Quantitative and Qualitative Changes of Rosemary (*Rosemarinus officinalis* L.) in Response to Mycorrhizal Fungi (*Glomus intraradices*) Inoculation under Saline Environments

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Abstract

Background: Arbuscular mycorrhizal fungi (AMF) have the potential to optimize the rhizospheric soil characteristics considerably thereby affecting plant growth.
Objectives: The aim is to investigate the effects of fungi inoculation (*Glomus intraradices*) on morphological, physiological and phytochemical traits of Rosemary (*Rosemarinus officinalis* L.) under salt stress.
Methods: A factorial experiment was conducted on the basis of randomized complete blocks design in three replications at the Institute of Medicinal Plants in the Academic Center for Education, Culture and Research (ACECR). The mycorrihzal fungi in two levels (inoculation and non-inoculation) and saline conditions in five levels (EC of 0, 2, 4, 6 and 8 $dS.m^{-1}$) were the two studied factors. The essential oil content and its components were measured with Clevenger-type apparatus and GC/Mass and GC.
Results: The results showed that the interaction effect of fungi inoculation and salinity was significant ($P\leq0.01$) on morphological and phytochemical traits. The highest number of shoots per plant in fungi inoculation and salinity of 2 $dS.m^{-1}$, number of leaves per plant in fungi inoculation and salinity of 6 $dS.m^{-1}$, stem diameter in non-inoculation and salinity of 8 $dS.m^{-1}$ was recorded, while the maximum leaf width in non-inoculation and salinity of 2 $dS.m^{-1}$, stems fresh weight in fungi inoculation and control and, roots fresh and dry weight in treatment of fungi inoculation for both traits and salinity of 8 $dS.m^{-1}$ and 6 $dS.m^{-1}$ was recorded, respectively. Also, interaction effect of fungi inoculation and salinity significantly increased essential oil content and its components.
Conclusion: The phytochemical and morpho-physiological traits of rosemary were improved due to inoculation of mycorrhizal fungi under saline conditions.

Keywords: ***Glomus intraradices,*** **Mycorrhizal inoculation, Quantitative and qualitative changes,** ***Rosemarinus officinalis*** **L., Salt stress**

Introduction

Rosemary (*Rosmarinus officinalis* L.) from Lamiaceae family is endemic plant to the Mediterranean regions and commonly cultivated in many parts of Iran [1]. Rosemary is an aromatic evergreen shrub that its abundant branches are soft and fluffy. The essential oil of plant has anti-microbial properties [2, 3]. It increases blood circulation and is effective on rheumatism and migraine [4].

Soil salinity as a worldwide problem restricts plant growth and production in many parts of the world especially in arid and semi arid regions. Environmental stresses especially salinity and drought has the most effect on medicinal plants [5]. The different results were dedicated from the effect of salinity stress on the quantitative and qualitative traits. For instance it was found that increasing of salinity stress decreased almost all of growth parameters in *Nigella sativa*, some growth traits and essential oil amount in chamomile [6, 9] and essential oil in Lemon Balm [7]. Also, effect of salinity on essential oil quality in Lemon verbena showed the increased amount of geranial as salinity level was increased [8]. Soil salinity causes the significant yield decrease through reducing nutrients uptake and increasing osmotic stress of plants [10, 11].

