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Original Article

Effect of Zeolite and Biofertilizers on the Essential Oil Yield and Some Physiological Characteristics of *Satureja hortensis* L. under Water Deficit Stress

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ABSTRACT

Garden or summer savory (*Satureja hortensis* L.) is one of the most important medicinal and aromatic plants that is cultivated in many parts of the world. Thus, in order to investigate the effect of zeolite and biofertilizer on essential oil yield, morphological and physiological characteristics of summer savory under water deficit stress, a split-factorial experiment was performed based on randomized complete block design with four replications at 2011 in Tehran, Iran. Irrigation regime with four levels (I1: 30, I2: 60, I3: 90 and I4: 120 irrigations after mm evaporation) and biofertilizer inoculation with three levels (B1: no inoculation, B2: inoculation with azospirillium + Mycorrhiza and B3: inoculation with Pseudomonas + Mycorrhiza) and zeolite with two levels (0 and 20 t/ha) were considered. The results showed that the highest essential oil yield was obtained under normal irrigation. The results showed that the biofertilizers were more effective on the content and yield of essential oil under normal irrigation conditions. Results also showed that the application of zeolite had a greater effect on increasing the essential oil yield in the absence of biofertilizer inoculation.

INTRODUCTION

The medicinal plant of savory (*Satureja hortensis* L.) belongs to the mint family and grows as a shrub [1]. Savory is a plant with numerous flowers up to 1.5 mm in size, lanceolate leaves and have secretory glands containing essential oil [2]. Savory is an aromatic plant that has various effects such as treating muscle aches, nausea and infectious diseases and diarrhea [3]. This plant has also been used in food as a flavoring [4]. This plant has been shown in laboratory studies to have antimicrobial, antioxidant, hypnotic and antispasmodic effects [5]. In addition, due to the presence of aromatic substances in the plant for food use, beverage preparation, industrial use, as well as due to its antibacterial and antifungal properties, it has always been highly regarded [6,7].

Drought stress plays a vital role in reducing crop yield all around the world. Various studies have shown that water stress has negative effects on yield and yield components of some medicinal plants as N. sativa [8], Plantago ovate [9], Mentha piperita [10]. The potential of medicinal and aromatic plants for growing under limited water conditions make them suitable alternative crops in such agro-ecosystems [11].

Several types of studies have shown a beneficial effect on crops by inoculation of PGPR include of Azotobacter, Azospirillum and Pseudomonas, [8,12, 13]. Inoculation of plants with PGPR and mycorrhiza can result in a significant change in various plant growth parameters [14]. The best performances of PGPR and mycorrhiza under field conditions are usually associated with non-optimal conditions for plant growth (limited fertilization and water), and they occur mainly in semi-arid agriculture [15].

Zeolites are a group of natural porous minerals that act as molecular sieves with their crystalline structure and, due to having open channels in their network, allow some ions to pass through and block the passage of some other ions [16]. Zeolite can also act as water moderators, in which they will absorb up to 55% of their weight in water and slowly released it under the plants demand. This property can prevent root rot and moderate drought cycles, [17]. Therefore, the objective of this study was to investigate the effects of biofertilizers and zeolite on *S. hortensis* L. under water deficit conditions.

MATERIALS AND METHODS

Field Experiment

The present study was conducted in the experimental field of the Islamic Azad University of Shahr-e-Qods Branch at Tehran, Iran (35˚ 68' N, 51˚11'E; 1417 m H) during 2011, with sandy loam soil, and 215 mm annual rainfall. The experiment was done as split-factorial, based on randomized complete block design (RCBD) with four replications. The factors consisted of drought stress with four levels (I_1, I_2, I_3 and I_4 refers to the irrigation after 30, 60, 90, and 120 mm evaporation from Class A pan evaporation, respectively) as main plot, biofertilizer inoculation with three types (B_1: non-inoculated; B_2: inoculated with *Azospirillum* and *Mycorrhiza*; B_3: inoculated with *Pseudomonas* and *Mycorrhiza*) and zeolite application with two levels (Z_1=non-application; Z_2=20 t/ha) as sub-plot. The species of the used *Mycorrhiza* was *Glomus mossae*. The weeds were controlled manually. All necessary cultural practices and plant protection was followed uniformly for all plant during the entire period of the experimentation.

