

Changes in essential oil and morpho-physiological traits of tarragon (*Artemisia dracunculus* L.) in responses to arbuscular mycorrhizal fungus, AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) inoculation under salinity

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ABSTRACT

This study aimed to evaluate the arbuscular mycorrhizal fungi (AMF) (*Glomus intraradices* N.C. Schenck & G.S. Sm.) inoculation and salinity effect on qualitative and quantitative changes in tarragon yield. Treatments included inoculation, and non-inoculation of AMF, and five salinity levels of irrigation water (with the electrical conductivity of 0, 2, 4, 6, and 8 dS m⁻¹). The results showed the plant height, SPAD value, number of leaves, dry mass of leaves and shoot per plant were reduced under salinity condition. The various levels of salinity decreased the content of tarragon essential oil and some its components consist of α -pinene, limonene, Z-ocimene, E-ocimene, and methyl chavicol while, it increased the content of bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and α -farnesene. AMF inoculation without salinity had the greatest positive effect on the evaluated traits of tarragon. Also, it improved the morpho-physiological traits under salinity due to alleviation of the harmful effects of salinity. Although the essential oil content was reduced with the AMF inoculation, the methyl chavicol amount was increased by the AMF inoculation under salinity condition.

Key words: arbuscular mycorrhizal fungi; *Artemisia dracunculus* L.; electrical conductivity; essential oils; *Glomus intraradices*; methyl chavicol; morpho-physiological traits; salinity

IZVLEČEK

SPREMEMBE V VSEBNOSTI ETERIČNIH OLJ IN MORFOLOŠKO-FIZIOLOŠKIH LASTNOSTI PEHTRANA (*Artemisia dracunculus* L.) KOT ODZIV NA INOKULACIJO Z ARBUSKULARNO MIKORIZNO GLIVO (*Glomus intraradices* N.C. Schenck & G.S. Sm.) V RAZMERAH SLANOSTI

Namen te raziskave je bil oceniti vpliv inokulacije z arbuskularno mikorizno glivo (*Glomus intraradices* N.C. Schenck & G.S. Sm.) in učinek slanosti na spremembe v kakovosti in količini pridelka pehtrana. Obravnavanja so obsegala inokulacijo in brez inokulacije z AMG ter pet stopenj slanosti vode za namakanje (z električno prevodnostjo 0, 2, 4, 6, in 8 dS m⁻¹). Rezultati so pokazali, da so se vrednosti znakov kot so višina rastlin, SPAD vrednost, število listov, suha masa listov in število poganjkov na rastlino zmanjšale v razmerah slanosti. Različne slanosti so zmanjšale vsebnosti eteričnih olj, med njimi α -pinena, limonena, Z-ocimena, E-ocimena in metil kavikola medtem, ko so se vsebnosti bornil acetata, eugenola, metil eugenola, kariofilena, germakrena in α -farnezena povečale. Inokulacija z AMG v razmerah brez slanosti je imela največje pozitivne učinke na vse ovrednotene lastnosti pehtrana. Izboljšala je tudi morfološke in fiziološke lastnosti v razmerah slanosti. Čeprav je bila vsebnost steričnih olj zmanjšana ob inokulaciji z AMG, se je vsebnost metil kavikola povečala v razmerah slanosti.

Ključne besede: arbuskularne mikorizne glive; *Artemisia dracunculus* L.; električna prevodnost; eterična olja; *Glomus intraradices*; metil kavikol; morfološko-fiziološke lastnosti; slanost

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1 INTRODUCTION

Tarragon (*Artemisia dracunculus* L.) is an herbaceous, perennial plant with alternate leaves of linear shape and light green color (Fernandez-Lizarazo et al., 2011). It is native to Russia and Siberia, alluvial valley areas, but today is spread also in the western areas of North America. However, tarragon is widespread also in parts of temperate Asia, as well as Central Asia and Eastern Europe (Fernandez-Lizarazo et al., 2011; Obolskiy et al., 2011). The tarragon leaves contain about 0.3 % essential oil of which methyl chavicol comprise approximately 70 % of its component. (Chopra et al., 1986; Verma et al., 2010). This plant possesses a wide range of health benefits, therefore, it widely used in traditional medicine & pharmaceutical industry. It's health benefits that can be pointed include appetizing, stomach tonic, diuretic, anti-scurvy, mild anti-worm and induce of menstruation. Tarragon is used in traditional medicine for the resolution of joint pain, hiccups and treatment of painful menstruation. Despite contradictory statements, it was recently reported the tarragon extract is anticancer agent for some cancer cells line such as human leukemic cancer cells, human endometrial cancer cells, human breast cancer cells, etc. (Obolskiy et al., 2011).