Among strategies to counteract this problem, application of arbuscular mycorrhizal (AM) fungi is more efficient on growth and yield of vigorous plants [12]. It should be noted that seven kinds of mycorrhiza: arbutoid mycorrhiza, ectomycorrhiza, endomycorrhiza or arbuscular mycorrhiza, ectendomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza, and orchidoid mycorrhiza have been recognized [13-15]. However, AM fungi are most commonly found in the rhizosphere roots of a wide range of herbaceous and woody plants [16]. The plant roots provide substances for the fungi and the fungi transfer nutrients and water to the plant roots [17, 18]. Endomycorrhizal fungi are inter and intracellular and penetrate the root cortical cells and form finger like branched structures called arbuscular and vesicles to be known as vesicular arbuscular mycorrhiza (VAM). In some cases no vesiclesare formed and they are known as arbuscular mycorrhiza (AM) [19, 20]. Most of arbuscular mycorrhizal fungi inoculation resulted increase in plant dry biomass, percent of colonization and the barbaloin content of *Aloe vera* [21]. The mycorrhizal symbiosis provides the plant with an increased ability for nutrient capture and cycling in soils with low nutrient availability [22]. Vazquez-Hernandez *et al*. (2011) cited that inoculation of papaya (*Carica papaya* L.) plants with mycorrhizal fungi increased the number of fruits and yield in these plants [23]. Therefore, the aim of this study is to investigate the effects of mycorrhiza inoculation on growth and phytochemical parameters of rosemary (*Rosmarinus officinalis* L.).

Methods and Materials

This experiment was carried out at the Medicinal Plants Institute (MPI) affiliated with the Academic Center for Education, Culture and Research (ACECR), (35° 54′ N and 50°

53′ E; 1461 m elevation) during 2013-2014. The soil was loam-silt with 0.071% nitrogen, 8.4 p.p.m phosphorous, 163.4 p.p.m potassium, EC 2.71 $dS.m^{-1}$, and pH 8.3.

In order to assess the effect of Arbuscular Mycorrhiza Fungi (AMF) on growth parameters and essential oil content of *Rosemarinus officinalis* L. plants under salt stress, *Glomus intraradices* was used for the inoculation of soil. This factorial experiment was done on the basis of randomized complete blocks design with 10 treatments and 3 replications. Two levels of inoculation and non-inoculation of mycorrhiza fungi (*Glomus intraradices*), and five levels of salinity (0, 2, 4, 6, and 8 $dS.m^{-1}$) were the two studied factors.

The studied parameters were plant height (cm), collar diameter of stem (mm), stem diameter (mm), number of leaves pre plant (leaves. $plant^{-1}$), leaf length (mm), leaf width (mm), number of shoots per plant (shoots.$plant^{-1}$), stems fresh and dry weight (g) , roots fresh and dry weight (g), leaves fresh, and dry weight (g), SPAD value, and content of essential oil (%), α-pinene (%), camphene (%), β-pinene (%), myrcene (%), limonene (%), and 1,8-cineole (%), linalool (%), camphor (%), borneol (%), α-terpineol (%), isophorane (%), isobornyl acetate (%) and trans-caryophylene (%).

Soil used for production of mycorrhizal inoculums was collected from field and mixed with sand (1:5 w/w) and 100 g fungi inoculums. Soil and sand were autoclaved before mixing at 120°C for 4 h. Plants were grown at 32°C under 16 h light and 8 h dark periods and were illuminated by white fluorescent light and sodium lamp with total irradiance of about 75 $\mu Em^{-2}s^{-1}$. Rorison's solution was used as nutrient medium. Finally, roots were removed from the soil, cut and then mixed with the soil. This inoculums included soil/sand mixture, extra radical hyphae, and spores and colonized roots. After 15 days of transplanting, they were treated with related saline water, i.e. control treatment and EC of 2, 4, 6, and 8 $dS.m^{-1}$ [24]. The effect of these bio-inoculants on different growth parameters of rosemary plants was recorded after 80 days of inoculation. Essential oils of the aerial parts were extracted by hydro-distillation method for 3 h using Clevenger-type apparatus [25]. The oils were dried over anhydrous sodium sulphate and kept at 4 °C until it was analyzed. GC analysis was carried out on a Younglin Instrument Acme 6000 M gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5 capillary column (30 m×0.25 mm; 0.25 μm film thicknesses). The oven temperature was held at 50ºC for 5 minutes, and then programmed at 3ºC min^{-1} to 240ºC and after that programmed at 15ºC min^{-1} to 300ºC (held for 3 minutes). Other operating conditions were: carrier gas, He with a flow rate of 0.8 mL min^{-1}; injector and detector temperatures was 290ºC, and split ratio, 1:10. GC/MS analysis was performed on a GC mentioned above coupled with an Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35-375 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils

were identified by comparison of their mass spectra and retention indices with those published in the literature [26, 27] and presented in the MS computer library. Each analysis was performed in triplicate.

