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Mycorrhiza inoculation effects on seedling establishment, survival and morphological properties of *Ziziphora clinopodioides* Lam.

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ABSTRACT

In this paper, the establishment and growth of medicinal species *Ziziphora clinopodioides* Lam. were studied through inoculation with two mycorrhizal fungi species, *Glomus mosseae* and *Glomus intraradices*, in arid/semi-arid Bahar-Kish rangelands, Iran in 2012 and 2013. The root colonization percentage of *Ziziphora*, as well as their establishment and growth were enhanced in 2013 using *G. mosseae*. In this year, less rainfall and higher temperature decreased the survival, growth and morphological traits of the studied plants. Growth and establishment of the inoculated plants using *G. intraradices* improved in 2013, while inoculation with *G. mosseae* showed more beneficial effects in 2012. In both years, the strain, the growth and establishment percentage of seedlings in inoculated treatments with mycorrhizal species were significantly improved. According to the results, *G. intraradices mycorrhizal* fungi is recommended as a biological fertilizer in increasing the forage production and the initial establishment of *Ziziphora* in arid and semi-arid rangelands.

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inoculation; *Mycorrhiza*; seedling establishment; semi-arid rangelands; *Ziziphora clinopodioides*

Introduction

Rangelands are environmentally stable sources, therefore, considered as one of the most valuable national resources in arid and semi-arid regions, such as Australia, Middle East, Africa, Central America, etc. The weather, landscape, and continuous exploitation of these rangelands depend mainly on the natural vegetation conservation. Thus, the improvement and rehabilitation of these lands, using nature-friendly setups, can play critical roles in soil and water conservation, and in local agriculture. The most vulnerable stage in the biological improvement of rangelands is the initial establishment of the plant seedlings. The well-established seedlings may not fail by poor soil fertility and environmental stresses, but they might be seriously affected by the adverse environmental conditions in the arid and semi-arid areas. Using novel technologies and plant interactions with other organisms may help in plant establishment. Plant inoculation with arbuscular mycorrhizal fungi was shown to improve natural fields and the regeneration of natural vegetation in degraded landscapes (Caravaca et al. 2003). *Retama sphaerocarpa*, *Rhamnus lycioides*, and *Olea europaea* subsp were inoculated with mycorrhizal fungi genes *Glomus* in pots. This setup

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Table 1. Soil physicochemical properties of the study area.

Nitrogen (%)	Organic matter (%)	Phosphorous (mg.kg^{-1})	Potassium (mg.kg^{-1})	Calcium (mg.kg^{-1})	Electrical conductivity EC (dS.m^{-1})	Soil acidity (pH)	Soil texture
0.14	1.88	25.47	306.67	2.1	0.04	7.53	Sandy loam

increased the plant's growth and establishment. In another study, woody legume inoculation with mycorrhizal fungi and Rhyzobioms helped the plant establishment in natural semiarid areas with water and soil nutrient deficiencies (Azcón-Aguilar et al. 2009). It was concluded that using the mycorrhizal fungi is an effective technique for rehabilitation and restoration of natural fields with degraded surface soil and vegetation cover. The lack of related research is mostly due to the research complexity and costs related to inoculation and extended areas in natural fields. As the most studies have been conducted on crop plants under field conditions, further studies on mycorrhizal effects on medicinal herbaceous plants are necessary in natural fields. As the resistance of *Ziziphora clinopodioides* Lam. to drought is very substantial, they can be used in the plantation in arid and semiarid areas that helps the environmental stability (Azizi 2004). Further, the aerial parts of *Ziziphora clinopodioides* Lam. are widely used in traditional medicine and pharmaceutical industries (Kheirkhah 2011). With an appropriate growth in arid and semiarid conditions, these plants can be considered as commercial sources (Naghdabadi et al. 2002). In Iran, the species grows in mountainous areas, debris and rocky slopes, and steppes at elevations ranging from 800 to 3700 meters above the sea level (Kheirkhah 2011). *Ziziphora clinopodioides* Lam is an indigenous plant in Bahar-Kish, Ghuchan, and is compatible with the dry environmental conditions. Mycorrhizal fungi are more efficient in plants with branchless roots like *Ziziphora clinopodioides* Lam. (Bagheri et al. 2012). Neighboring mycorrhizal fungi with such plants leads to increase in the absorption zone and the plant resistance to water shortage conditions. The present study characterized the capability of mycorrhizal fungi as a symbiotic and compatible fungus with nature in order to increase the initial establishment and production of *Ziziphora clinopodioides* Lam. Parallel with the improvement of plant establishment on the basis of ecological principles, the pot method was applied as a technique for the improvement and restoration of arid and semi-arid rangelands.

