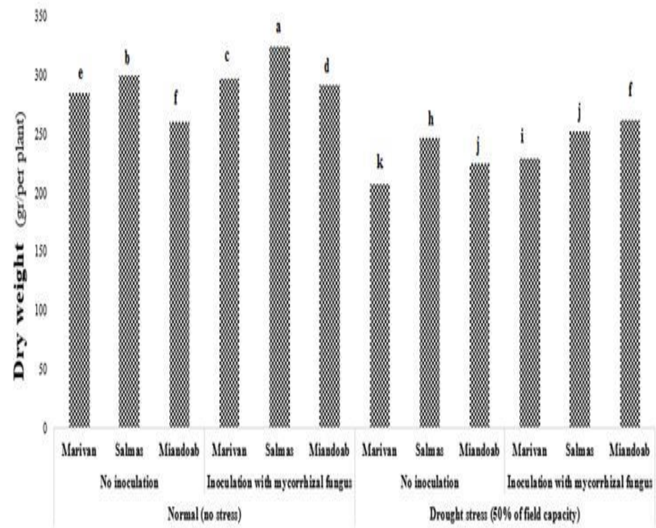

Figure 2: comparison of the mean triple effect (drought stress and mycorrhizal fungus across cultivars) for the dry weight trait (gr/plant)

Identical letters indicate no significant difference at the level of P≤ 0.05

Plant height

Plant height is among the morphological traits which are influenced by genes and the environment and is among the significant traits due to its connection to lodging. Thus, selecting the genotypes with a proper plant height can play a significant role in increasing yield (Tabatabaii et al., 2017). Results of mean comparison between the various levels of factor a (normal and 50% FC) indicated that the 50% field capacity treatment led to a 7% increase in plant height compared to normal irrigation (no stress) (Table 2). Inoculation with the mycorrhizal fungus was revealed to have a significant impact on this trait, resulting in a 7.46% increase in plant height. Moreover, the Miandoab cultivar had the highest, and the Marivan cultivar had the lowest mean plant height at 73.7cm and 58.3cm, respectively (Table 2).

Number of branches

The number of main branches is determined by some factors such as plant density and package preparation. Not only does the number of main branches influence the growth and development of aerial organs, but it also impacts flowering yield (Yazdi Samadi & Poustini, 1994). A comparison of the mean a*b*c triple effects on the trait of the number of branches (diagram 3) indicated that the combinations of normal irrigation with mycorrhizal fungus inoculation in the Salmas cultivar and normal irrigation without mycorrhizal fungus inoculation in the Salmas cultivar had the largest numbers of branches at 7.1 and 7.07, respectively. Meanwhile, the combinations of normal irrigation and mycorrhizal fungus inoculation in the Marivan cultivar, 50% FC, and mycorrhizal fungus inoculation in the Marivan cultivar, and 50% FC stress without mycorrhizal fungus inoculation in the Marivan cultivar yielded the lowest number of branches. In the absence of intra-plant competition, the number of main branches increases, which results in competition in the plant. This competition leaves a positive impact on the yield while reducing the number of secondary branches. This might be the reason why although an increased number of primary branches reduces the photosynthesis potential (due to the increased density and reduced light received by the leaves and branches), it does not harm yield since it reduces the number of secondary branches.

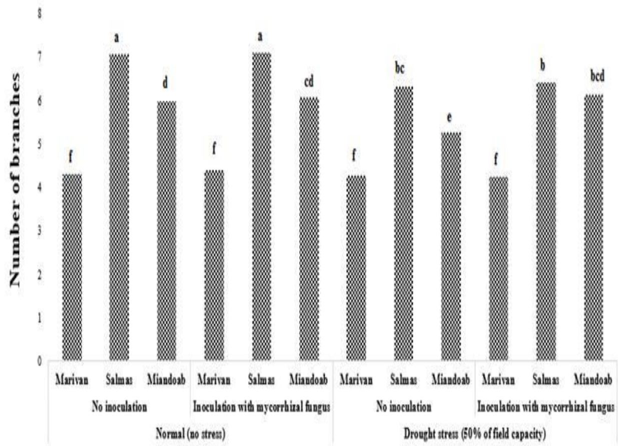

Figure 3: comparison of the mean triple effect (drought stress and mycorrhizal fungus across cultivars) for number of branches trait (number)

Identical letters indicate no significant difference at the level of P≤ 0.05

Number of flowers

The 50% FC treatment resulted in a 4.9% decline in the number of flowers compared to the normal irrigation (no stress) treatment (Table 2). Inoculation with mycorrhizal fungus resulted in a 2.86% increase in the said trait. Moreover, the Miandoab and Salmas cultivars had the largest and smallest mean number of flowers at 53 and 50.17, respectively (Table 2). The largest and smallest mean number of flowers were associated with the combinations of normal irrigation in the Miandoab cultivar and 50% FC stress in Marivan cultivar at 54.5 and 48.67, respectively (Table 3).