Analysis of variance of the results was done using the SPSS software (ver. 17), and means in the results were compared by "Duncan's multiple range tests" at $P \leq 0.01$.

Results

The result indicated that the interaction effect of mycorrhizal fungi inoculation and salinity had significant effect on the morphological traits including number of shoots per plant, stem diameter, number of leaves per plant, leaf width, stems fresh weight, roots fresh and dry weight, while it was not significant for the plant height, collar diameter of stem, leaf length, stems dry weight, leaves fresh and dry weight and SPAD value (Table 1). Concerning the mean comparisons, the maximum number of shoots per plant was obtained at fungi inoculation treatment under EC of 2 $dS.m^{-1}$. However, the minimum number of shoots was observed in plants treated by fungi inoculation and EC of 8 $dS.m^{-1}$. The greatest stem diameter was evident in the treatment of non-inoculation and EC of 8 $dS.m^{-1}$ and the least with inoculation and 4 $dS.m^{-1}$. The highest number of leaves was found in fungi inoculation and EC of 6 $dS.m^{-1}$ and the lowest number in non-inoculation and EC of 8 $dS.m^{-1}$. The maximum and minimum leaf width was obtained in non-inoculation of mycorrhizal fungi and salinity of 2 $dS.m^{-1}$, and also, fungi inoculation with salinity level of 8 $dS.m^{-1}$, respectively. The maximum and minimum amount of roots fresh weight (25.8 g and 8.13 g) were obtained in fungi inoculation at salinity level of 6 $dS.m^{-1}$ and the treatment of fungi inoculation at 4 $dS.m^{-1}$, respectively. The maximum amount of roots dry weight (12.63 g) in fungi inoculation and salinity level of 8 $dS.m^{-1}$ and the lowest amount (4.93 g) in fungi inoculation and salinity level of 2 $dS.m^{-1}$ were found. The highest stems fresh weight (12.06 g) was attained in fungi inoculation and salinity level of 0 $dS.m^{-1}$, while the lowest (3.8g) of that was reported in fungi inoculation and 8 $dS.m^{-1}$ (Table 2).

According to variance analysis on phytochemical traits, the interaction effect of mycorrhiza fungi inoculation and salinity was significant on essential oil, α-pineneand 1, 8-cineole. Considering interaction effects of mycorrhiza fungi inoculation and salinity, the highest content of essential oil (2.00 ± 0.11%) was obtained under fungi inoculation and EC of 2 $dS.m^{-1}$, while, the lowest value was observed in mycorrhiza inoculation and 6 and 8 $dS.m^{-1}$ (Table 3). The maximum content of α-pinene (29.13 ± 1.15%) by non-inoculation and salinity of 2 $dS.m^{-1}$ and the lowest of that (24.80 ± 0.38%) in plants inoculated by fungi and 6 $dS.m^{-1}$ was attained (Table3). According to mean comparisons, the greatest amount of 1, 8-cineole (8.41 ± 0.88%) was obtained in non-inoculation and 8 $dS.m^{-1}$and the lowest value of that was recorded in fungi inoculation and 2 $dS.m^{-1}$ (Table 3). The inoculation of mycorrhiza fungi improved the camphene content and the highest content (8.17 ± 0.23%) was related to 8 $dS.m^{-1}$. However, non-inoculation was resulted in highest content of

Table 1- Analysis of variance for effects of *Mycorriliza* fungi and salinity on measured traits in rosemary (*Rosemarinus officinalis* L.)