Measurements

Following parameters were recorded for each sample: plant height and width, stem diameter and dry weight per m^2. The dry weight of whole plants was calculated in each plot after drying the plants in the oven (under 75 ˚C for 48 h), [18]. Essential oil percentage determined by Clevenger-type apparatus according to the European Pharmacopeia method [19].

Relative water content (RWC)

The following formula was employed to calculate RWC [20]:

RWC=100 (Wf-Wd)/ (Wt-Wd) (Eq.1)

Where, Wf; fresh weight, Wd; dry weight, and Wt; turgor weight of leaf disks.

Leaf proline was measured by the Bates method (1973). In this way, 10 mL of 10 mL sulfosalicylic acid solution was added to 100 mg of leaf powder and after 24 hours, this solution was centrifuged at 13000 rpm for 10 minutes. Take 2 mL of the supernatant and add 2 mL of ninhydrin. Then 1 mL of glacial acetic acid was added and the tubes were immersed in boiling water for 1 hour. After cooling, 4 mL of toluene was added to each tube to form two phases, then the supernatant was removed and its absorbance was read at 520 nm [21].

$$\mu\text{mole proline/g f.w. material}=\frac{\frac{\text{(toluene)ml proline/ml µg)}}{\text{µmoleµg/ 115.5}}}{\text{(g sample/5)}}$$

Leaf chlorophyll content was determined by the Arnon method [22].

One gram of fresh plant material was poured into a porcelain mortar, then crushed using liquid nitrogen and crushed well. Twenty mL of 80% acetone was added to the sample, then placed in a centrifuge at 6000 rpm for 10 minutes. The upper isolated extract from the centrifuge was transferred to a glass balloon. Some of the sample was poured into a balloon in a spectrophotometer and then the absorbance was read separately at 663 nm for chlorophyll a, 645 nm for chlorophyll b and 470 nm for carotenoids.

Finally, using the following formulas, the amount of chlorophyll a, b and carotenoids in mg/g wet weight of the sample was obtained [22].

Chlorophyll a = (19.3 * A663 - 0.86 * A645) V/100W

Chlorophyll b = (19.3 * A645 - 3.6 * A663) V/100W

Carotenoids = 100(A470) - 3.27(mg chl. a) – 104 (mg chl. b)/227

V: volume of filtered solution (centrifuge supernatant)

A: light absorption at wavelengths of 663, 645 and 470 nm

W: wet weight of the sample in grams

Statistical Analysis

Data of all measured parameters were subjected to variance analysis using MSTAT-C statistical software. Also, Duncan's multiple range tests were used to compare treatment means at a 0.05 probability level. The regression analysis was done by SPSS *ver. 21*.

Table 1 The results of the soil analysis

Soil properties	Content
Soil texture	Silt. Loam
Sand (%)	58.72
Silt (%)	22.09
Clay (%)	19.9
EC (ds/m)	0.75
pH	7.90
P(mg/m)	38
K(mg/m)	21
Organic Matter (%)	0.88
Depth of Sampling(cm)	0-30

RESULTS AND DISCUSSION

The results indicated that the effect of water stress, biofertilizer and zeolite were significant on biological yield, essential oil content, essential oil yield, plant height, plant width and stem diameter. Besides, the interaction of irrigation × biofertilizer on biological yield, essential oil content and essential oil yield, the interaction of irrigation × zeolite on biological yield and essential oil yield and the interaction of biofertilizer × zeolite on biological yield were significant (Table. 2).

Biological yield decreased in response to water deficit stress. The results showed that the difference between biofertilizer treatments was greater with increasing stress intensity. This means that the application of biofertilizer treatments was more effective under severe stress conditions i.e., irrigation after 120 mm (Fig. 1). Khalid (2006) reported that drought stress has significant effects on dry weight in *Ocimum basilicum L.* and *O.americanum L*. [23]. Zeolite consumption also had a greater effect on increasing biological yield under higher stress conditions. Therefore, it seems that zeolite under water stress conditions can partially compensate the decrease in biological yield (Fig. 2). Zeolite provides an ideal physical root zone media and the addition of zeolite has improved the nutrient status especially selective retention of NH_4^+ and K^+ ions [24]. Overally, under water stress stoma's become blocked or half-blocked and this leads to a decrease in absorbing CO_2; Therefore, the plants consume a lot of energy to absorb water, which cause a reduction in producing photosynthetic matters [25]. The interaction of biofertilizer and zeolite also showed that in the absence of biofertilizer, zeolite was more effective in increasing biological yield (Fig. 3).