Saline soil and saline irrigation are one of the most important environmental stresses, particularly in arid and semi-arid regions (Greenway and Munns, 1980; Kuznetsov and Shevyakova, 1997). Salt stress during vegetative and reproductive stage reduces crops biomass and yield (Aslam et al., 1993). Salinity affects plant growth and metabolism through disturbing several physiological processes of plants (Levitt, 1980; Hoshida et al., 2000). The first effect of salinity on plants is reducing water uptake even when the soil is completely wet, due to the decreased soil solution potential. Even more, cell membrane function may be damaged due to intracellular ion homeostasis disruption in plants exposed to salinity. Under these circumstances, some physiological disorders occur and programmed cell death imposed ultimately (Fan et al., 2013). Moreover, salt stress in plants leads to nutrients imbalance, reduction of the photosynthesis efficiency, increasing free radicals production such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^{\cdot}) and singlet oxygen (1O_2), and metabolic toxicity which causes the destruction of the cell membrane (Greenway and Munns, 1980; Fan, et al., 2013; Sankar, et al., 2007; Agarwal and Shaheen: 2007).

Plants responses to salinity stress are complex thus, salinity tolerance mechanisms are not clear yet. During the evolution and adaptation to salinity stress, various molecular mechanisms have been developed in plants for confronting the salinity. Some plants mechanisms

cope with salinity by regulating ion homeostasis with accumulation of compatible solutes for osmotic adjustment, free radical scavenging, alterations in membrane structures, and phytohormone biosynthesis stimulation. It was also reported that the ion homeostasis was regulated via selective accumulation of ions, ion exclusion as well as limited ions uptake by root, ion transport to the leaves and that distribution at cellular and plant level (Fan et al., 2013; Hasegawa et al., 2000; Jaleel et al., 2007).

Some microorganisms such as plant growth promoting rhizobacteria and fungi, especially arbuscular mycorrhizal fungi (AMF), can improve the plant growth and crop yield in saline soils via alleviating destructive effects of salinity stress (Cho et al., 2006). Arbuscular mycorrhizal fungi inhabit the rhizosphere and have a symbiotic association with the roots of most terrestrial plant species (Gini et al., 2003; Smith and Read 1997). The most common AMF in saline soil are species from the genus *Glomus* (Ho, 1987; Wang et al., 2004). Molecular studies revealed that about 80 % of fungi spores in saline soil belong to a single species, *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker (Wilde et al., 2009). Wu et al. (2010) reported that root inoculation of *Citrus tangerina* Tanaka by *G. geosporum* and *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker could improve growth, photosynthesis rate, root architecture, and ionic balance under 100 mM NaCl. Kapoor et al. (2002) reported AMF diminished the adverse effects of salinity and thereby increased coriander (*Coriandrum sativum* L.) growth. However, the root inoculation of sour orange (*C. aurantium* L.) and sweet orange (*C. sinensis* (L.) Osbeck) by *G. intraradices* under the 30, and 60 mM salt had no effect on salinity tolerance, but the Cl^- concentration increased in root (Zou et al., 2013).

AMF can significantly improve resistance of host plants to varied biotic and abiotic stresses. Arbuscular mycorrhiza leads to better nutrients mobility in nutrient poor soils (Marschner and Dell, 1994). Thus, the growth of plants coexisted with AMF is enhanced due to the increased nutrients uptake, especially N and P (Marschner and Dell, 1994). However, the role of AMF in stress conditions and salinity may not be limited only to this nutritional effect (Ruiz-Lozano and Azcon, 2000). The tolerance mechanisms to salinity in plant symbiosis with AMF is consisted with improved osmotic potential adjustment, enhanced water use efficiency, production of plant growth hormones, reduced detrimental effect of oxidative stress, mitigation of toxic ion effects or improved nutritional status (Auge, 2001). According to reports on the alleviating detrimental salinity effects via the AMF root

inoculation, this study was aimed for evaluation the qualitative and quantitative changes of tarragon yield

under AMF (*Glomus intraradices*) inoculation and salinity condition.

2 MATERIALS AND METHODS

2.1 Plant preparation and growth conditions

This study was conducted in an experimental greenhouse of the Medicinal Plants Institute (MPI) affiliated with the Academic Center for Education, Culture and Research (ACECR) in Karaj as a factorial experiment in the randomized complete block design (RCBD) with 4 replications during 2015. The research station is located at 1472 m a. s. l., 35° 54' N and 50° 53' E. The same rooted transplants of tarragon (*Artemisia dracuncululus* L. var. *sativa*) were provided from MPI seed bank (1096-MPISB), and were transferred into pots. Plants were grown in a plastic greenhouse for 3 months (from March 21 to June 20, 2015) with a photon flux density about 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h light and 8 h dark period and the average temperature of 21/16 °C for day/night. The soil texture was loam-silt, its physio-chemical properties contains 0.08 % nitrogen, 36.2 ppm phosphorus, 49.8 ppm potassium, 7.9 pH, and electrical conductivity (EC) 1.2 dS m^{-1} .