Source of variance	d.f.	Plant height	Collar diameter of stem	Number of shoots per plant	Stem diameter	Number of leaves per plant	Leaf length	Leaf width	Stems fresh weight	Stems dry weight	Roots fresh weight	Roots dry weight	Leaves fresh weight	Leaves dry weight	SPAD value
Rep. (block)	2	13.24^{ns}	1.65^{ns}	0.301^{ns}	0.056^{ns}	0.04^{ns}	47.43^{**}	2.48^{*}	8.82^{**}	0.19^{ns}	0.036^{ns}	29.95^{**}	37.06^{**}	27.37^{**}	5.51^{ns}
Mycorrhiza (M)	1	94.34^{**}	0.8^{ns}	3.07^{*}	0.19^{ns}	0.61^{**}	16.88^{**}	1.62^{ns}	0.84^{ns}	9.63^{**}	0.005^{ns}	0.000003^{ns}	12.28^{*}	23.58^{**}	375.52^{**}
Salt treatment (S)	4	635.43^{**}	1.04^{ns}	44.31^{**}	1.94^{**}	0.28^{**}	36.61^{**}	9.7^{**}	31.9^{**}	15.72^{**}	0.257^{**}	15.17^{**}	90.62^{**}	39.52^{**}	25.12^{ns}
M×S	4	9.03^{ns}	0.51^{ns}	6.00^{**}	0.43^{*}	1.36^{**}	4.66^{ns}	2.14^{*}	8.06^{**}	0.5^{ns}	0.327^{**}	22.58^{**}	0.39^{ns}	0.21^{ns}	23.33^{ns}
Error	18	7.26	0.57	0.65	0.14	0.015	6.46	0.55	0.29	0.73	0.024	1.009	2.21	1.5	17.85
CV (%)		7.83	16.17	15.1	18.56	2.44	8.08	19.41	7.77	20.18	5.88	11.41	7.66	9.89	14.1

Table 1- Continued

Source of variance	d.f.	Essential oil content	α-pinene	Camphene	β- Pinene	Myrcene	Limonene	1,8-Cineole	Linalol	Camphor	Borneol	α-Terpineol	Isophorane	Isobornyl acetate	Trans-caryophylene
Rep.(block)	2	0.18^{**}	8.39^{*}	0.0^{ns}	0.0^{ns}	0.0003^{ns}	0.194^{*}	0.21^{ns}	0.00001^{ns}	0.22^{ns}	0.36^{*}	0.00008^{ns}	0.002^{ns}	0.00004^{ns}	0.00001^{ns}
Mycorrhiza (M)	1	0.002^{ns}	0.04^{ns}	1.32^{**}	0.73^{**}	3.65^{**}	0.12^{ns}	1.01^{ns}	0.02^{ns}	0.139^{ns}	0.09^{ns}	0.097^{**}	0.28^{**}	5.94^{**}	0.47^{**}
Salt treatment (S)	4	0.205^{**}	6.67^{**}	0.54^{**}	0.15^{**}	0.68^{**}	0.082^{ns}	0.49^{**}	0.08^{**}	0.168^{**}	0.13^{ns}	0.013^{**}	0.061^{**}	1.83^{**}	0.91^{**}
M×S	4	0.186^{**}	6.17^{*}	0.058^{ns}	0.03^{ns}	0.53^{**}	0.119^{ns}	1.03^{*}	0.124^{**}	0.07^{ns}	0.207^{ns}	0.0058^{*}	0.069^{**}	0.56^{*}	0.15^{*}
Error	18	0.011	1.4	0.035	0.014	0.079	0.044	0.23	0.008	0.17	0.57	0.0015	0.0075	0.143	0.043
CV (%)		10.94	4.3	2.43	10.57	7.19	4.9	6.74	3.86	4.26	5.17	16.99	5.48	8.36	13.23

