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RESEARCH ARTICLE

Effects of Salicylic Acid and Microorganisms on Morphological and Physiological Characteristics (*Satureja hortensis* L.) under Drought Stress

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ABSTRACT

Objective: As a critical limitation of plant growing, drought stress has always received a lot of attention from botanical researchers. This study intends to investigate the role of salicylic acid and the Mycorrhiza and Azotobacter bio-fertilizer on *Satureja hortensis* L. under drought stress.

Materials and Methods: Salicylic acid and bio-fertilizers have been shown to improve drought tolerance in growing plants. To evaluate the synergistic effect of salicylic acid, *Azotobacter chroococcum* bacteria, and arbuscular mycorrhizal fungi under drought stress (-3.5 atm: W1), (- 6.5 atm: W2), and (-10 atm: W3), a 2-year (2016 to 2018) field experiment was organized based on split-plot factorial statistical in a randomized complete block design, with three replicates.

Results: The main findings of this study showed that the combinable use of bio-fertilizers and salicylic acid diminished the disadvantageous effects of drought stress. Co-application of bio- fertilizers and salicylic acid significantly increased chlorophyll a and b (22% and 31.5%), carotenoid (30.7%) contents, aerial fresh (38.3%) and dry (64.1%) weights, root fresh (55.8%), and dry (45%) weights, auxin (15%), percentage of essential oil (30.7%) in *S. hortensis* while it decreased the proline content (48.8%) under severe stress as compared to the control groups, which confirmed the efficacy of this approach and its role in drought tolerance.

Conclusion: The results demonstrated that this new suggested treatment could effectively alleviate drought stress symptoms and improve *S. hortensis* growth under spreading drought conditions and limited water resources.

Keywords: Arbuscular Mycorrhizal Fungi, Bio-Fertilizers, Drought Stress, Phytohormone, *Satureja hortensis*

INTRODUCTION

Medicinal plants are plants whose quality of materials is far more important and necessary than their quantity. Therefore, in order to achieve maximum quality, knowledge and awareness of the factors affecting the growth and development of medicinal plants is very important. Knowledge of environmental, plant and agronomic factors has an important role in the success of medicinal plants.[1] Among the factors affecting the growth, development and production of active ingredients of medicinal plants and aromatic plants, the lack of which more than other inputs affects the reduction of production. Although extensive and comprehensive research has been done on the effect of water stress on crops, the behavior of medicinal plants in such conditions has not been well studied.[1] To understand the existence and survival of medicinal plants in arid and semi-arid regions, which also cover a large part of our country, extensive research is needed on plants with medicinal value and the application of various treatments.[2]

The use of Azotobacter has been considered as a biological fertilizer in agriculture due to its ability to stabilize molecular nitrogen in cooperation with plants as well as the production of growth-promoting hormones. In addition to the significant potential it has shown to improve the growth of host plants, this bacterium has been considered for other reasons such as the wide range of host plants, species diversity, and modulating the effects of environmental stresses. It is effective[3]. There are successful reports of the use of this bio-fertilizer to combat dehydration in plants[4]. Also, since the global approach in the production of medicinal plants is effective in improving the quantity and quality of material, it seems that the healthy nutrition of plants through the use of bio-fertilizers is most in

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line with the objectives of the production of medicinal plants.[5] Salicylic acid (SA) is a phenolic compound in plants that is considered a hormone-like regulator and plays an important protective role in defense mechanisms against biological and environmental stresses. Induction of flowering, growth and development, ethylene synthesis, orifice closure and respiration are important roles of SA[6]. This substance has increased resistance to water shortage in wheat.[7] In view of the above, this study intends to investigate the role of SA and the Mycorrhiza and Azotobacter bio-fertilizer on *Satureja hortensis* under drought stress.

MATERIALS AND METHODS

Plant Material

In order to study the effect of the application of Azotobacter (Az), Arbuscular Mycorrhizal Fungi (AMF) and SA on quantitative and qualitative properties of savoury (*S. hortensis* L) in drought stress conditions of this study in Zamanabad the village of Shahr-e Ray (Tehran province) for two cropping years (2016 and 2017); the crushed randomized complete block design (RCBD) was performed in three replications. Main plot of three irrigation levels (soil moisture potential -3.5 atm or crop capacity (FC), potential of -6.5 atm as medium stress and potential of -10 atm as severe stress), sub-factors including biological fertilizers of Az and Mycorrhiza with inoculation levels with Az strain, combination of seeds with AMF and combined use of Azotobacter-mycorrhiza (AM) and SA with non-foliar application and foliar application (with a concentration of 0.6 mM) were assigned. The physical and chemical characteristics of farm soil are shown in Tables 1 and 2.

Table 1. Physical properties the soil of field.

Texture	Clay(%)	Silt (%)	Sand (%)	S.P. (%)
Clay	35.71	37.78	25.51	35.29

S.P.: Poorly graded sand

Measurement of Photochemical Activity

Chlorophyll and Carotenoids

To measure the photosynthetic pigments (e.g., chlorophyll a, b and total chlorophyll), fully expanded mature leaves were sampled and dissolved in acetone (80%) and after being centrifuged, the absorbance of each sample was measured using a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) in wavelengths of 663.2, 646.8 and 470 nm for chlorophyll a, chlorophyll b and total chlorophyll content, and according to the following relations the amount of pigments were calculated based on the µg/g fresh weigh.[8]

Extraction of Essential Oil

Precisely weighed *S. hortensis* powder (200 g) was mixed with distilled water (3000 ml). The mixture was heated, and kept at a low boil for 4.5 h till the amount of oil in the vessel no longer increased, and then the heating was stopped. After 1 h, the volume of essential oil was recorded. The percentage of essential oil yield was calculated using the formula volume of essential oil divided by the weight of the sample powder. The volatile extract obtained was kept at 4 °C after drying with anhydrous sodium sulphate. The upper yellow oil was used as the sample for further analysis.