2.2 Treatments

Treatments included inoculation and non-inoculation with AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) as the first factor, and five salinity levels of irrigation water (with the EC of 0, 2, 4, 6, and 8 dS m^{-1}) as the second factor. The leaching fraction equal 0.5 was used in irrigation practices in order to less salt accumulation. The electrical conductivity of the solutions was measured by EC meter (HI9811, Hanna, USA) and the salinity levels were kept constant throughout the experiment period for irrigation water. Same amount of irrigation water were applied for each treatment during the growing period. The salinity treatments began 30 days after transplanting via irrigation water with an interval every two days until the harvest time. The treatment solutions were made with saline water and distilled water depending on target salinity, while the control treatment of salinity was prepared with double distilled water. Natural saline water was obtained from Hoz-e-Soltan Lake in Qom, Iran. The major ions of the saline water were: 128 $\text{g l}^{-1} \text{Na}^+$, 218.7 $\text{g l}^{-1} \text{Cl}^-$, 1.23 $\text{g l}^{-1} \text{K}^+$, 19.5 $\text{g l}^{-1} \text{Mg}^{2+}$, 0.086 $\text{g l}^{-1} \text{Ca}^{2+}$, and 48.8 $\text{g l}^{-1} \text{SO}_4^{2-}$.

For this experiment, 40 uniform plastic pots (20 cm upper diameter \times 15 cm bottom diameter \times 18 cm height) as experimental plots were divided into subgroups with or without AMF inoculation. Inocula consisted of soil possessing fungal spores, hyphae and mycelium. According to the method of Tommerup

(1992), fungus identification was checked using light microscope (AXIO Imager; Carl Zeiss, Jena, Germany). AMF inoculum was multiplied in the open pots culture of sweet corn (*Zea mays* L. convar. *saccharata* var. *rugosa* Bonaf.) as a host and after six months of plant growth, the shoots were eliminated and the underground parts were stored for two months in polyethylene bags at 5 °C. Thirty grams of the AMF inoculum (root fragments with 85 % of colonized roots length) was added to 3.0 kg of autoclaved (121°C, 0.11 MPa, 1 h) soil for AMF inoculation (Carretero et al., 2008). Non-mycorrhizal treatments received the same amount of autoclaved AMF inoculum.

2.3 Essential oils analysis

The harvested plant materials of tarragon were air-dried in a shaded place at a convenient temperature (24 ± 2 °C) during 6 days. Essential oils of the aerial parts were extracted by hydro-distillation method for 3 h using Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and kept on 4 °C until further analysis (British Pharmacopoeia, 1988). The extracted essential oils were identified by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) analysis. GC/MS analysis was carried out on an Agilent instrument coupled with a Agilent 5973N Mass system equipped with flame ionization detector (Hewlett-Packard Company, USA) and a SGE BPX5 capillary column (30 m \times 0.25 mm; 0.25 μm film thicknesses, Kinesis Ltd., UK). Temperature program included: an oven temperature held for 5 minutes at 50 °C and enhanced to 240 °C with 3 °C per min rate. Then, enhancement of temperature was programmed up to 300 °C with 15 °C per min rate and this temperature was held for 3 minutes. Other operating conditions include: carrier gas was He with a flow rate of 0.5 ml min^{-1} ; injector and detector temperatures were 290 °C, and split ratio, 1:25. Mass spectra were taken at 70 eV (Socaci et al., 2008). The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature and presented in the MS computer library (Adams, 2001).

2.4 Measurements and statistical analysis

The sampling was conducted in the onset of flowering stage as all plants were harvested 93 days after transplanting. For future accuracy and to reduce errors, samples were picked in four replicates randomly from the separate experimental plot. The analyzed morpho-

physiological traits were plant height, the number of leaves, SPAD value, leaf dry mass, shoot dry mass. The SPAD values were recorded using a SPAD-502 meter (Konica-Minolta, Japan). All the data were subjected to

statistical analysis (one-way ANOVA) using SAS software (Ver. 9.2). The difference between treatments means was compared by Duncan's multiple range test at 5 % confidence interval.

3 RESULTS AND DISCUSSION

Results showed that the AMF inoculation had a significant effect on the plant height ($p \leq 0.05$), number of leaves, SPAD value, and leaf and shoot dry mass ($p \leq 0.01$). Also, analysis of variance showed that different levels of salinity had a significant ($p \leq 0.01$) effect on

these traits. Between the salt stress and AMF inoculation was observed a significant interaction in the number of leaves ($p \leq 0.05$), leaf and shoot dry mass ($p \leq 0.01$) (Table 1).