Table 2 - Interaction and mean effects of different treatments on measured traits*

Mycorrhiza Fungi	Salinity level ($dS.m^{-1}$)	Plant height (cm)	Collar diameter of stem (mm)	Number of shoots per plant ($Shoots.plant^{-1}$)	Stem diameter (mm)	Number of leaves per plant ($Leaves.Plant^{-1}$)	Leaf length (mm)	Leaf width (mm)	Stems fresh weight (g)	Stems dry weight (g)	Roots fresh weight (g)	Roots dry weight (g)	Leaves fresh weight (g)	Leaves dry weight (g)	SPAD value (SPAD)
Non-inoculation	0	43.3^{a}	4.83	8^{a}	1.62^{b}	221.67^{a}	35.16^{a}	4.66^{a}	10.15^{a}	6.12^{a}	19.8^{a}	11^{a}	23.52^{a}	15.26^{a}	30.84
	2	41.7^{ab}	4.8	8.2^{a}	1.75^{b}	202.33^{ab}	32.33^{b}	4.95^{a}	8.34^{b}	5.53^{ab}	14.4^{b}	9.93^{a}	21.95^{a}	13.76^{b}	28.72
	4	39.15^{b}	4.83	4.85^{b}	1.88^{b}	189.67^{b}	31^{bc}	4.63^{a}	7.05^{c}	4.38^{b}	13.91^{b}	7.51^{b}	20.05^{b}	12.95^{b}	31.51
	6	29.08^{c}	4.99	3.58^{c}	1.91^{b}	179^{b}	30.08^{cd}	2.55^{b}	5.09^{d}	3.25^{c}	14.04^{b}	8.18^{b}	18.08^{c}	11.33^{c}	31.73
	8	18.93^{d}	3.94	2^{d}	3.04^{a}	121.33^{c}	28.66^{d}	2.33^{b}	4.58^{d}	2.07^{d}	10.89^{c}	7.41^{b}	13.5^{d}	8.55^{d}	26.98
Non-inoculation		32.67^{b}	4.84	5^{b}	2.12	156^{b}	30.7^{b}	3.59	6.87	3.67^{a}	14.33	8.81	18.78^{b}	11.47^{b}	26.42^{b}
Inoculation		36.22^{a}	4.52	5.6^{a}	1.95	210^{a}	32.2^{a}	4.06	7.21	4.8^{a}	14.88	8.81	20.06^{a}	13.26^{a}	33.49^{a}
Non-inoculation	0	42.75	4.52	7.33^{bc}	1.6^{d}	263^{b}	33	4.33^{ab}	8.25^{bc}	5.13	14.83^{b}	8.46^{cd}	22.76	14.3	26.7
	2	40.96	5.18	6.33^{cd}	1.66^{cd}	213^{c}	31.3	5.06^{a}	7.86^{cd}	4.6	16.85^{bcd}	10.08^{bc}	21.73	13.16	22.3
	4	37.53	4.93	4.66^{e}	2.26^{bc}	114.3^{de}	30.66	4.93^{ab}	6.83^{ef}	4.06	13.65^{bcd}	8.04^{d}	19.26	12.06	29.26
	6	25.5	5.43	4.33^{e}	1.63^{cd}	110^{de}	30.16	1.33^{c}	6.08^{g}	2.9	13.8^{bcd}	10.2^{b}	17.5	10.46	28.1
	8	16.62	4.16	2.33^{f}	3.37^{a}	79.3^{e}	28.33	2.306^{c}	5.36^{g}	1.63	12.49^{cd}	7.23^{d}	12.63	7.43	25.73
Inoculation	0	43.92	5.13	8.66^{ab}	1.63^{cd}	95^{de}	37.33	5^{ab}	12.06	7.1	13.96^{bcd}	7.9^{d}	24.26	16.23	34.98
	2	42.5	4.43	10.0^{a}	1.82^{cd}	191.6^{c}	33.33	4.83^{ab}	8.83^{b}	6.1	11.18^{de}	4.93^{e}	22.16	14.36	35.13
	4	40.77	4.73	5.03^{de}	1.5^{d}	128.3^{d}	31.33	4.33^{ab}	7.26^{de}	4.7	8.13^{e}	6.76^{d}	20.83	13.83	33.76
	6	32.67	4.55	2.83^{f}	2.13^{bcd}	333.3^{a}	30	3.76^{b}	4.1^{h}	3.6	25.8^{a}	11.8^{ab}	18.66	12.2	35.36
	8	21.25	3.73	1.66^{f}	2.7^{b}	300^{ab}	29	2.36^{c}	3.8^{gh}	1.8	15.33^{bc}	12.63^{a}	14.36	9.66	28.23