Root length

The 50% FC treatment resulted in a 21.6% increase in root length compared to the normal (no stress) treatment (Table 2). Inoculation with mycorrhizal fungus resulted in a 5.77% increase in the said trait. Moreover, the Marivan and Salmas cultivar had the largest and smallest mean root lengths at 10.4cm and 8.85cm, respectively (Table 2). The largest and smallest mean root lengths were associated with the combinations of 50% FC treatment in the Marivan cultivar and normal irrigation in the Salmas cultivar at 11.13cm and 7.917cm, respectively (Table 3).

Essential oil yield

The 50% FC treatment resulted in a 31.9% increase in essential oil yield compared to the normal (no stress) treatment (Table 2). Inoculation with mycorrhizal fungus resulted in a 5.36% increase in the said trait. Moreover, the Marivan and Salmas cultivar had the highest and lowest essential oil yields at 29.58 and 27, respectively (Table 2). The highest and lowest essential oil yields were observed in the combinations of 50% FC treatment in the Marivan cultivar and normal irrigation in the Salmas cultivar at 34 and 23, respectively (Table 3).

Essential oil content

A comparison of the mean triple effects of a*b*c on the essential oil content (Diagram 4) indicated that the combination of 50% FC with mycorrhizal fungus inoculation in the Marivan cultivar had the highest mean essential oil content at 52.07%, while the combinations of 50% FC without mycorrhizal fungus inoculation in Marivan cultivar and 50% FC without mycorrhizal fungus inoculation in Miandoab cultivar had the lowest mean essential oil content.

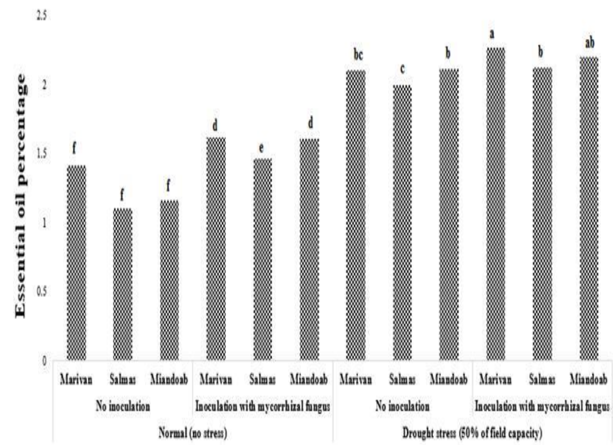

Figure 4: comparison of the mean triple effect (drought stress and mycorrhizal fungus across cultivars) for essential oil content (%)

Identical letters indicate no significant difference at the level of P≤ 0.05

Root colonization

A comparison of the mean triple effects of a*b*c on rot colonization (Diagram 5) indicated that the combination of normal irrigation and mycorrhizal fungus inoculation in the Marivan cultivar had the greatest mean root colonization at 52.07, while the combination of 50% FC without mycorrhizal fungus inoculation in Marivan cultivar yielded the lowest mean root colonization. The drought stress was revealed to significantly reduce colonization in inoculated plants. The colonization percentage in fungal species depends on the type of the plant and type of fungus (Khayatnezhad and Gholamin 2021, Ma, Khayatnezhad et al. 2021, Zheng, Zhao et al. 2021, Zhu, Liu et al. 2021, Zhu, Saadati et al. 2021). Savaghebi et al. (2010)

demonstrated that wheat root colonization increased as a result of mycorrhizal fungus inoculation, which is consistent with the results of the present study. The reason for increased root colonization under drought stress is the influence of the mycorrhizal fungus on water absorption in its coexisting plants which indicates the better compatibility and high capability of the fungal species in roots under stress conditions. These species increase the efficiency of plants under drought stress by increasing the plant's water absorption (Sun and Khayatnezhad 2021, Tao, Cui et al. 2022, Wang, Ma et al. 2022, Zhang, Khayatnezhad et al. 2022).