Table 1: Analysis of variance for the effects of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation and salinity on morpho-physiological traits of tarragon

S.O.V.	Df.	Mean Square				
		Plant height	Number of leaves	Leaf dry mass	Shoot dry mass	SPAD value
Replication	3	8.99 ^{ns}	91.6 ^{ns}	0.005 [*]	0.005 [*]	9.76 ^{ns}
AMF inoculation	1	190.14 [*]	11262.4 ^{**}	0.22 ^{**}	0.51 ^{**}	106.27 ^{**}
Salinity (S)	4	109.9 ^{**}	1828.4 ^{**}	0.03 ^{**}	0.075 ^{**}	48.92 ^{**}
AMF × (S)	4	47.68 ^{ns}	154.4 [*]	0.026 ^{**}	0.139 ^{**}	16.69 ^{ns}
Error	27	26.07	51.37	0.0016	0.0078	7.22
CV (%)		8.84	6.47	7.22	10.02	7.34

ns: non-significant differences; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

The plant height and SPAD value were increased for 7.8, and 9.3 percentage by AMF inoculation, respectively (Figure 1), but their amount were decreased under salinity condition. In comparison with the control treatment, the plant height and SPAD values were significantly reduced with increasing salinity to 4 and 6 dS m⁻¹, what indicated that the leaf chlorophyll was more susceptible to raise of salinity (Figure 2, 3). These results also confirm the finding of other studies (Bernstein et al., 2010; Dolataadian et al., 2011; Amira and Qados, 2011; Mukhtar balal et al., 2011). Reduced osmotic potential in salinity condition is resulted in arrest of cell division and elongation (Jacoby, 1994). In addition, Na⁺ and Cl⁻ accumulation have toxic effects on the cell division and photosynthetic system, a reason for reduced plants growth. Also, salinity stress reduced

biosynthesis and transport of cytokinin and gibberellin, but ABA biosynthesis was increased. These factors are contributing to the reduction of plant height under salt stress compared to control (Jacoby, 1994). Destruction of chloroplasts, chlorophyll photo-oxidation and prevented chlorophyll biosynthesis are the main reasons for the decline in content of photosynthetic pigments under salinity conditions (Sultan, 2005). It has also been reported that reduced chlorophyll amount is a consequence of increased chlorophyllase activity under salt stress (Reddy and Vora, 2005). Glutamate is a precursor for proline and chlorophyll biosynthesis. Thus, increased proline production in salt stress decreases glutamate availability in the chlorophyll biosynthesis (Draskiewicz, 2000).

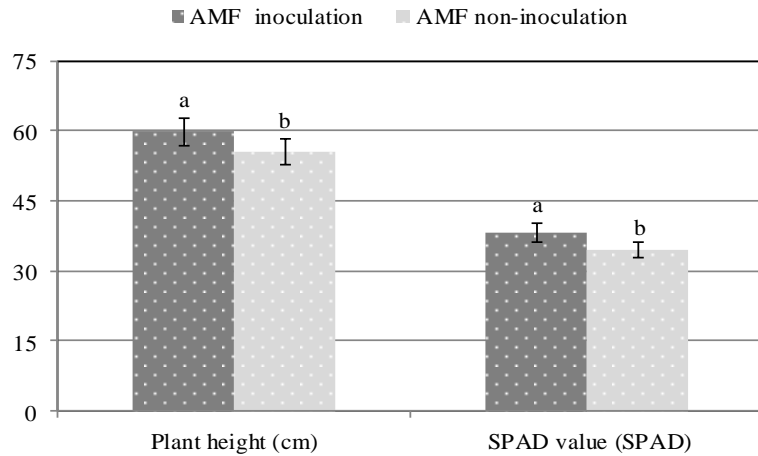


Figure 1: Effect of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on plant height (cm), and SPAD value (SPAD). The vertical bars represent standard errors of the means

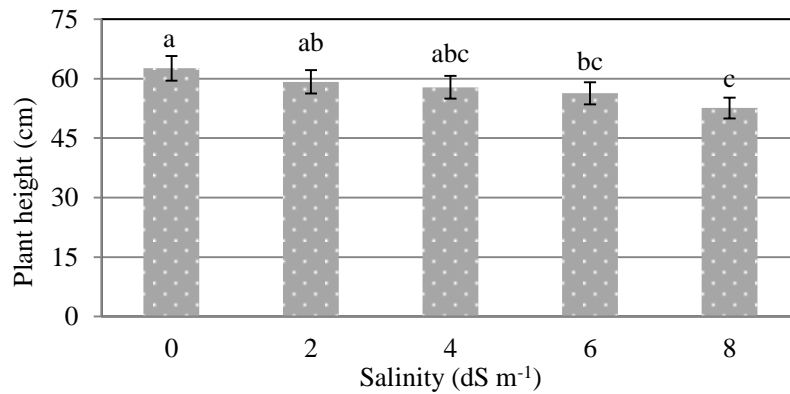


Figure 2: Effect of irrigation water salinity on plant height (cm). The vertical bars represent standard errors of the means