Analysis of Essential Oil

A GCMS-QP2010 Plus Mass Spectrometer (Shimadzu, Kyoto, Japan), equipped with a DB-5 MS capillary column (30.0 m × 0.25 mm; film thickness, 0.25 μm) and a mass spectrometry (MS) detector was used for GC-MS analysis. The injector temperature was 250 °C. The oven temperature was programmed from 50 °C (1 min isothermal) to 180 °C at a rate of 5 °C/min and then to 250 °C at a rate of 10 °C/min, and then kept for 6 min. The interface was kept at 280 °C. The mass spectra were obtained at 70 eV. The sector mass analyser was set to scan from 30 to 550 amu. Helium was used as a carrier gas with a flow rate of 1 mL/min. Essential oil (0.1 mL) of the sample was injected (in split mode 20:1). Volatile oil components were calculated as a relative percentage of the total oil using peak area. Retention index of all the components were determined by Kovats method using n-alkanes (C6–C32) as standards. Identification of individual constituents was accomplished by comparing their MS spectra by matching the mass spectral data with those from the NIST (NIST 08, NIST 08s; National Institute of Standards and Technology, Gaithersburg, MD, USA), and by comparison of their MS spectra and GC retention indices with those of standard compounds available in the laboratory and also by comparison with some other relevant references.[9]

Auxin

In order to measure the amount of auxin (IAA), 1 gr of leaf tissue from the leaves near the top of the stem (leaf+stem) and the root was boiled separately in 10 ml of 80% ethanol, and after grinding, it was passed through filter paper and then an amount of 1 ml A litre of the obtained extracts was poured into separate test tubes and 2 ml of Salkowski's reagent was added to each test tube. (In order to prepare Salkowski's reagent, 0.5 M ferric chloride ($FeCl_3$) solution was first prepared. Then, one ml of this solution was mixed with 50 ml of 35% perchloric acid and after stirring the mixture, Salkowski's reagent was prepared). Then, the tubes were placed in a Bain-Marie at 40-50°C for 15 minutes until the complete reaction and the presence of IAA in the extract was revealed with a pink colour. At the end, the

Table 2. Chemical properties the soil of field.

K (Available)	P (Available)	Zn (ppm)	Fe (ppm)	Organic C (%)	Total N (%)	H	EC (dS m^{-1})
580	8.16	0.37	3 .18	1.33	0.09	0.48	1. 02

EC: Electrical conductivity

optical absorbance of the samples was measured at 530 nm by a Pharmacia LKB-Novaspac spectrophotometer. The amount of IAA in the samples was calculated using a standard curve in the range of 0 to 40 mg/L. Pure IAA was used to draw the standard curve.[10]

Proline

Proline content was estimated using ninhydrin reaction.[11] A portion (0.5 g) of shoot was homogenized with 10 mL of 3% (w/v) sulphosalicylic acid and passed through Whatman filter paper (no. 2; Whatman, Maidstone, UK). Ninhydrin reagent (2 mL) (Sigma, St. Louis, Missouri, USA) and glacial acetic acid (2 mL) were added to 2 mL of the filtered extract. The mixture was then incubated at 100°C for 1 h and the reaction was terminated by placing it on ice. The reaction mixture was extracted with 4 mL toluene and the absorption of chromophore was measured at 520 nm, against toluene as blank, using the spectrophotometer. Proline content was calculated using L-proline (Sigma) as a standard curve. Plants were harvested at the time of flowering and the samples were dried in the drying at 105 °C up to constancy for the required time and then weighed to measure dry weight. After determining the dry weight, 10 g of the leaves and flowering twigs of each sample was sent to the laboratory. The percentage of essential oil by water distillation was measured by Clevenger Model 500HM6. Essential oil yield was calculated by multiplying the percentage of essential oil by the dry weight of the vegetative body.

Statistical Analysis

Data analysis with software SAS 9.4 and comparison of the mean of treatments with least significant difference (LSD) test was performed at a probability level of 0.05%. Graphs were drawn using Excel software.

RESULTS

Aerial Fresh Weight (AFW) and Aerial Dry Weight (ADW)

This study showed that AMF, Az, SA, and Az+AMF with SA had improved AFW and ADW in all irrigation levels. On the other hand, drought stress reduced AFW and ADW by 40.8% and 55.4% under the W3 drought than the control (Table 3). The inoculation with Az+AMF+0.6 mM SA spraying in every irrigation regime had the most positive effect on *S. hortensis* AFW and ADW compared with control. AFW and ADW plants sprayed with 0.6 mM of SA in some of the treatments did not show any significant difference comparing to non-sprayed plants in the same treatments. Nevertheless, inoculated with Az+AMF enhanced the AFW and ADW 17.1% and 34.4% at W1 conditions, while inoculation with Az+AMF+SA causes increased AFW and ADW 23.6% and 57.3%. Also, under drought stress conditions (W2 and W3) application of bio-fertilizers, SA either or together showed no significant difference compared with the control (13.6 and 8.1 g) for ADW. The highest AFW (103 g) and ADW (28.8 g) were observed from the inoculation with Az+AMF and 0.6 mM SA spraying under W1 irrigation conditions (Table 3).

Root Fresh Weight (RFW) and Root Dry Weight (RDW)

Drought stress diminished the RFW and RDW. The RFW and RDW decreased under the mild drought stress W2 (24.7% and 22.8%) and severe drought stress W3 (52.6% and 49.2%) as compared with the control (3.63 and 1.4 g). Under W2 and W3 conditions, the RFW and RDW indicated a non-significant difference between means with application Az, AMF, SA, and compound with SA as compared with the control treatments, while using of AMF+SA, Az+AMF alone and with SA caused a significant improvement in the root fresh and dry weight in W1 irrigation conditions. Also, the greatest the root fresh (6.2 g) and dry (2.2 g) weight was seen in plants treated with Az+AMF and sprayed with SA in W1 condition (Table 4).