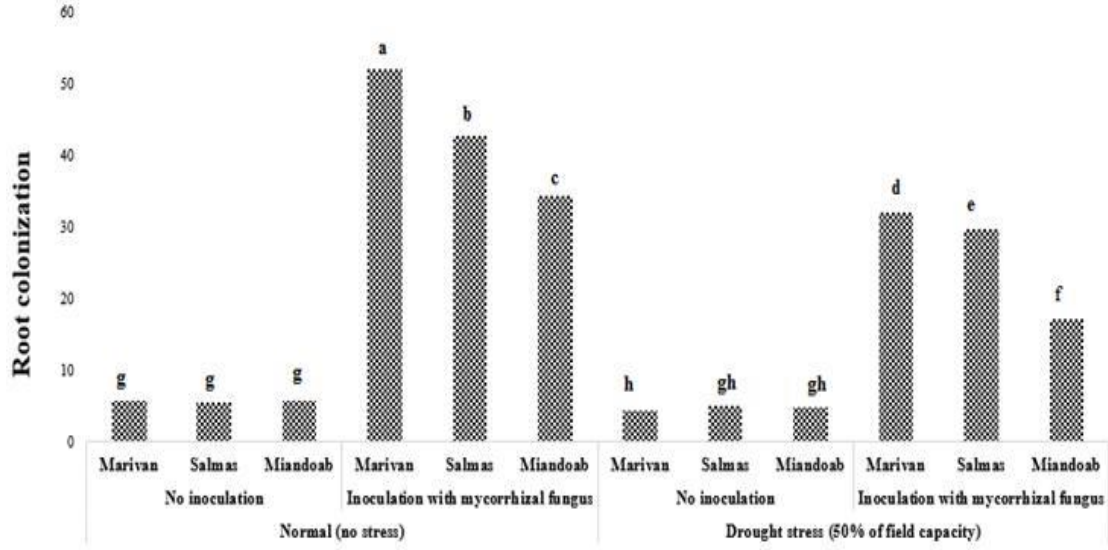

Figure 5: comparison of the mean triple effect (drought stress and mycorrhizal fungus across cultivars) for root colonization Identical letters indicate no significant difference at the level of P≤ **0.05**

Table 1: Results of analysis of variance of quantitative and qualitative traits evaluated

Source	df	Mean of Squares								
		Wet weight	Dry weight	Plant height	Number of branches	Number Of flowers	Root length	Essential oil function	Essential Oil percentage	Root colonization
Replication	2	270.8ns	0.421ns	12.58ns	0.515*	2.583ns	0.413ns	1.028ns	0.0001ns	3.116ns
Factor a	1	395012**	28168.1**	186.7*	1.266*	61.36*	31.379**	544.4**	4.928**	751.6**
Error	2	90.75	1.194	5.028	0.035	2.028	0.07	3.861	0.001	0.441
Factor b	1	14280**	4259.7**	245.4**	0.312**	20.25**	2.896**	21.77**	0.495**	7963.18**
AB	1	380.3**	12.25**	11.11ns	0.128*	2.25*	0.112ns	0.0001ns	0.105**	610.9**
Factor c	2	65374.7**	2344.3**	769.3**	18.26**	24.08**	7.468**	20.19**	0.093**	175.9**
AC	2	28718.1**	1304.6**	0.778ns	0.276**	1.361*	0.534**	4.194**	0.013*	13.61**
BC	2	433.1**	331.84**	16.77ns	0.168*	0.583ns	0.047ns	0.528ns	0.005ns	181.99**
ABC	2	210.58**	168.47**	8.111ns	0.174*	0.75ns	0.125ns	1.083ns	0.018*	9.075**
Error	20	8.272	1.415	5.972	0.031	0.306	0.047	0.411	0.004	0.267
C. V %		3.51	2.45	3.63	3.14	1.07	2.27	2.3	3.49	2.56

Table 2 : Mean of simple effects evaluated

	wet weight	Dry weight	Plant height	Number of branches	Number of flowers	Root length	Essential oil function	Essential oil percentage	Root colonization
Factor A									
Normal (no stress)	673.89	293.09	65.06	5.82	52.89	8.61	24.33	1.39	24.74
Drought stress (50% of field capacity)	464.39	237.15	69.61	5.45	50.28	10.48	32.11	2.13	15.60
Percentage decrease and increase compared to the control	-31.1	-19.1	+7	-6.4	-4.9	+21.6	+31.9	+53.1	-36.9
Factor B	wet weight	Dry weight	Plant height	Number Of branches	Number Of flowers	Root length	Essential Oil function	Essential Oil percentage	Root colonization
No inoculation	549.22	254.24	64.72	5.54	50.83	9.26	27.44	1.65	5.30
Inoculation with Mycorrhizal fungus	589.06	276.00	69.94	5.73	52.33	9.83	29.00	1.88	35.04
Percentage decrease and increase compared to the control	6.7	7.8	7.46	3.24	2.86	5.77	5.36	12.45	84.87
Factor B	wet weight	Dry weight	Plant height	Number of branches	Number Of flowers	Root length	Essential Oil function	Essential oil percentage	Root colonization
Marivan	651[a]	254.6[b]	58.3[c]	4.3[c]	51.58b	10.40a	29.58 a	1.85a	23.62a
Salmas	548.7[b]	280.9[a]	70[b]	6.7[a]	50.17c	8.85c	27.00 c	1.67c	20.85b
Miandoab	507.7[c]	259.7[b]	73.7[a]	5.8[b]	53.00a	9.37b	28.08 b	1.77b	16.05c
Identical letters indicate no significant difference at the level of P≤ 0.05									

Table 3 : Comparison of the average AC side effect for the evaluated traits

AC		Number of flowers	Root length	Essential oil function
Normal (no stress)	Marivan	52.50 b	9.683 c	25.17 c
	Salmas	51.67 c	7.917 e	23 d
	Miandoab	54.50 a	8.232 d	24.83 c
Drought stress (50% of field capacity)	Marivan	50.67 d	11.13 a	34 a
	Salmas	48.67 e	9.792 c	31 b
	Miandoab	51.50 c	10.52 b	31.33 b
Identical letters indicate no significant difference at the level of P≤ 0.05				

CONCLUSION

Results of the present study revealed that acceptable results could be achieved at 50% field capacity irrigation and consumption of mycorrhizal fertilizers to achieve optimal water and nutrient consumption. Thus, the growth traits can be enhanced in the plants through proper water resource management and the use of mycorrhizae.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mani Alizadeh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.

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