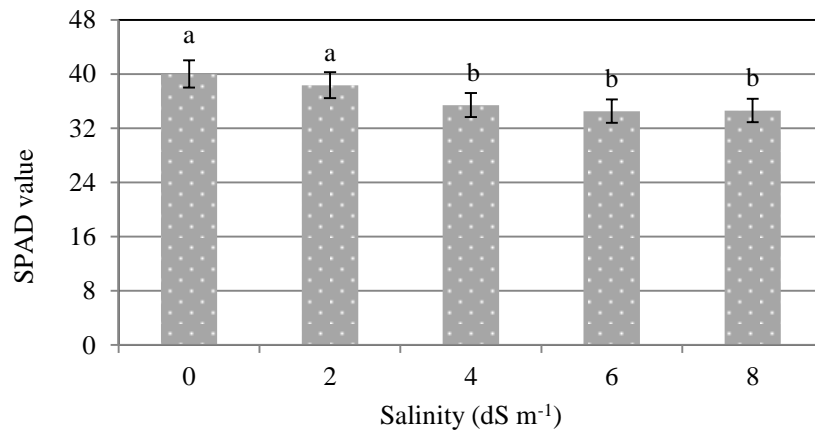


Figure 3: Effect of irrigation water salinity on SPAD value (SPAD). The vertical bars represent standard errors of the means

The number of tarragon leaves was increased by AMF inoculation. However, the number of leaves was reduced through increasing salt concentration under either inoculation or non-inoculation of AMF. The highest number of leaves (with an average of 146.28) was observed in AMF inoculation without the salinity. However, the lowest of it (67.91) was obtained at the 6 dS m⁻¹ salinity without using the AMF (Figure 4). These findings confirm the results of other studies (Bernstein et al., 2010; Amira and Qados, 2011; Mukhtar balal et al., 2011). During salinity stress, plant leaf area was reduced due to smaller leaves formation and leaf abscission. Thus, the photosynthetic capacity diminished and the supply of assimilates for optimal growth was reduced. In addition, the rapid leaves senescence under salt stress caused the reduction of the leaf area durability (Munns, 1993).

Use of the AMF could ameliorate the tarragon leaf and shoot dry mass so that AMF inoculation in non-saline condition produced the greatest leaf dry mass (0.76 g) and its minimum (0.39 g) was observed in the 8 dS m⁻¹ salinity without AMF inoculation (Figure 5). Similar to leaf dry mass, the highest shoot dry mass was obtained when the AMF inoculation was used in no-salinity stress (1.33 g) conditions and it's the least amounts was gained in the 8 dS m⁻¹ salinity without AMF inoculation (0.62 g). Also, leaf and shoot dry mass were increased with increasing salinity to 4 dS m⁻¹ without AMF inoculation. In contrast, increasing salinity to more than 4 dS m⁻¹ led to reduced leaf dry mass compared to the control (Figure 6). These findings are consistent with other studies (Kapoor et al., 2002; Ben Khaled et al., 2003; Rabie and Almadini, 2005; Gupta and Rutaray, 2005; Saleh and Al-Garni, 2006; Porrás-Soriano et al., 2009).

Plant dry mass reduction in saline conditions is a response to spent metabolic energy for coping with the salt stress (Parida and Das, 2005). Main factors that influenced the plant dry mass consisted of reduced leaf area, increased chlorophyll destruction, reduced photosynthesis rate, toxic effects of Na⁺ and Cl⁻ accumulation, decreased water uptake, and imbalance in nutrients (Sankar et al., 2007; Agarwal and Shaheen, 2007; Verma and Mishra, 2005). However, some authors reported that phosphorous nutrition can reduce the detrimental effects of salinity stress on plant growth. Therefore, AMF with increased phosphorous uptake can ameliorate for the harmful effects of salinity stress. On the other hand, the potassium content was increased in AMF inoculated plants. Thus, it protected host plant against adverse effect to sodium through enhanced potassium to sodium ratio (Marschner and Dell, 1994; Ruiz-Lozano and Azcon, 2000; Jeffries et al., 2003). Also, it was reported that use of AMF in lettuce is the reason for the roots extension. In addition, the photosynthesis rate and water use efficiency were improved, while the evapotranspiration was reduced (Ruiz-Lozano et al., 1996).

The tarragon essential oil content in this study was linearly reduced by the increased salinity. The AMF inoculation in various salinity levels reduced the essential oil content more than when AMF was not used, especially under the 8 dS m⁻¹ salinity. Therefore, the greatest content (1.15 %) of essential oil was obtained in the AMF inoculation under no salinity condition, while its minimum (0.2 %) was acquired in the AMF inoculation along with the 8 dS m⁻¹ salinity (Figure 7).