The essential oil content range varied between 1.47 ~ 1.93%, which was obtained from irrigation after 120 and 60 mm evaporation, respectively. The highest essential oil content was found in the use of biofertilizer (*Pseudomonas* and *Mycorrhiza*) (1.82%) which is superior to control (non-inoculated) (table 3). The mean comparison indicated that the highest essential oil yield was obtained under normal irrigation. The results showed that the effect of biofertilizers on the essential oil content and yield was greater under normal irrigation conditions (Fig. 4, 5). The results of the interaction of biofertilizer and zeolite on the essential oil yield showed that the application of zeolite had a greater effect on increasing the essential oil yield in the absence of biofertilizer inoculation (Fig. 6). Ghassemi-Golezani et al. (2018) reported the water stress caused an increase in essential oil percentage of Ajowan seeds. On the other hand, essential oil yield decreased as a result of large reductions in the seeds per plant and seed yield [25]. Gholizadeh *et al* (2010) reported that zeolite effect on fresh weight and essential oil content in Moldavian Balm (*Dracocephalum moldavica L.*) [28]. Moreover, Biofertilizer could enhance the growth and production of *S. hortensis L*. Total extraction of soil moisture by biofertilizer inoculated plants is greater and water can be extracted from deeper layers in the soil profile [14]. Therefore, plant biological yield, and essential oil yield increase in inoculated plants were attributed primarily to improved utilization of soil moisture. These effects could result in more water uptake, especially under water stress conditions.

Plant height, plant width and stem diameter of *S. hortensis* decreased significantly under drought stress. The greatest reduction was observed in severe water deficit stress (irrigation after 120 mm evaporation). It was also seen that as increasing water stress, resulted to decrease plant height, plant width, stem diameter and biological yield and essential oil content. Reduction in water and nutrient elements uptake by the roots under water stress; will result in lower assimilation and biomass production in the plants [24]. Haj Seyed Hadi reported that irrigation treatments have significant effects on biological yield and plant height in black cumin seed yield [8]. On the other hand, the highest mentioned traits were found in the full irrigated treatment. Application of zeolite and biofertilizer (*Pseudomonas*+*Mycorrhiza*) resulted to increase plant height, plant width and stem diameter significantly. The result of regression analysis indicated linear regression between irrigation regime and essential oil yield was significant (Table. 4) with negative relationship correlation r=-0.961, adjusted coefficient of determination R^2=0.923 (Fig. 7). Reduction of essential oil yield due to decreased soil moisture may be due to the negative effect of drought stress on growth and the function of the vegetative body of the plant.

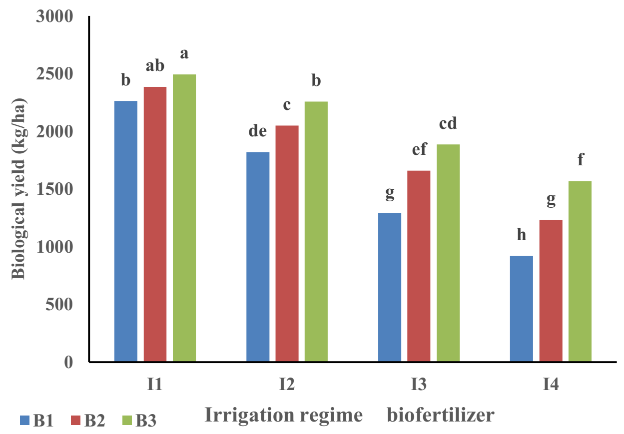

Fig. 1 The mean comparison of irrigation and biofertilizer interaction on biological yield