Chlorophyll and Carotenoid Contents

The results of this work showed various levels of drought (W2 and W3) caused a reduction in the concentration of photosynthetic pigments. The lowest chlorophyll a, b, and carotenoid were obtained in W3 stress, which indicated a decrease of 40%, 39.4%, and 48%, respectively, than W1 irrigation conditions (Table 5). Moreover, it has been reported that the chlorophyll decline may be due to the chloroplast disintegration and the instability of the chlorophyll protein complex. The chlorophyll a, b significantly enhanced with the application of AMF and Az+AMF alone or with SA at W1 and W3 watered conditions in comparison with control groups, but under the W2 situation, just the Az+AMF+SA treatment had increased the chlorophylls a and b contents. Carotenoid was significantly enhanced by the

Table 3. Comparison of two-year means of aerial fresh weight (AFW) and aerial dry weight (ADW) of *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels.

Treatment		**AFW (g)**			**ADW (g)**		
Bio-fertilizers	SA (mM)	W1	W2	W3	W1	W2	W3
Non-inoculation	0	83.3 $^{c-f}$	71.2 $^{e-h}$	49.3 ^{j}	18.3 $^{d-f}$	13.6 $^{f-i}$	8.1 ^{i}
	0.6	85.7 $^{b-e}$	72.7 $^{d-h}$	53.8 ^{ij}	18.6 $^{c-f}$	14.6 $^{f-h}$	10.6 ^{hi}
Azotobacter (Az)	0	87.5 $^{b-d}$	73.2 $^{d-h}$	60 $^{h-j}$	18.6 $^{c-f}$	15.3 $^{e-h}$	10.6 ^{hi}
	0.6	87.5 $^{b-d}$	73.7 $^{d-h}$	61 $^{h-j}$	21.1 $^{b-e}$	15.3 $^{e-h}$	11.1 ^{hi}
Mycorrhiza (AMF)	0	88.7 $^{a-d}$	75.3 $^{d-h}$	61.3 $^{h-j}$	21.6 $^{b-d}$	16 $^{d-h}$	11.1 ^{hi}
	0.6	95.5 $^{a-c}$	78.7 $^{d-g}$	65.2 $^{g-i}$	24 $^{a-c}$	17.1 $^{d-g}$	12.1 $^{g-i}$
Co-inoculation with Az+AMF	0	97.7 ^{ab}	79.5 $^{d-g}$	65.5 $^{g-i}$	24.6 ^{ab}	17.3 $^{d-g}$	13.1 $^{f-i}$
	0.6	103 ^{a}	80.3 $^{c-g}$	62.8 $^{g-i}$	28.8 ^{a}	17.8 $^{d-g}$	13.3 $^{f-i}$

Means followed by the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*); SA: Salicylic acid.

Table 4. Comparison of two-year means of root fresh weight (RFW) and root dry weight (RDW) of *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels.

Treatment		**RFW (g)**			**RDW (g)**		
Bio-fertilizers	SA (mM)	W1	W2	W3	W1	W2	W3
Non-inoculation	Control	3.63 $^{d.g}$	2.73 $^{f.j}$	1.72 ^{j}	1.40 $^{d.g}$	1.08 $^{g.j}$	0.71 ^{k}
	0.6	3.77 $^{d.f}$	2.79 $^{f.i}$	1.9 $^{l.j}$	1.42 $^{c.f}$	1.08 $^{g.j}$	0.83 ^{jk}
Azotobacter (Az)	0	3.85 ^{de}	2.79 $^{f.i}$	2.32 $^{h.j}$	1.48 $^{c.e}$	1.15 $^{f.j}$	0.92 $^{i.k}$
	0.6	3.88 ^{de}	2.82 $^{f.i}$	2.35 $^{h.j}$	1.55 $^{c.e}$	1.23 $^{e.i}$	0.95 $^{h.k}$
Mycorrhiza (AMF)	0	4.27 ^{cd}	3.18 $^{e.h}$	2.38 $^{h.j}$	1.63 ^{cd}	1.26 $^{d.i}$	0.95 $^{h.k}$
	0.6	5.02 ^{bc}	3.43 $^{d.g}$	2.41 $^{h.j}$	1.78 ^{c}	1.28 $^{d.i}$	0.96 $^{h.k}$
Co-inoculation with Az+AMF	0	5.37 ^{ab}	3.48 $^{d.g}$	2.62 $^{g.j}$	2.10 ^{ab}	1.33 $^{d.h}$	1 $^{h.k}$
	0.6	6.20 ^{a}	3.51 $^{d.g}$	2.68 $^{g.j}$	2.27 ^{a}	1.33 $^{d.h}$	1.03 $^{g.k}$

Means followed by the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*); SA: Salicylic acid.

application of AMF+SA and Az+AMF alone or with SA at W1 conditions (Table 5). The highest mean of the carotenoid (0.35 mg g^{-1}) was achieved in the W1 irrigation level and integrated application of Az+AMF and SA. Under the W2 irrigation condition, integrated application of Az+AMF with SA had a significant effect on carotenoid content compared to the control, but under W3 drought stress application of bio-fertilizers and SA showed no significant effect between treatments (Table 5).

Proline

The results of the analysis of 2-year data showed the impact of drought stress, bio-fertilizers, SA, and their interaction was significant on the proline content. As shown in Table 6, with rising drought stress, the amount of proline improved. Outcomes revealed that Az+AMF treatments decreased the proline content under well-watered and water deficiency situations. As a result, the highest proline content (47.7 µg g^{-1}) was obtained under W3 conditions. This treatment showed 15.8% increases compared to the control treatment (40.9 µg g^{-1}).

Auxin

As shown in the results of Table 6, the interaction between treatments on IAA content was significant. IAA content of the *S. hortensis* plants was reduced in W2 and W3 stress situations, while we achieved a higher IAA content in all treatments inoculated with Az+AMF.

Essential Oil

The result of our study showed plants inoculated with any of the bio-fertilizers alone or together improved the quantity of essence percentage under all irrigation conditions. Plants

Table 5. Comparison of two-year means of chlorophylls and carotenoid contents of *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels.