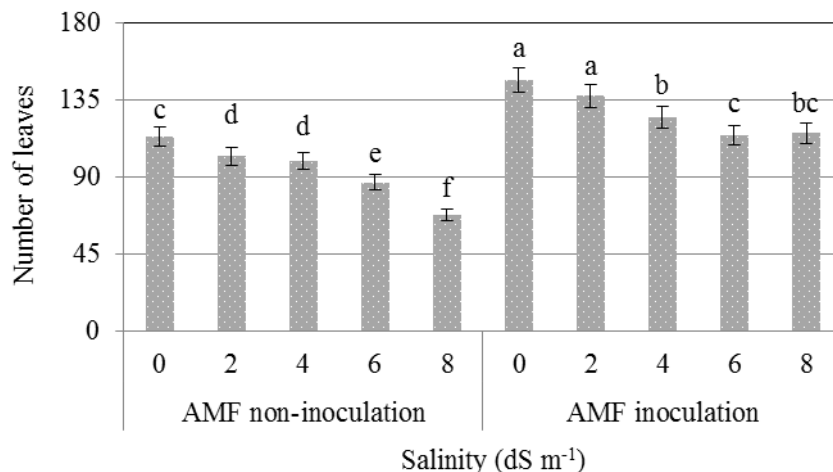


Figure 4: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on number of leaves. The vertical bars represent standard errors of the means

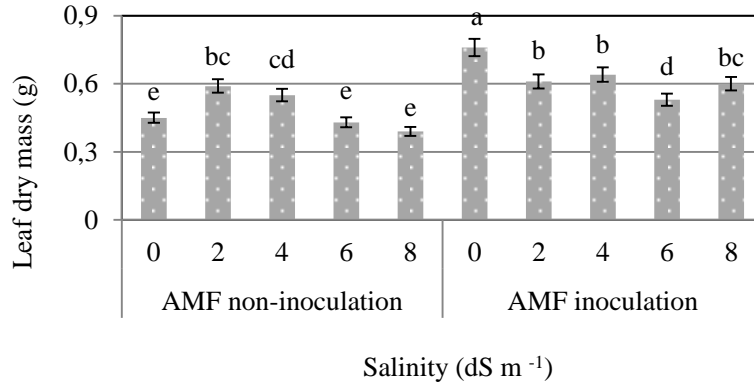


Figure 5: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on leaf dry mass (g). The vertical bars represent standard errors of the means

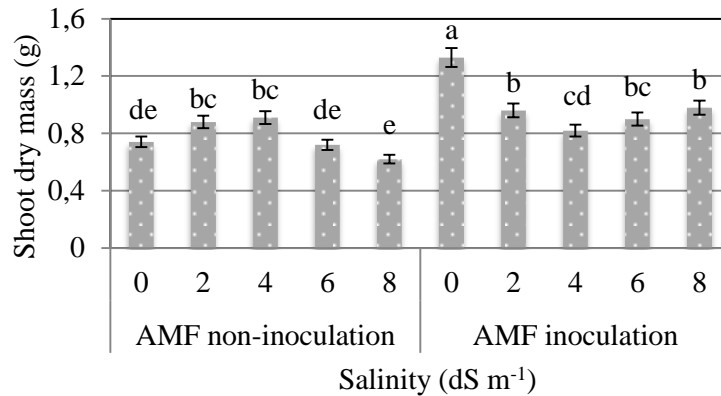


Figure 6: Effect of irrigation water salinity and AMF inoculation on shoot dry mass (g). The vertical bars represent standard errors of the means

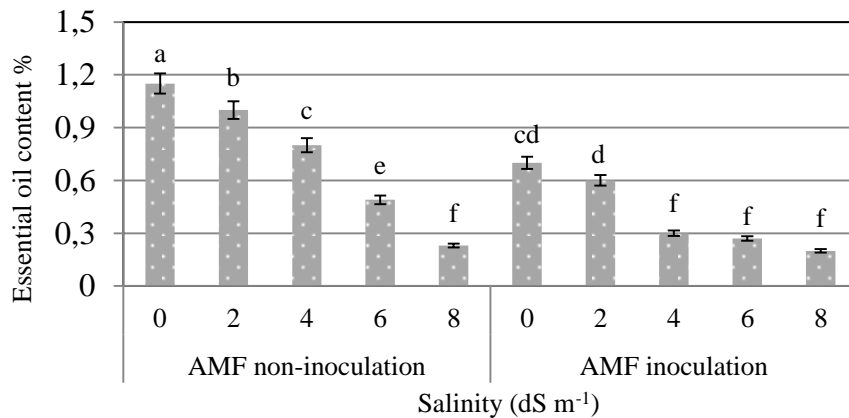


Figure 7: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on essential oil content (%). The vertical bars represent standard errors of the means