Materials and methods

The study area

The two-year study was conducted in Bahar-Kish rangelands of Ghuchan, Khorasan-e-Razavi province, Iran, in 2012–2013. The mean precipitation in this area, mainly snow in winter, was 346 mm based on the data from the meteorological Neyshabur station. The climate of the area was considered as hyper cold semi-arid. In the case of topography, the area had steep slopes with generally faced to the northern direction at elevations ranging 2000–3200 m above the sea level. To conduct the experiment, a one-hectare plot was selected and fenced to prevent animals from entering the study area. The altitude of the study plot ranged between 2400 and 2500 m from the sea level and was located toward the northern slope direction. As the effect of slope and geographical direction was not a part of the present study, the site was selected somehow that the effects of such factors were negligible. In order to determine the soil physicochemical properties of study area, soil sampling was selected from depths 0 to 30 cm, and transferred to the laboratory for physical and chemical analysis (Table 1).

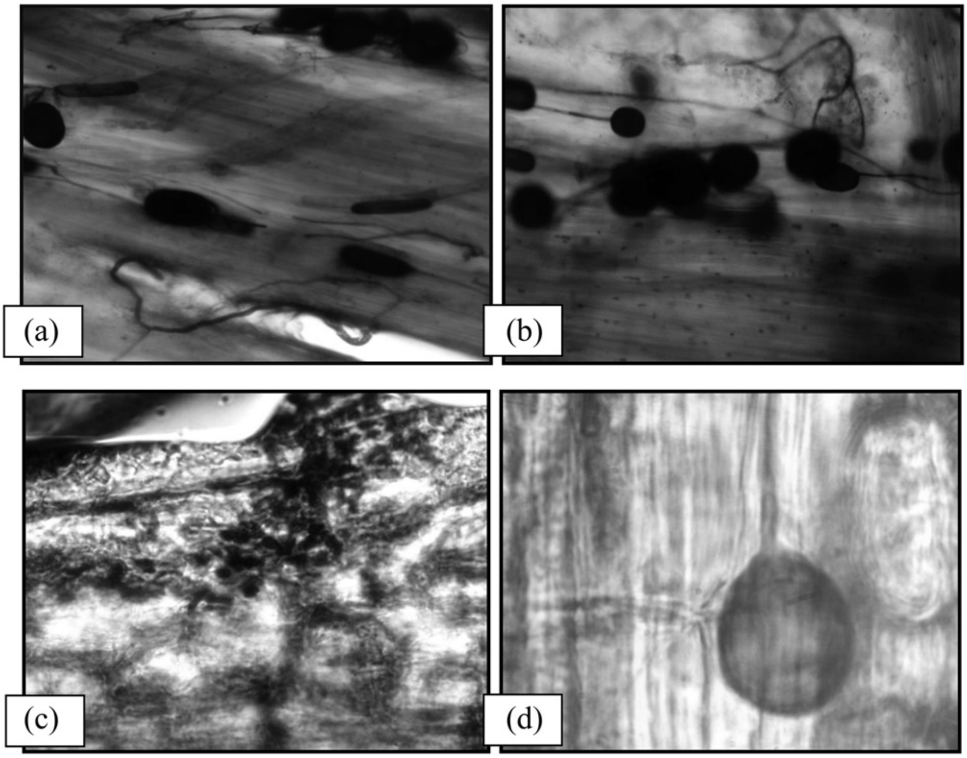

Figure 1. (a) and (b) the contaminated roots of *Ziziphora* by numerous vesicles and hypha, (c) the contaminated root by numerous arbuscules, and (d) the contaminated root by a vesicle and its producer.

Plantation and mycorrhizal inoculation

Medicinal *Ziziphora clinopodioides* Lam. was first planted in seedling trays containing 160 seeds in a research greenhouse at Ferdowsi University of Mashhad (January 2012). After a month, the seedlings were transferred to paper pots with 9 cm × 7 cm dimensions and a capacity of 160 g soil. Separate pots, containing 50 live spores per gram soil, were inoculated using two mycorrhizal species *G. mosseae* and *G. intraradices* separately with a ratio of 1:10 mycorrhizal substance and soil. To obtain this, the seedlings were first rolled in mycorrhizal soil and planted in the pots. With this setup, the inoculation substance consisted of soil + spore + plant roots + mycorrhizal fungi hyphae. When plants reached the heights of ∼3–5 cm, their pots were transferred outside the greenhouse, and after a week, they were transferred to the study site in Bahar-Kish, Ghuchan in 2013. They were then planted in split plots with three repetitions in an area of about 3600 square meters.