	Treatment		Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})
	Bio-fertilizers	SA (mM)			
W1	Non-inoculation	Control	0.5 $^{d.f}$	0.38 $^{e.g}$	0.25 $^{d.f}$
		0.6	0.51 $^{c.e}$	0.4 $^{d.f}$	0.25 $^{d.f}$
	Azotobacter (Az)	0	0.55 $^{b.d}$.	0.41 $^{c.e}$	0.27 $^{c.e}$
		0.6	0.56 $^{a.c}$	0.43 $^{c.d}$	0.27 $^{c.e}$
	Mycorrhiza (AMF)	0	0.56 $^{a.c}$	0.43 $^{c.d}$	0.28 $^{c.d}$
		0.6	0.58 ^{ab}	0.45 ^{bc}	0.30 ^{bc}
	Co-inoculation with Az+AMF	0	0.6 ^{ab}	0.48 ^{ab}	0.33 ^{ab}
		0.6	0.61 ^{a}	0.5 ^{a}	0.35 ^{a}
W2	Non-inoculation	Control	0.43 $^{g.j}$	0.33 $^{h.j}$	0.19 ^{gh}
		0.6	0.45 $^{f.i}$	0.33 $^{h.j}$	0.19 ^{gh}
	Azotobacter (Az)	0	0.45 $^{f.i}$	0.35 $^{g.i}$	0.2 ^{gh}
		0.6	0.46 $^{e.h}$	0.35 $^{g.i}$	0.21 $^{f.g}$
	Mycorrhiza (AMF)	0	0.48 $^{e.g}$	0.35 $^{g.i}$	0.22 $^{f.g}$
		0.6	0.48 $^{e.g}$	0.36 $^{f.h}$	0.23 $^{e.g}$
	Co-inoculation with Az+AMF	0	0.48 $^{e.g}$	0.36 $^{f.h}$	0.23 $^{e.g}$
		0.6	0.5 $^{d.f}$	0.38 $^{e.g}$	0.25 $^{d.f}$
W3	Non-inoculation	Control	0.33 ^{k}	0.23 ^{l}	0.13 ^{i}
		0.6	0.38 $^{j.k}$	0.25 ^{l}	0.13 ^{i}
	Azotobacter (Az)	0	0.38 ^{jk}	0.25 ^{l}	0.14 ^{i}
		0.6	0.38 ^{jk}	0.26 ^{kl}	0.15 ^{i}
	Mycorrhiza (AMF)	0	0.4 ^{ij}	0.3 ^{jk}	0.15 ^{i}
		0.6	0.4 ^{ij}	0.31 ^{ij}	0.16 ^{hi}
	Co-inoculation with Az+AMF	0	0.4 ^{ij}	0.31 ^{ij}	0.17 ^{hi}
		0.6	0.41 $^{h.j}$	0.33 $^{h.j}$	0.17 ^{hi}

Means followed by the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*); SA: Salicylic acid.

Table 6. Comparison of two-year means of proline and auxin (IAA) of *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels.

Treatment		Proline (µg g^{-1} FW)			IAA (µg g^{-1} FW)		
Bio-fertilizers	SA (mM)	W1	W2	W3	W1	W2	W3
Non-inoculation	Control	40.9 ^{c}	45.5 ^{b}	47.7 ^{a}	0.85 $^{d.f}$	0.82 ^{fg}	0.80 ^{g}
	0.6	40.6 ^{c}	44.3 ^{b}	45.1 ^{b}	0.87 $^{c.e}$	0.85 $^{d.f}$	0.83 $^{e.g}$
Azotobacter (Az)	0	35.2 ^{de}	36.5 ^{d}	40.2 ^{c}	0.89 $^{a.d}$	0.87 $^{c.e}$	0.83 $^{e.g}$
	0.6	33.9 ^{ef}	35.8 ^{d}	39.5 ^{c}	0.90 $^{a.d}$	0.88 $^{b.d}$	0.85 $^{d.f}$
Mycorrhiza (AMF)	0	30.1 ^{hi}	31.4 ^{gh}	33.8 ^{ef}	0.90 $^{a.d}$	0.89 $^{a.d}$	0.87 $^{c.e}$
	0.6	28.8 ^{lj}	30.3 ^{gh}	32.7 ^{fg}	0.93 ^{ab}	0.90 $^{a.d}$	0.89 $^{a.d}$
Co-inoculation with Az+AMF	0	25.5 ^{kl}	27.0 ^{jk}	28.7 ^{ij}	0.93 ^{ab}	0.92 $^{a.c}$	0.90 $^{a.d}$
	0.6	22.1 ^{n}	23.3 ^{mn}	24.4 ^{lm}	0.95 ^{a}	0.93 ^{ab}	0.92 $^{a.c}$

Means followed by the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*); SA: Salicylic acid.

sprayed with the SA and inoculated with Az+AMF showed the highest percentage of essence 29.2%, 29.7% and 30.7%, respectively under well-watered and stress conditions (W2, W3) compared to control. However, the application of SA with every of Az, AMF, or by their combination significantly enhanced the percentage of the essence under all of the irrigation levels compared to the plants treated with SA alone. Therefore, the highest percentage of essence (1.8 v/w) was induced by spraying 0.6 mM of SA+Az+AMF together as inoculation at W3 drought (Figure 1).