* Means in each column followed by the same letter are not significantly different ($P \leq 0.01$)

Table 3- Changes in content of essential oil and its components of *Rosemarinusofficinalis* L. by inoculation of mycorrhiza fungi under saline conditions.

Mycorrhiza Fungi	Salinity level ($dS.m^{-1}$)	Essential oil (%)	α-pinene (%)	Camphene (%)	β-Pinene (%)	Myrcene (%)	Limonene (%)	1,8-Cineole (%)
non-inoculation	0	1.00 ± 0.09^{d}	28 ± 2.1^{a}	6.9 ± 0.4	1.03 ± 0.04	3.85 ± 0.025^{cde}	3.83 ± 0.22	7.21 ± 0.29^{bc}
	2	1.20 ± 0.02^{c}	29.13 ± 1.15^{a}	7.85 ± 0.07	1.01 ± 0.05	3.73 ± 0.08^{cdef}	4.38 ± 0.33	7.07 ± 0.06^{bc}
	4	1.00 ± 0.09^{d}	27.7 ± 1.75^{a}	7.57 ± 0.07	0.95 ± 0.08	5.12 ± 0.61^{a}	4.35 ± 0.36	7.01 ± 0.53^{bc}
	6	1.20 ± 0.03^{c}	27.8 ± 0.13^{a}	7.43 ± 0.14	0.97 ± 0.07	4.07 ± 0.08^{bc}	4.28 ± 0.16	7.12 ± 0.03^{bc}
	8	1.00 ± 0.11^{d}	25.34 ± 1.11^{b}	7.79 ± 0.04	0.79 ± 0.16	4.47 ± 0.28^{b}	4.2 ± 0.04	8.41 ± 0.88^{a}
Inoculation	0	1.20 ± 0.05^{c}	28.25 ± 2.35^{a}	7.55 ± 0.08	1.34 ± 0.12	3.43 ± 0.24^{ef}	4.34 ± 0.32	6.98 ± 0.17^{bc}
	2	2.00 ± 0.11^{a}	28.5 ± 1.47^{a}	7.97 ± 0.13	1.38 ± 0.14	3.21 ± 0.35^{f}	4.33 ± 0.34	6.53 ± 0.57^{c}
	4	1.40 ± 0.17^{b}	28.15 ± 1.29^{a}	8.09 ± 0.19	1.37 ± 0.13	3.5 ± 0.2^{def}	4.28 ± 0.2	7.19 ± 0.4^{bc}
	6	0.80 ± 0.11^{e}	24.8 ± 0.38^{b}	7.86 ± 0.07	1.36 ± 0.13	4.02 ± 0.06^{bcd}	4.18 ± 0.05	7.56 ± 0.19^{b}
	8	0.80 ± 0.05^{e}	27.9 ± 1.18^{a}	8.17 ± 0.23	0.86 ± 0.12	3.6 ± 0.15^{cdef}	4.54 ± 0.13	6.72 ± 0.78^{c}

* Means in each column followed by the same letter are not significantly different ($P < 0.01$)
Data are presented as mean ± SE.