Results showed that the effect of salinity on essential oil components was also dependent on the salt concentration. The content of α -pinene, limonene, *Z* and *E*-ocimene were increased at 2 and 4 dS m⁻¹ salinity, but their content decreased when the salt concentration increased to over 4 dS m⁻¹ compared to the control treatment. The amounts of these compounds were increased by AMF inoculation in non-salinity stress, but using AMF in 2 and 4 dS m⁻¹ salinity reduced their content. Use of AMF had no significant difference with AMF non-inoculation in 6 and 8 dS m⁻¹ salinity. The highest content of α -pinene (1.03 %), limonene (3.22 %), *Z* and *E*-ocimene (6.35 and 7.31 %) were obtained under 2 dS m⁻¹ salinity without AMF inoculation and the lowest amount of these compounds were obtained in the 4 dS m⁻¹ salinity with AMF application (0.1, 0.47, 0.91, and 0.77 %, respectively) (Table 3).

The content of methyl chavicol had no significant difference with the control up to 6 dS m⁻¹ salinity, but it was significantly reduced at the 8 dS m⁻¹ salinity. AMF application in control and under 8 dS m⁻¹ salinity increased methyl chavicol content. However, in the other grades of salinity there was not a significant difference between AMF inoculation and non-inoculation. The maximum and minimum methyl chavicol content were gained in AMF inoculation and control treatment (88.8 %), and non-application of AMF under 8 dS m⁻¹ salinity (64.3 %) (Table 3).

The amount of bornyl acetate was increased under salinity with AMF inoculation. Its highest content (0.73 %) was observed in the AMF inoculation with the 8 dS m⁻¹ salinity and its minimum content (0.08 %) was obtained in AMF non-inoculation with control treatment of salinity. The content of eugenol, methyl eugenol, caryophyllene, germacrene, and α -farnesene was increased with increasing the level of salinity. Thus, their maximum content (0.9, 4.24, 1.33, 2.54, and 2.64 %, respectively) was observed under the 8 dS m⁻¹ salinity. Moreover, their minimum content (0.11, 0.61, 0.13, 0.41, and 0.06 %, respectively) was obtained in

the control without AMF inoculation. The amounts of these compounds were elevated with increasing the salinity up to 6 dS m⁻¹ when AMF inoculated. But their content was significantly reduced when the salinity increased to the 8 dS m⁻¹ (Table 3).

In summary, the various salinity levels decreased the tarragon essential oil content and the content of α -pinene, limonene, *Z*-ocimene, *E*-ocimene, and methyl chavicol, while the content of bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and α -farnesene was increased. Similar results were obtained also by other authors (Ashraf and Orooj, 2006; Tabatabaie and Nazari, 2007; Aziz et al., 2008; Belaqqiz et al., 2009; Baatour et al., 2010). Salinity could directly influence essential oil content and its components via changing the activity of the enzymes which are responsible for the terpenoids or phenylpropanoid biosynthesis and by altering the abscisic acid to cytokinins ratio. Furthermore, impaired photosynthesis and carbohydrate production indirectly affect the essential oil content and its components (Safarnejad et al., 2006; Turkan, 2011). It is reported that under the salinity stress, the biosynthesis of monoterpene biosynthesis is more affected than sesquiterpenes due to energy shortages thus, monoterpene biosynthesis is more vulnerable to salinity stress (Turner and Croteau, 2004). However, that the reduced ratio of oxygenated monoterpene to sesquiterpenes is caused by changes in cell bioenergetics under environmental stress. Another factor involved in this issue is the difference in the position of the compounds biosynthesis in using oxygen and energy resources (Dudareva et al., 2004).

The AMF inoculation in various levels of salinity resulted in reduced content of essential oils and a number of its major components, while the content of methyl chavicol, bornyl acetate, and caryophyllene was increased. Ahmadi-Khoei et al. (2013) reported similar results that the stimulation and changes in essential oil and phenol biosynthesis through fungal inoculation are possible.

Table 2: Analysis of variance for effects of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation and salinity on phytochemical traits of tarragon

S.O.V.	Df.	Mean Square					
		Essential oil content	α -pinene	limonene	Z-ocimene	E-ocimene	methyl chavicol
Replication	3	0.002 ^{ns}	0.007 ^{**}	0.026 ^{**}	0.18 ^{ns}	0.018 ^{ns}	5.92 ^{ns}
AMF inoculation	1	1.02 ^{**}	0.67 ^{**}	1.3 ^{**}	37.71 ^{**}	42.47 ^{**}	306.42 ^{**}
Salinity (S)	4	0.68 ^{**}	0.12 ^{**}	0.35 ^{**}	16.58 ^{**}	24.94 ^{**}	194.9 ^{**}
AMF \times (S)	4	0.074 ^{**}	0.28 ^{**}	0.81 ^{**}	21.31 ^{**}	26.8 ^{**}	160.51 ^{**}
Error	27	0.005	0.0008	0.0038	0.067	0.066	27.68
CV (%)		12.39	3.97	5.18	7.71	7.33	6.74

ns: non-significant differences; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