Thymol Content

Results of the analysis of the data showed that the effect of drought stress, fertilizers Az and AMF, spraying of SA and their interaction on Thymol content was significant. Plants inoculated with any one of the bio-fertilizers alone or together, their Thymol content has been statistically increased compared to the control treatment. Drought stress increased the content of thymol. The W3 drought conditions enhanced the content of thymol by 20.5% compared to control. However, the application of Az, AMF, or their combination showed an increased Thymol content under all irrigation regimes. Furthermore, SA spray increased the content of Thymol 16.4%, 15% and 5.6%, respectively at W1, W2 and W3 levels of irrigation, however, this increase wasn not significant in the W3 irrigation level. Also, SA had a significant effect on Thymol content in non-inoculated and inoculated plants with either the combination of Az+AMF, just Az, or just AMF in all irrigation treatments. Based on the mean comparison, the highest Thymol content (4.71%) was obtained by the combination treatment (Az+AMF + SA) at W3 drought and the lowest (3.5%) was in untreated plants (control) in W1 irrigation level (Figure 2).Thymol Content Results of the analysis of the data showed that the effect of drought stress, fertilizers Az and AMF, spraying of SA and their interaction on Thymol content was significant. Plants inoculated with any one of the bio-fertilizers alone or together, their Thymol content has been statistically increased compared to the control treatment. Drought stress increased the content of thymol. The W3 drought conditions enhanced the content of thymol by 20.5% compared to control. However, the application of Az, AMF, or their combination showed an increased Thymol content under all irrigation regimes. Furthermore, SA spray increased the content of Thymol 16.4%, 15% and 5.6%, respectively at W1, W2 and W3 levels of irrigation, however, this increase wasn not significant in the W3 irrigation level. Also, SA had a significant effect on Thymol content in non-inoculated and inoculated plants with either the combination of Az+AMF, just Az, or just AMF in all irrigation treatments. Based on the mean comparison, the highest Thymol content (4.71%) was obtained by the combination treatment (Az+AMF + SA) at W3 drought and the lowest (3.5%) was in untreated plants (control) in W1 irrigation level (Figure 2).

Carvacrol Content

The results presented in (Figure 3) show significant effects of the irrigation treatments and inoculated with Az+AMF, as drought stress W3 increased carvacrol content 77.1%. As well as plants treated with Az, AMF and the combination of Az+AMF had greater carvacrol content in all irrigation levels W1, W2 and W3 compared with control. Foliar application of SA in non-inoculated plants under W1 irrigation treatment was not statistically significant on carvacrol content, while spraying SA revealed significant enhancement under W2 and W3 conditions. However, under all irrigation treatments, carvacrol content in plants treated with either the combination of SA and Az+AMF increased. Therefore, the highest carvacrol content (38.5%) was achieved in plants inoculated with the Az+AMF+SA under W3 drought stress.

DISCUSSION

Drought stress had adverse effects on the studied traits of the *S. hortensis* medicinal plant, the application of SA and microorganisms improved these adverse effects of stress. Drought stress reduced the morphological characteristics of the *S. hortensis* medicinal plant. It reported the drought stress diminished RDW and ADW in two Thymus species but, inoculation with AMF in low and intense drought stress enhanced the *T. daenensis* species root and shoot dry weight.[12] The influence of drought stress on decreasing the ADW and RDW of plants can be related to that water shortage, lowers the absorption, transferring, and utilization of nutrients, which results in a decline in carbon storage and biomass.[13] It has been reported that the seedlings of valerian inoculated with AMF, Az, and combination of Az+AMF had higher ADW and RDW under good and deficit irrigation conditions than the corresponding non-inoculation seedlings that is in line with this result.[14]

The treatment with bio-fertilizers can counteract the drought stress influences on plant production, particularly at severe drought stress. It is recognized as a positive impact of bio-fertilizers on improving plants' nutritional conditions under stressful environments conditions. Plant growth promoting *Rhizobacteria* have a key role in stimulating the nitrogen uptake and the effectiveness of it in the functioning of photosynthesis and plant growth.[15] It was found that the use of PGPR increased microbial activity because they can produce an exo-polysaccharide, which leads to improved soil physical and chemical conditions such as soil structure, soil aggregation, and soil penetrance following improved soil moisture-holding capacity.[16] It reported improvement of the components of yield and productivity in wheat using the application of PGPR (soil application) alongside SA (foliar spraying) that might be by collaboration with IAA and/or cytokinin synthesis, the stimulation of cell division, and photosynthesis.[17] It has been reported in the plant (*Valeriana officinalis L.*) that rising drought stress

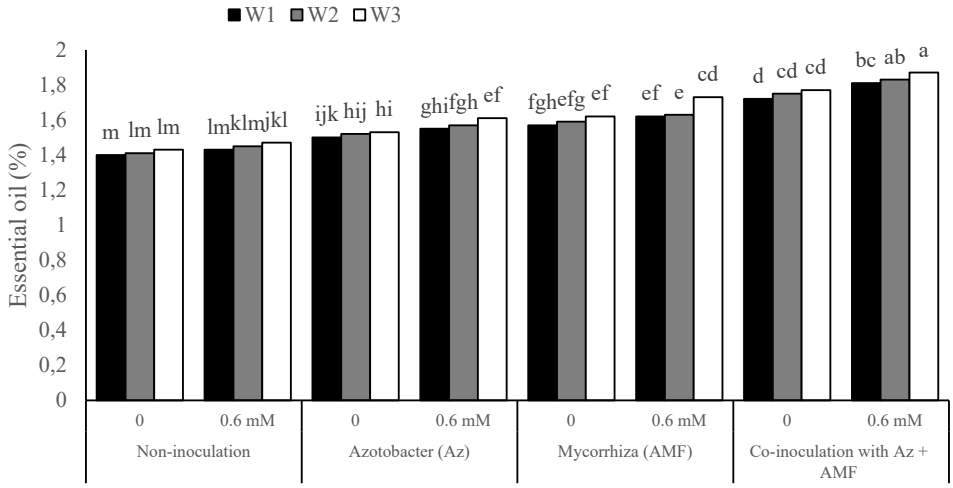

Figure 1. Comparison of two-year means of essential oil *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels. The letters a-m and f indicate that the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*).