Table 3- continued

Mycorrhiza Fungi	Salinity level ($dS.m^{-1}$)	Linalol (%)	Camphor (%)	Borneol (%)	α-Terpineol (%)	Isophorane (%)	Isobronyl acetate (%)	Trans-Caryophylene (%)
non-inoculation	0	2.07 ± 0.09^{e}	9.32 ± 0.54	5.91 ± 0.13	1.49 ± 0.11^{a}	4.79 ± 0.08^{cd}	5.38 ± 0.43^{a}	2.26 ± 0.34^{a}
	2	2.24 ± 0.01^{cd}	9.5 ± 0.45	5.52 ± 0.24	1.3 ± 0.02^{bc}	4.8 ± 0.07^{cd}	4.4 ± 0.06^{cd}	1.23 ± 0.17^{ef}
	4	2.18 ± 0.04^{cde}	9.55 ± 0.42	5.65 ± 0.4	1.32 ± 0.03^{b}	5.79 ± 0.42^{b}	3.45 ± 0.53^{de}	4.41 ± 0.08^{de}
	6	2.05 ± 0.1^{e}	9.99 ± 0.1	5.62 ± 0.59	1.3 ± 0.02^{bc}	6.76 ± 0.91^{a}	3.31 ± 0.6^{e}	1.46 ± 0.06^{de}
	8	2.6 ± 0.17^{a}	10.26 ± 0.23	5.84 ± 0.09	1.26 ± 0.0^{bc}	4.99 ± 0.02^{cd}	3.83 ± 0.34^{e}	0.89 ± 0.34^{f}
Inoculation	0	2.42 ± 0.08^{b}	9.32 ± 0.44	5.32 ± 0.41	1.23 ± 0.02^{c}	4.28 ± 0.33^{de}	5.28 ± 0.38^{ab}	2.15 ± 0.29^{ab}
	2	2.12 ± 0.07^{de}	9.69 ± 0.33	5.39 ± 0.33	1.22 ± 0.02^{cd}	4.89 ± 0.03^{cd}	5.29 ± 0.39^{ab}	1.83 ± 0.13^{bc}
	4	2.15 ± 0.05^{de}	9.72 ± 0.62	5.53 ± 0.24	1.12 ± 0.07^{e}	5.4 ± 0.23^{bc}	4.84 ± 0.16^{abc}	1.71 ± 0.07^{cd}
	6	2.4 ± 0.07^{b}	9.87 ± 0.04	6.07 ± 0.21	1.24 ± 0.01^{bc}	3.87 ± 0.54^{e}	4.73 ± 0.11^{bc}	1.43 ± 0.07^{de}
	8	2.31 ± 0.035^{bc}	10.7 ± 0.65	5.65 ± 0.4	1.14 ± 0.06^{de}	3.91 ± 0.52^{e}	4.68 ± 0.08^{bc}	1.38 ± 0.1^{de}

* Means in each column followed by the same letter are not significantly different ($P \leq 0.01$)
Data are presented as mean ± SE.

myrecene (5.12 ± 0.61), linalol (2.6 ± 0.17), α-Terpineol (1.49 ± 0.11), isophorane (6.76 ± 0.91), isobronyl acetate (5.38 ± 0.43) and caryophylene (2.26 ± 0.34) under different salinity levels (4, 8, 0, 6, 0 and 0 $dS.m^{-1}$, respectively and non-inoculation of mycorrhiza fungi) (Table 3).

Discussion

The results showed that the inoculation of mycorrhiza fungi (*Glomusin traradices*) under salinity conditions had positive effect on the growth and phytochemical traits of rosemary (*Rosemarinus officinalis* L.). It improved the morphological and phytochemical traits including number of shoots per plant, stem diameter, number of leaves per plant, leaf width, stems fresh weight, root fresh and dry weight and essential oil content and its components.