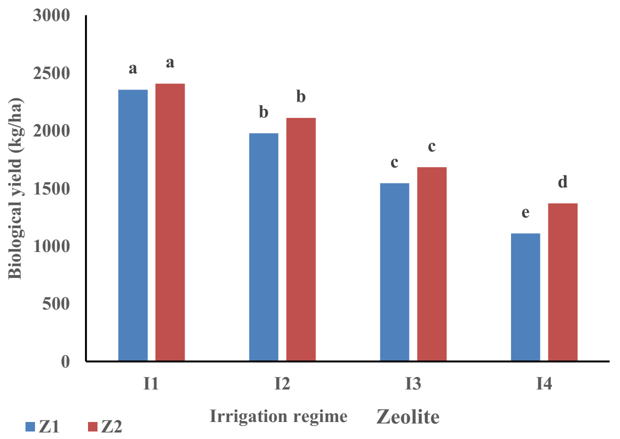

Fig. 2 The mean comparison of irrigation and zeolite interaction on biological yield

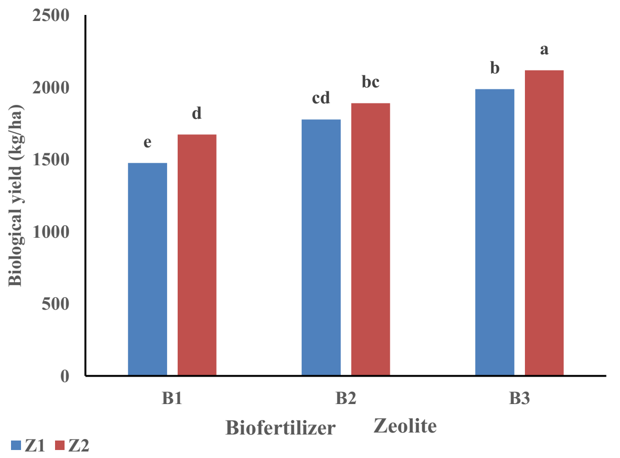

Fig. 3 The mean comparison of biofertilizer and zeolite interaction on biological yield

Adverse effects of drought stress in reducing essential oil yield of basil has been reported by Hasani *et al*. [29] and Refaat and Saleh [30]. The results indicated that the effect of water stress, biofertilizer and zeolite were significant on the content of chlorophyll a, b, total chlorophyll, proline content and RWC (at 1% levels).

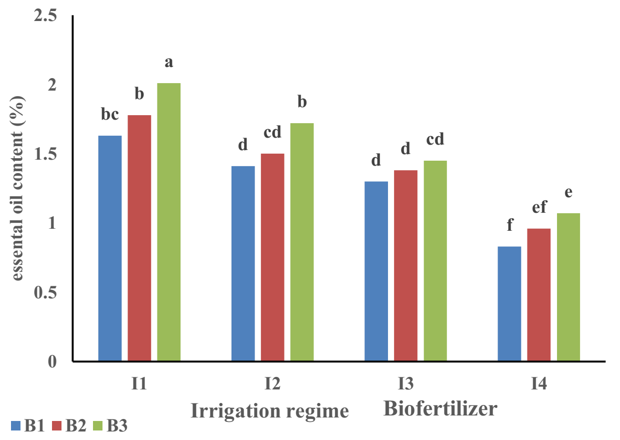

Fig. 4 The mean comparison of irrigation and biofertilizer interaction on essential oil content

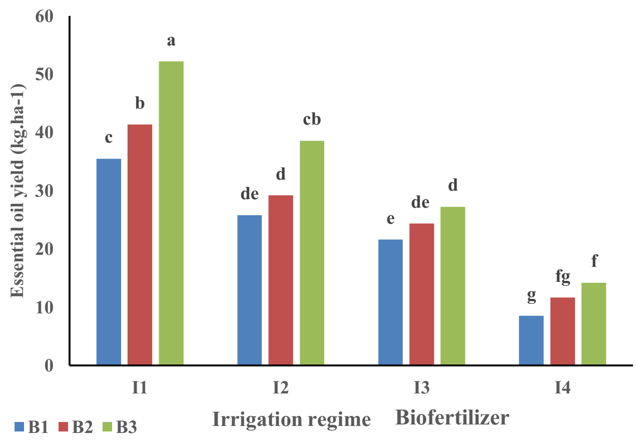

Fig. 5 The mean comparison of irrigation and biofertilizer interaction on essential oil yield