Table 2: Continued

S.O.V.	Df.	Mean Square					
		germacrene	caryophyllene	methyl eugenol	eugenol	bornyl acetate	α -farnesene
Replication	3	0.108 ^{**}	0.005 ^{ns}	0.004 ^{ns}	0.0085 ^{**}	0.0008 ^{ns}	0.011 ^{ns}
AMF inoculation	1	0.526 ^{**}	0.251 ^{**}	2.04 ^{**}	0.083 ^{**}	0.34 ^{**}	1.42 ^{**}
Salinity (S)	4	4.38 ^{**}	0.665 ^{**}	16.06 ^{**}	0.642 ^{**}	0.24 ^{**}	6.14 ^{**}
AMF \times (S)	4	0.78 ^{**}	0.075 ^{**}	2.19 ^{**}	0.118 ^{**}	0.004 ^{**}	0.93 ^{**}
Error	27	0.022	0.002	0.011	0.0015	0.0009	0.024
CV (%)		11.15	5.8	5.11	9.089	7.57	10.95

ns: non-significant differences; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

Table 3: Comparison of the means percentage in essential oil components under the salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation of tarragon

AMF	Salinity	α -pinene	limonene	Z-ocimene	E-ocimene	methyl chavi col	bornyl aceta te	eugenol	methyl eugeno l	caryophyllene	germacrene	α - farnes ene
Non- inoculati on	0	0.72 ^c	2.16 ^c	4.98 ^c	5.5 ^c	75.9 ^{bc}	0.08 ^g	0.11 ^g	0.61 ^f	0.13 ^f	0.41 ^f	0.07 ^f
	2	1.03 ^a	3.22 ^a	6.35 ^a	7.31 ^a	83.4 ^{ab}	0.21 ^f	0.11 ^g	0.65 ^f	0.3 ^e	0.57 ^{ef}	0.8 ^d
	4	0.95 ^b	2.57 ^b	6.29 ^{ab}	6.38 ^b	76.4 ^{bc}	0.28 ^e	0.13 ^g	0.75 ^f	0.31 ^e	0.72 ^e	0.59 ^{de}
	6	0.58 ^d	0.71 ^e	1.33 ^e	1.11 ^f	75.9 ^{bc}	0.42 ^d	0.68 ^b	2.72 ^c	0.92 ^{bc}	1.81 ^c	1.99 ^b
	8	0.29 ^f	1.34 ^d	2.63 ^d	2.38 ^d	64.3 ^d	0.5 ^{bc}	0.9 ^a	4.24 ^a	1.33 ^a	2.54 ^a	2.64 ^a
Inoculation	0	0.88 ^b	2.54 ^b	5.96 ^b	6.65 ^b	88.8 ^a	0.23 ^f	0.18 ^f	0.61 ^f	0.21 ^{ef}	0.55 ^{ef}	0.51 ^e
	2	0.13 ^g	0.44 ^f	0.93 ^f	1 ^f	80.8 ^b	0.42 ^d	0.34 ^e	1.45 ^e	0.59 ^d	0.99 ^d	1.14 ^c
	4	0.10 ^g	0.47 ^f	0.91 ^f	0.77 ^f	83.4 ^{ab}	0.49 ^c	0.5 ^d	1.96 ^d	0.88 ^c	1.67 ^c	1.79 ^b
	6	0.39 ^e	0.92 ^e	1.64 ^e	1.57 ^e	71. ^{cd}	0.54 ^b	0.73 ^b	4.13 ^a	1.43 ^a	2.2 ^b	2.58 ^a
	8	0.3 ^{ef}	1.35 ^d	2.43 ^d	2.38 ^d	79.5 ^b	0.73 ^a	0.62 ^c	3.08 ^b	1.02 ^b	1.78 ^c	1.96 ^b

Means with the same letters in each column indicate no significant difference between treatments at the 5 % level of probability.

4 CONCLUSION

The morpho-physiological characteristics and essential oil content of tarragon were reduced under salinity stress, but some content of essential oil components were increased such as bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and α -farnesene. In general, the AMF inoculation in no salinity condition had the most positive effect on tarragon morpho-

physiological traits and methyl chavicol amount. Although, the essential oil content was reduced with the AMF inoculation, but methyl chavicol amount as a major tarragon essential oil component was increased by the AMF inoculation under salinity condition. Therefore, we can conclude that the AMF inoculation caused to alleviate salinity stress harmful effects.

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