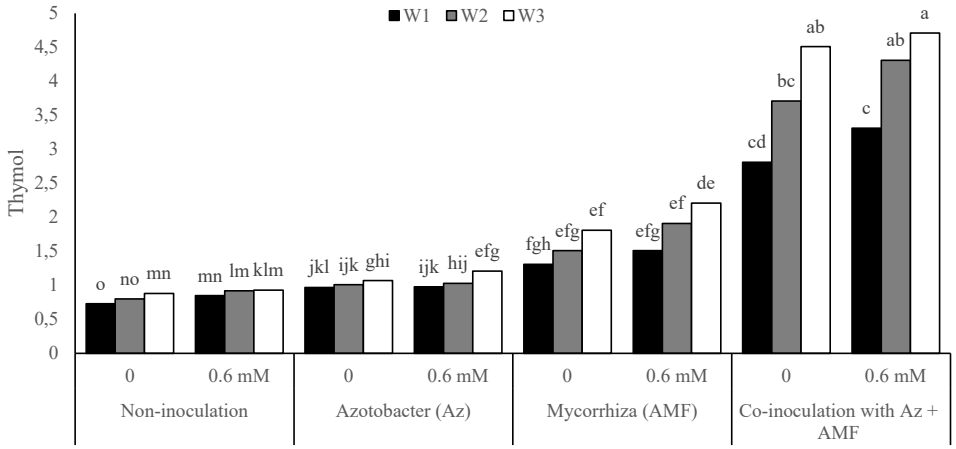

Figure 2. Comparison of two-year means of thymol content oil *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels. The letters a-m indicate that the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*).

caused decreasing RDW, while bio-fertilizers enhancement the RDW than in the control (without bio-fertilizers). The PGPR stimulated the root growth by improving the nutrient conditions, increasing microbial activity, and influencing other useful symbiotic relationships.[18] It has been reported that the plant (*Scutellaria integrifolia L.*) inoculation with AMF can cause an impressive root growth and increment the power of the plant to overcome difficult conditions such as phosphorus deficiency.[19] Furthermore, increased RDW of the marjoram plant inoculated with bacteria was attributed to increment lateral roots and root nutrients sorption capacity.[4]

Some of the PGPR, which produce *Rhizobiotoxin* via diminishing ethylene production in the plants, enhanced root growth.[20] The proper ratio between nitrogen and phosphorus improves root yield too, and has a useful result on plant growth. Since the role of Az has been proven in incrementing the solvability of phosphorus from inorganic insoluble compounds, thus, Az can provide a correct level between nitrogen and phosphorus by producing suitable hormones or decreasing ethylene. It can lead to increment root and plant growth.[21] Foliar spray of SA leads to stimulation of root and shoot growth, and as well as strengthened the effect of PGPR on root and shoot growth.[22] Therefore, PGPRs can sustain plant grow under drought stress circumstances. SA, by cooperation in absorption and translocation of essential elements in the plant, caused an increasing plant growth.[23] Besides, foliar spray of SA acts as a cofactor in adjusting the physiological attributes, thus enhancing leaf water content and photosynthetic processes that lead to improved root hairs performance to uptake water and raise plant growth. As a result, it was observed that the application of PGPR with

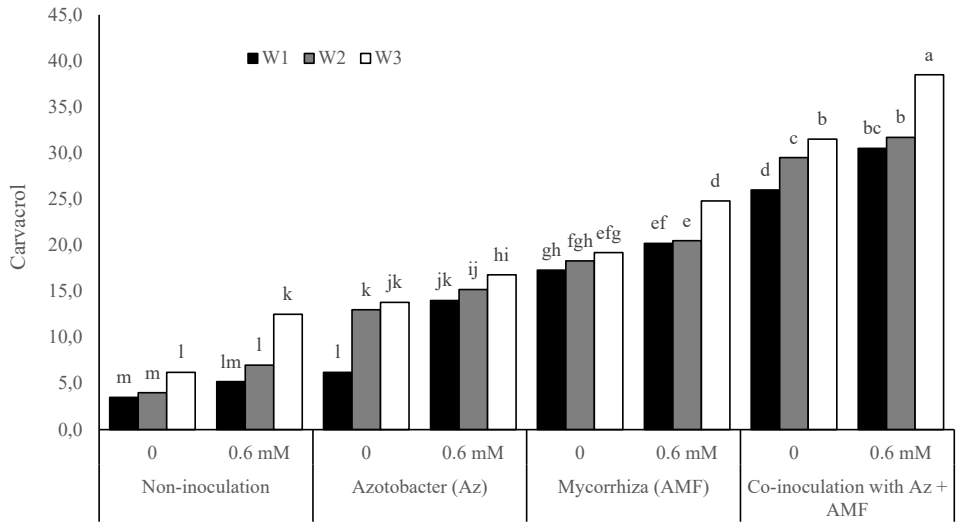

Figure 3. Comparison of two-year means of carvacrol content *Satureja hortensis* by different bio-fertilizers and SA treatments under different irrigation levels. The letters a-m indicate that the same letter in each column are not significantly different according to the LSD test (p=0.05). Control: W1 (Field capacity); *Medium stress*: W2 (6.5 *atm*); *Severe stress*: W3 (10 *atm*).

SA was effective in alleviating the detrimental effects of water deficit and improving the nutritional situation of plants.[24]

Decreased chlorophyll content under the water deficit condition agrees with other findings.[24] The reduction of chlorophyll a and b concentration may be related to the oxidative burst induced through drought stress, which can decompose these pigments via producing oxygen radicals in cells.[25] About this context, it was reported that application of the PGPR, SA, or their combination in wheat plants mitigated significantly injurious impacts of water deficiency.[24] In plants inoculated with PGPR, SA helped to improve the physiological attributes and photosynthetic activity via raised CO_2 assimilation rates because of enhanced stomata conductance, RWC and photosynthetic pigment concentrations under water shortage.[26] Besides, increased chlorophyll concentration in leaves using PGPR alone or with SA could be related to higher accessibility of nutrients and improved organic matter in the Rhizosphere.[23]

Carotenoid concentration enhancement under water deficiency, in addition to protecting the photosynthetic apparatus, probably improved the cells' defensive system, therewith decreasing the destruction induced via oxidative stress.[27] On this subject, a reduction was seen in carotenoid contents in the thymus species under drought stress conditions,[12] but AMF with inoculation increased its contents, which is in line with our result. PGPRs could stimulate the manufacture of carotenoids via activating the plastidial methylerythritol phosphate (MEP) pathway as the precursor for the synthesis of carotenoids.[28] Thus, enhancing the carotenoid concentration stimulates defence mechanisms activated with these PGPRs to ameliorate plant endurance to stress status.[29]