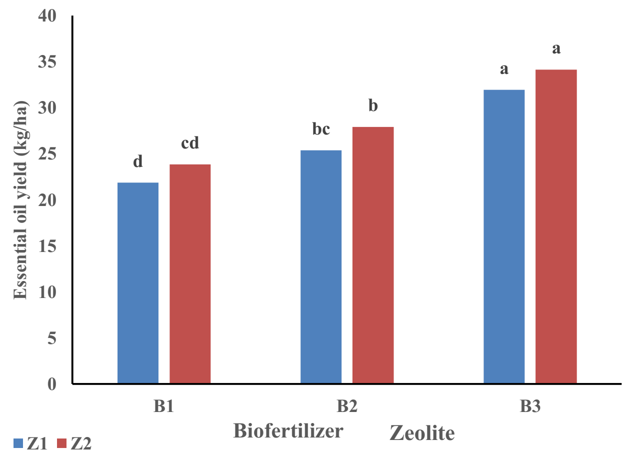

Fig. 6 The mean comparison of biofertilizer and zeolite interaction on essential oil yield

Ineraction of irrigation × biofertilizer and biofertilizer × zeolite also was significant on RWC (Table. 5). The results showed that by increasing the amount of stress, the content of chlorophyll a, b and finally total chlorophyll decreased. So that with increasing the irrigation after 90 mm evaporation, the amount of chlorophyll content decreased significantly (Table. 6).

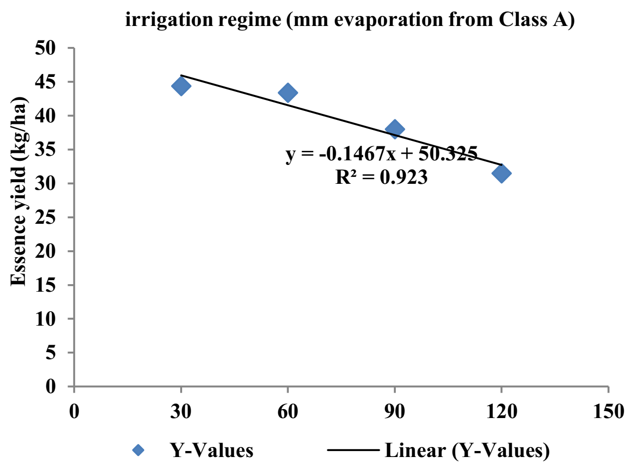

Fig. 7 Relationship between essential oil yield and irrigation regime

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The results also showed that inoculation treatments with tested bacteria and fungi increased the chlorophyll content so that treatments A_2 (*Azospirillum* and *Mycorrhiza*) and A_3 (*Pseudomonas* and *Mycorrhiza*) had the highest chlorophyll content. The use of zeolite treatment also increased the amount of chlorophyll content by 5% (Table. 6). Chlorophyll content in living plants is one of the important factors in maintaining photosynthetic capacity [31]. In this study, the amount of chlorophyll due to drought stress decreased sharply. It seems that the decrease in chlorophyll content due to drought stress is due to the increased production of oxygen radicals, which cause free radicals to decay and decompose this pigment [32, 33].

The results showed that increasing the irrigation intervals of more than 60 mm of evaporation increased the proline concentration in the plant. The use of bacterial and fungal inoculation treatments also increased the amount of proline in the plant. The application of zeolite at a rate of 20 t/ha reduced the amount of proline in the plant (Table. 6). Proline content increased with increasing intensity of drought stress. Proline molecules include hydrophilic and hydrophobic parts. Soluble proline can affect the solubility of various proteins and prevent albumin abnormalities. This property of proline is because interaction between proline and the level of hydrophobic proteins is established and due to the increase in the total level of hydrophilic protein molecules, their stability increases and inhibits their change in nature. Enzymes are also affected and protected by this proline mechanism due to their protein structure [34], which plants probably increase their proline for the reasons mentioned.

The results of the water content of cellular tissues in leaves showed that increasing the amount of stress decreased RWC. The use of *Pseudomonas* and *Mycorrhiza* as well as the application of zeolite treatment increased the amount of RWC (Table. 6). The results showed that the application of biofertilizers increased RWC in all irrigation conditions. In conditions of severe stress (120 mm), the use of biofertilizers was more effective in increasing RWC (Fig. 8). The effect of zeolite on increasing RWC was greater under non application of biofertilizer (Fig. 9). The rate of RWC in plants with high resistance against drought is higher than in others. In other words, the plant having higher yields under drought stress should have high RWC.