Number of shoots per plant was increased by the inoculation of mycorrhiza under salinity of 2 $dS.m^{-1}$. Gupta *et al*. (2002) reported that inoculation of wild mint (*Mentha arvensis* L.) by *Glomus fasciculatum*in increased its shoot growth [28]. Such benefits have been interpreted as the result of a positive influence of AM fungi on nutrient uptake of their host plants, especially for nutrients such as P that have poor mobility in the soil [29-32]. Inoculation of mycorrhiza fungi had positively stimulated the growth of rosemary stem and also the diameter was increased even in salt stress. This finding is in agreement with the finding of Francineyde *et al*. (2014) on *Libidi biaferrea* (Mart. ex Tul.) that inoculation of mycorrhiza improved stem diameter in these seedlings [33]. To define the mycorrhizal efficiency, it is important to consider variables that represent the physiology of the photobiont [34- 36].

Our results showed that the inoculation of fungi raised the number of leaves per plant in comparison with control and non-inoculation. These results are similar to the results of Lauro *et al*. (2014) on pomegranate seedlings and Kumar *et al*. (2011) on *Sida cordifolia* L. [37, 38]. The improved quantity might be due to enhanced photosynthesis associated with increased P uptake and high amounts of assimilates were produced to support both symbiosis and leaves yield [39]. The leaf width showed the highest increase by non-inoculation of fungi. The greatest value of stems fresh weight was obtained at fungi inoculation and non saline conditions. These results are in accordance with the results of Nisha *et al*. (2013) [40]. They suggested that application of arbuscular mycorrhiza fungi improved stems fresh weight of *Cyamopsis tetragognoloba* (L). Under saline soil, greater CO_2 assimilation could adequately provide carbohydrates for the fungal partner and results in more benefits to plants from AM association [41].

The roots fresh and dry weight increased with application of mycorrhiza fungi under the salinity level of 6 and 8$dS.m^{-1}$. The results of this study are in line with Ozdemir *et al*. (2010) findings on grapevine genotypes (*Vitis* spp.) [42]. Mycorrhizal hyphae extend beyond the depletion zone around roots and capture nutrients that are several centimeters away from root surfaces and thus suppress the adverse effect of different salinity stress [43].

Inoculation of fungi mycorrhiza under saline soil improved the content of essential oil

and its components. Previously, it was reported that fungi inoculation directly increases the essential oil content in shoots of *Origanum* sp. [44] as well as sweet basil [45]. The fungi inoculation in plants grown under such conditions reduces the negative effects of Na^+ and Cl^- ions by maintaining vacuolar membrane integrity, which facilitates compartmentalization within vacuoles and selective ion intake thereby preventing ions from interfering in metabolic pathways of growth [46]. The increased oil yield was associated with a larger number of peltate glandular trichomes, the main site of essential oil synthesis [47].

The main components percent of essential oil, α-pinene and 1,8-cineole, were improved by fungi inoculation under saline treatments. There are results showing the efficiency of AM inoculation in improving not only the content of the plants essential oil but also on the content and quality of the main components of essential oils, as observed for *Mentha arvensis* L. [48], *Coriandrum sativum* L. [49], and *Ocimum basilicum* L. [50]. Previous studies have shown that the various components of primary and secondary metabolites optimize their synthesis via association with AM (54). Many researchers reported that AM could enhance the ability of plants to cope with salinity stress by improving mineral nutrient absorption, maintaining ion balance protecting enzyme activities, and facilitating water uptake [51, 52, 53].

Conclusion

The results provided evidence that the inoculation of mycorrhiza fungi (*Glomus intraradices*) positively improved the morphological and phytochemical traits of rosemary (*Rosemarinus officinalis* L.) under different level of salinity. Thus, may be this ability is due to more extension of mycorrhizal hyphae and more availability of macro and micro nutritional elements in soil. The best results were obtained in application of mycorrhizal fungi and salinity level of 2 and 6 $dS.m^{-1}$.

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