The use of bio-fertilizers in severe drought stress can be more efficient in reducing proline accumulation, in comparison with non-treated plants.[14] Proline acts as an essential signalling part against drought stress to stimulate mitochondria performing and change cell replication, increasing the expression of special recovery genes related to drought stress.[30] Increasing proline content helps to sustain membrane structure by protecting the cell redox potential and diminishing the reactive oxygen species (ROS) level through reducing lipids peroxidation.[31]

It has been revealed that T. aestivum that has a higher potential for accumulate proline shows higher percentages of endurance.[32] Also, the lowest proline content (22.1 µg g^{-1}) was related to treatment inoculation with Az+AMF+SA. This treatment showed 45.9% decreases compared to the control. A similar observation reported that water stress statistically enhanced proline in two Thymus species but, all treatments inoculated with AMF resulted in reduced proline in every level of water stress.[12] The results of other research studies stated that plants treated with the combination of PGPR and SA significantly decreased the proline accumulation and brought the amounts near to the irrigation control treatment.[33] Although the *S. hortensis* plants were in water deficiency conditions (W2) and treated with Az+AMF or combination (Az+AMF+SA) did not show statistically significant differences regarding proline content compared to W1 treatments that were similar with the Hafez et al.[24] results. Besides, application SA on plants under stress conditions and inoculation with Az+AMF caused significant decreases of proline than non- application of SA (27 and 28.7 µg g^{-1}). The Synergistic effects between PGPR and SA decreased the proline accumulation under water shortage situations due to the osmotic regulation, scavenging ROS and sustaining the integrity of subcellular constructions in plant cells, moderating of the adverse effects of water shortage.[34]

Lowering IAA content under stress may result from diminished IAA synthesis or a raise in the destruction of IAA by stimulating the activity of the oxidase enzyme.[35] However, PGPRs with leak phytohormone in the soil can moderate the endogenous levels of phytohormone IAA.[1] Results exhibited that with rising drought stress levels, the IAA content decreased, which was similar to the previous report by Khan, et al.[33] The highest content of IAA (0.95 µg g^{-1} FW) was obtained with the use of Az+AMF and SA under irrigation W1 conditions, and the lowest content of this trait (0.80 µg g^{-1} FW) was related to the treatment of non-inoculation under W3 irrigation condition. Khan et al.[33] reported that spraying of SA was also efficient and improved the IAA content in both the shoot and rhizosphere. The application PGPR, SA, and their combined treatment raised the IAA significantly.[33] When plants were inoculated with AZ+AMF, AMF alone, and with SA, Az+SA under stress conditions showed no significant difference compared to plants under the W1 conditions. All the treatments have ameliorative results, but the treatment with SA exhibited a lessened influence in this context, which could be due to the fundamental difference between the PGPR and SA in the biosynthesis or modulation of phytohormones. In general, IAA production is due to the direct operation of PGPR on crops, that enhances the number of root and absorption surface area of the plant. Therefore, under drought stress follows efficient absorption of nutrients and water by plant roots.[36] High levels of IAA are produced via the intermediate indole-pyruvic acid pathway.[37] The presence of the ipdC gene in the bacteria gives it the capacity to provide enough contents of IAA that could be utilized for plant growth increment.[38]

Previous studies have proved that carvacrol, thymol, and their precursors, p-cymene, and - terpinene are the main components of the *S. hortensis* oil.[39] As well as the concentrations of carvacrol and thymol in Iranian accessions of *S. hortensis* are deficient. Although the essential oil content and its composition in *S. hortensis* is genetically controlled, but variations in phytochemical characters have different origins.[40] Biosynthesis of essential oil components is relevant to producing valuable terpenes that can be considered a defensive response to different stresses.[41] It has been reported that environmental factors such as water stress and inoculation with biofertilizers could significantly influence the biosynthetic pathway and accumulation of natural compounds. As well as was demonstrated that with the use of AMF or rhizobacteria, the production of valuable terpenes in the plant would also be altered.[4] Mohammadi et al.[42] reported that inoculation of *S. hortensis* with certain *P. fluorescens* Migula strains can significantly increase the plant biomass and some essential oil yields under water stress conditions.

CONCLUSION

The findings of this work demonstrated that the percentage and composition of the essential oil in *S. hortensis* increased significantly under W2 and W3 treatments, while decreased plant FW and DW, chlorophylls and carotenoid contents, and IAA. Application of these rhizobacteria and fungi in soil and spraying SA improved the growth and tolerance of plants under stress by increasing proline, carvacrol, and thymol. The improved growth in response to biofertilizers inoculation at each level of irrigation could be ascribed to the biosynthesis IAA, thereby regulating root development and defence system metabolism to improve drought-tolerance.

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REFERENCES

1. Achard P, Gusti A, Cheminant S, et al. Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr Protoc*. 2009;19(14):1188-1193.
2. Anjum S, Wang L, Farooq M, Khan I, Xue L. Methyl jasmonate induced alteration in lipid peroxidation, antioxidative defence system and yield in soybean under drought. *J Agron Crop Sci*. 2011a;197(4):296-301.
3. Elewa TA, Sadak MS, Saad AM. Proline treatment improves physiological responses in quinoa plants under drought stress. *Biol Res*. 2017;14(1):21-33.
4. Banchio E, Bogino PC, Zygadlo J, Giordano W. Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana L. Biochem Syst Ecol*. 2008;36(10):766-771.
5. Smith SE, Facelli E, Pope S. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant and Soil*. 2010;326:3-20.
6. Brundrett MC, Tedersoo L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol*. 2018;220(4):1108-1115.
7. Bakry B, El-Hariri D, Sadak M, El-Bassiouny H. Drought stress

mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil. *J Appl Sci Res*. 2012;8(7):3503-3514.

8. Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr Protoc Food Anal Chem*. 2001,1:F4-3. doi.org/10.1002/0471142913.faf0403s01.
9. Pang YX, Fan ZW, Wang D, et al. External application of the volatile oil from *Blumea balsamifera* may be safe for liver-A study on its chemical composition and hepatotoxicity. *Molecules* 2014;19:18479–18492.
10. He Y, Oyaizu H, Suzuki S. Indole-3-acetic acid production in Pseudomonas fluorescens HP72 and its association with suppression of creeping bentgrass brown patch. *Curr Microbiol*. 2002;47:138–143.
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil*. 1973;39:205.
12. Arpanahi AA, Feizian M, Mehdipourian G, Khojasteh DN. Arbuscular mycorrhizal fungi inoculation improve essential oil and physiological parameters and nutritional values of *Thymus daenensis Celak* and *Thymus vulgaris* L. under normal and drought stress conditions. *Eur J Biol*. 2020;100:103217.
13. Hu Y, Schmidhalter U. Drought and salinity: A comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci*. 2005;168:541-549.
14. Javan Gholiloo M, Yarnia M, Ghorttapeh AH. Evaluating effects of drought stress and bio-fertilizer on quantitative and qualitative traits of valerian (*Valeriana officinalis* L.). *J Plant Nutr.* 2019;42(13):1417-1429.
15. Kader M, Mian, M. Hoque, M. Effects of Azotobacter inoculant on the yield and nitrogen uptake by wheat. *J Biol Sci*. 2002;2(4):259-261.
16. Kasim WA, Osman ME, Omar MN, Abd El-Daim IA, Bejai S, Meijer J. Control of drought stress in wheat using plant-growth-promoting bacteria. *J Plant Growth Regul*. 2013;32(1):122-130.
17. Asl KK, Hatami M. Application of zeolite and bacterial fertilizers modulates physiological performance and essential oil production in dragonhead under different irrigation regimes. *Acta Physiol Plant*. 2019;41(1):17.https://doi.org/10.1007/s11738-018-2801-x.
18. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*. 2003;255:571-586.
19. Joshee N, Mentreddy S, Yadav AK. Mycorrhizal fungi and growth and development of micropropagated *Scutellaria integrifolia* plants. *Ind Crops Prod*. 2007;25(2):169-177.
20. Schippers B, Bakker A, Bakker P, Van Peer R. Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. *Plant and Soil*. 1990;129(1):75-83.
21. Copetta A, Lingua G, Berta G. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza*. 2006;16(7):485-494.
22. Hafez E, Gharib H. Effect of exogenous application of ascorbic acid on physiological and biochemical characteristics of wheat under water stress. *Int J Plant Prod*. 2016;10(4):579-596.
23. Khan N, Bano A, Babar MA. The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria. *Symbiosis*. 2017;72(3):195-205.
24. Hafez E, Omara AED, Ahmed A. The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. *Agron*. 2019;9(9):524.
25. Anjum SA, Xie X-Y, Wang L-C, Saleem MF, Man C, Lei W. Morphological, physiological and biochemical responses of plants to drought stress. *Afr J Agric Res*. 2011b;6(9):2026-2032.
26. Hafez EM, Ragab AY, Kobata T. Water-use efficiency and ammonium-N source applied of wheat under irrigated and desiccated conditions. *Int J Plant Soil Sci*. 2014;3(10):1302-1316.
27. Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proc Natl Acad Sci*.2012;109(14):5535-5540.
28. Baslam M, Esteban R, García-Plazaola JI, Goicoechea N. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. *Appl Microbiol Biotechnol*. 2013;97(7):3119-3128.
29. Kamali S, Mehraban A. Nitroxin and arbuscular mycorrhizal fungi alleviate negative effects of drought stress on Sorghum bicolor yield through improving physiological and biochemical characteristics. *Plant Signaling Behav*. 2020;15(11):1813998.
30. Jayant KS, Sarangi S. Effect of drought stress on proline accumulation in peanut genotypes. *Int J Adv Res*. 2014;2(10):301-309.
31. Shinde S, Villamor JG, Lin W, Sharma S, Verslues PE. Proline coordination with fatty acid synthesis and redox metabolism of chloroplast and mitochondria. *Plant Physiol*. 2016;172(2):1074-1088.
32. Zhou W, Li Y, Zhao B-C, et al. Overexpression of TaSTRG gene improves salt and drought tolerance in rice. *J Plant Physiol*. 2009;166(15):1660-1671.
33. Khan N, Bano A, Curá JA. Role of beneficial microorganisms and salicylic acid in improving rainfed agriculture and future food safety. *Microorg*. 2020;8(7):1018.
34. Hafez E, Seleiman M. Response of barley quality traits, yield and antioxidant enzymes to water-stress and chemical inducers. *Int J Plant Prod*. 2017;11(4):477-490.
35. Muhammad N, Hakim Quraishi U, Chaudhary HJ, Munis MFH. Indole-3-acetic acid induces biochemical and physiological changes in wheat under drought stress conditions. *Philipp Agric Sci*. 2016;99:19-24.
36. Vacheron J, Desbrosses G, Bouffaud M-L, et al. Plant growth-promoting rhizobacteria and root system functioning. *Fron Plant Sci*. 2013;4:356. https://doi.org/10.3389/fpls.2013.00356.
37. Ahmed A, Hasnain S. Auxins as one of the factors of plant growth improvement by plant growth promoting rhizobacteria. *Pol J Microbiol*. 2014;63(3):261-266.
38. Rosier A, Medeiros FH, Bais HP. Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. *Plant and Soil*. 2018;428(1-2):35-55.
39. Vernet P, Gouyon R, Valdeyron G. Genetic control of the oil content in *Thymus vulgaris* L: A case of polymorphism in a biosynthetic chain. *Genetica*. 1986;69(3):227-231.
40. Pank F, Pfefferkorn A, Kruger H. Evaluation of a summer savory collection (*Satureja hortensis* L.) with regard to morphology, precocity, yield components and essential oil and carcacrol content, Zeitschrift Fur Arznei Und Gewurzpflanzen. 2004;p.72.
41. Selmar D, Kleinwächter M. Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiol*. 2013;54(6):817-826.

42. Mohammadi H, Dashi R, Farzaneh M, Parviz L, Hashempour H. Effects of beneficial root pseudomonas on morphological, physiological, and phytochemical characteristics of *Satureja hortensis (Lamiaceae)* under water stress. *Braz J Biol*. 2017;40(1): 41-48.

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