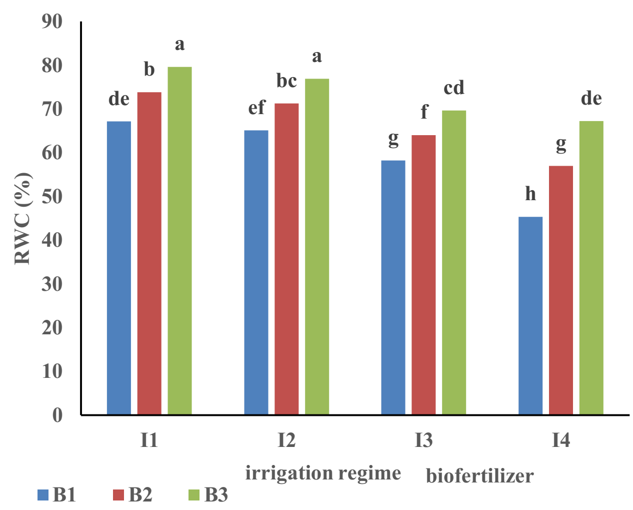

Fig. 8 The mean comparison of irrigation and biofertilizer interaction on RWC

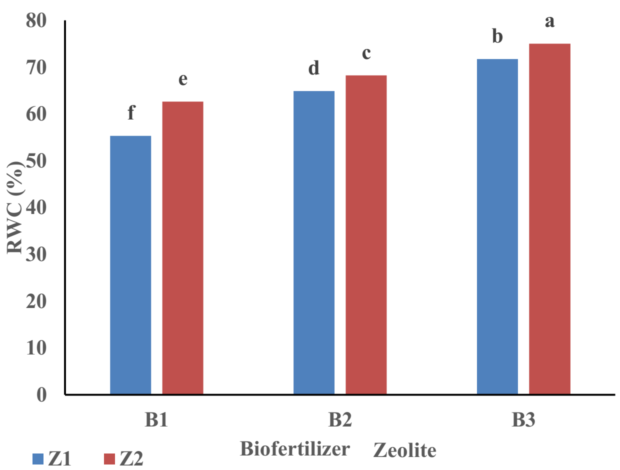

Fig. 9 The mean comparison of biofertilizer and zeolite interaction on RWC

Table 2 The variance analysis of factors effects on studied characteristics

S.O.V	D.F	Mean Square					
		Biological yield	Essential oil content	Essential oil yield	Plant height	Plant width	Stem diameter
Replication	3	501217.344 ns	0.988 ns	341.854 ns	40.032 ns	23.882 ns	0.004 ns
I	3	2877869.066 **	2.123 *	305.841 ns	141.121**	84.542 **	0.013 **
Error	9	204891.659	0.360	441.429	10.557	6.261	0.001
B	2	2113717.885 **	0.924 **	2572.327 **	510.112 **	307.689 **	0.049 **
I × B	6	232341.024 **	0.282 **	391.711**	4.184 ns	2.507 ns	0.0001 ns
Z	1	559218.010 **	0.297**	711.036 **	137.760**	82.696 **	0.013 **
I × Z	3	102257.622 **	0.084 ns	120.793 **	2.241 ns	1.343 ns	0.0001 ns
B × Z	2	60946.698 *	0.007 ns	18.687 ns	11.753 ns	6.969 ns	0.001 ns
I × B × Z	6	27612.642 ns	0.071 ns	48.132 ns	0.688 ns	0.402 ns	0.0001 ns
Error	60	30286.647	0.037	21.847	7.857	4.758	0.001
CV (%)	-	7.60	9.42	9.99	12.25	11.53	8.24

**and *: significant at 1 and 5%, respectively; ns: non-significant. I: irrigation regime, B: Biofertilizer, Z: Zeolite.

Table 3 The simple effects of treatments on agronomical traits

Irrigation regime	Essential oil content (%)	Plant height (cm)	Plant width (cm)	Stem diameter (cm)
I_1	1.93±0.03 a	42.1±0.06 a	29.2±0.06 a	0.55±0.001 a
I_2	1.81±0.02 b	35.5±0.07 b	27.5±0.07 ab	0.31±0.001 b
I_3	1.58±0.02 c	32.6±0.06 b	25.7±0.06 bc	0.29±0.002 b
I_4	1.47±0.02 d	28.2± 0.05c	23.1±0.07 c	0.27±0.001 b
biofertilizer				
B_1	1.43±0.01 c	35.4±0.06 b	25.2±0.07 b	0.36±0.001 b
B_2	1.65±0.02 b	40.3±0.05 a	29.1±0.06 a	0.38±0.001 a
B_3	1.82±0.01 a	39.5±0.06 a	28.4±0.06 a	0.41±0.001 a
zeolite				
Z_1	1.58±0.01 b	38.2±0.05 b	24.5±0.05 b	0.43±0.001 b
Z_2	1.69±0.01 a	41.1±0.04 a	26.3±0.05 a	0.45±0.001 a

Means within the same column and row factors, followed by the same letter are not significantly different (p<0.05). †: indicate the estimation of Standard Error of Means (SEM).

Table 4 ANOVA of regression analysis of essential oil yield and irrigation regimes relationship

Model	D.f	Mean Square	R	R Square	Adjuster R. Square
Regression	1	96.888 *	0.961 *	0.923	0.885
Residual	2	4.019	-	-	-
Total	3	-	-	-	-

Table 5 Mean squares of tested treatments effect on studied characteristics

S.O.V	D.F	Mean Square				
		Proline content	Total Chlorophyll	Chlorophyll b	Chlorophyll a	RWC
Replication	3	0.094 *	0.007 ns	0.154 *	6.831 ns	116.001 ns
I	3	0.331 **	0.025 **	0.537 **	24.036 **	724.283 **
Error	9	0.024	0.002	0.039	1.783	64.192
B	2	1.204 **	0.089 **	1.943 **	87.340 **	1751.183 **
I × B	6	0.010 ns	0.001 ns	0.016 ns	0.739 ns	21.783 *
Z	1	0.319 **	0.023 **	0.527 **	23.404 **	473.482 **
I × Z	3	0.005 ns	0.0001 ns	0.008 ns	0.342 ns	9.545 ns
B × Z	2	0.029 ns	0.002 ns	0.045 ns	2.045 ns	30.073 *
I × B × Z	6	0.002 ns	0.0001 ns	0.003 ns	0.121 ns	4.069 ns
Error	60	0.018	0.001	0.030	1.344	8.181
CV (%)	-	3.54	5.47	4.85	5.06	4.41

**and *: significant at 1 and 5 %, respectively; ns: non-significant. I: irrigation regime, B: Biofertilizer, Z: Zeolite

Table 6 The simple effects of treatments on physiological traits

Water stress	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Proline content (mM/g FW)
I_1	1.55±0.005† a	0.39±0.002 a	1.94±0.002 a	10.32±0.08 b
I_2	1.52±0.006 a	0.38±0.001 a	1.90±0.002 a	11.05±0.10 b
I_3	1.45±0.005 b	0.34±0.001 b	1.79±0.001 b	12.85±0.09 a
I_4	1.35±0.004 c	0.34±0.001 b	1.69±0.001 b	13.12±0.08 a
Biofertilizer				
B_1	1.50±0.004 b	0.35±0.001 b	1.85±0.001 b	11.24±0.08 b
B_2	1.51±0.004 b	0.36±0.001 a	1.87±0.001 a	11.58±0.07 b
B_3	1.56±0.004 a	0.36±0.001 a	1.87±0.001 a	12.68±0.07 a
Zeolite				
Z_1	1.48±0.003 b	0.34±0.001 a	1.81±0.002 b	11.88±0.07 a
Z_2	1.53±0.002 a	0.34±0.001 a	1.89±0.001 a	10.25±0.06 b

Means within the same column and row factors, followed by same letter are not significantly difference ($p<0.05$). †: indicate the estimation of Standard Error of Means (SEM).

Under water deficit, the cell membrane is subjected to changes such as an increase in penetrability and a decrease in sustainability [35].

CONCLUSION

The results show that severe drought stress (irrigation after 90 and 120 mm of evaporation) reduced biological yield and essential oil yield. However, the use of biofertilizers, especially Pseudomonas + Mycorrhiza, as well as the application of 20 t/ha zeolite under stress conditions improved these characteristics. Based on the results, the use of the mentioned biofertilizers and zeolite along with irrigation after 60 mm evaporation is effective in reducing water consumption and can achieve acceptable yield.

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