Evaluation of established plants

The evaluation of the seedlings establishments was done through two stages. First, the established bases were counted in 2013, then, in the seed producing stage before leaf shedding, the heights of plants were measured along with re-counting the established bases in the same year. Nine plants from each treatment were selected randomly and removed from the soil. The stems and the root lengths were measured from the collar to the root tip using a ruler. The samples were put in paper pockets separately and air-dried first, then oven dried at 70 °C for 24 hr. The dry weights of the roots, stems, and leaves were recorded using a digital scale with an accuracy of 0.01 g.

Table 2. Variations in establishment and colonization of *Ziziphora* seedlings after treatments with two mycorrhizal species, *G. intraradices* and *G. mosseae*.

Sources of variations	Degree of freedom	Colonization percentage	Establishment percentage
Repetition	2	1.8 ns	96.6 ns
Mycorrhizal treatments	2	21615.5**	1520.5**
Main error	4	6.2 ns	149.9 ns
Time	2	277.53**	8562.4**
Time × Mycorrhizal treatments	4	249.8**	652.7**
Minor error	12	14.52 ns	49.93 ns

**,*and ns are: mean significant at 0.01, 0.05 and not significant, respectively.

To determine the root colonization percentage of *Ziziphora clinopodioides* Lam. with the mycorrhizal treatment, a piece of the fresh root of the plant with an approximate weight of 0.2 g was selected randomly and cut into 1 cm pieces after being carefully washed with water. The samples were transferred into containers with 10% potassium hydroxide (KOH) solution and kept at the ambient temperature for 48 hr. The root pieces were then washed and put in 0.1 M hydrochloric acid (HCl) solution for 2 min to neutralize the basal environment. Philips and Himan modified method and Jiuwanti Monte method were, respectively, used to stain the roots and determine the percentage of colonization of the roots occurred by mycorrhizal fungi (Phillips and Hayman 1970; Giovannetti and Mosse 1980). Analysis of variance and the mean comparisons were done using statistical software SPSS version 18 (SPSS Inc., Chicago, IL) and Minitab version 16 (manufactured by Minitab Inc.) on the basis of Duncan multiple range test at the 5% confidence level.

Results and discussion

Root colonization percentage of Ziziphora *treated with mycorrhiza fungi*

Figure 1 shows that *Ziziphora* has a symbiotic association with mycorrhizal fungi. According to the results in Table 2, the effect of the inoculation with two mycorrhizal species on the root colonization of *Ziziphora clinopodoides* Lam. was significant ($p \leq .01$). This colonization was substantially different in 2012 compared to 2013. The reciprocal effect of mycorrhiza and time on the percentage of colonization and establishment of *Ziziphora* was shown to be substantial ($p \leq .01$).

The mycorrhizal colonization was distinguishably evident using both fungi species at the beginning and at the end of the first year, compared to the control plants with no inoculation treatment (Figure 2). At the end of the year 2012, the mean colonization percentage was shown to be higher than both the percentages of the beginning of the year 2012 as well as the end of the second growth season 2013 for the two fungi.

The root colonization percentages of both fungi at the end of the growth season 2012 are higher than the ones of the beginning of the growth season 2012 by about 7 and 16% for *G. mosseae* and *G. intraradices*, respectively. This trend is the same for the root colonization effect of *G. intraradices* at the end of the growth season 2013; however, the root colonization of *G. mosseae* was weakened at the end of 2013 compared to the beginning of 2012. At the end of 2013, the root colonization percentage of the studied plant species by *G. intraradices* was approximately 13% higher than the percentage when treated by the *G. mosseae* fungus.

Establishment of Ziziphora *treated with mycorrhizal fungi*

In a general term, the establishment of *Ziziphora* seedlings decreased significantly (about 50%) in both years with/without applying the mycorrhiza fungi (Figure 3). Compared to the control, mycorrhizal inoculation did not affect the seedling establishment significantly at the beginning of the growth season of the first year (Figure 3).

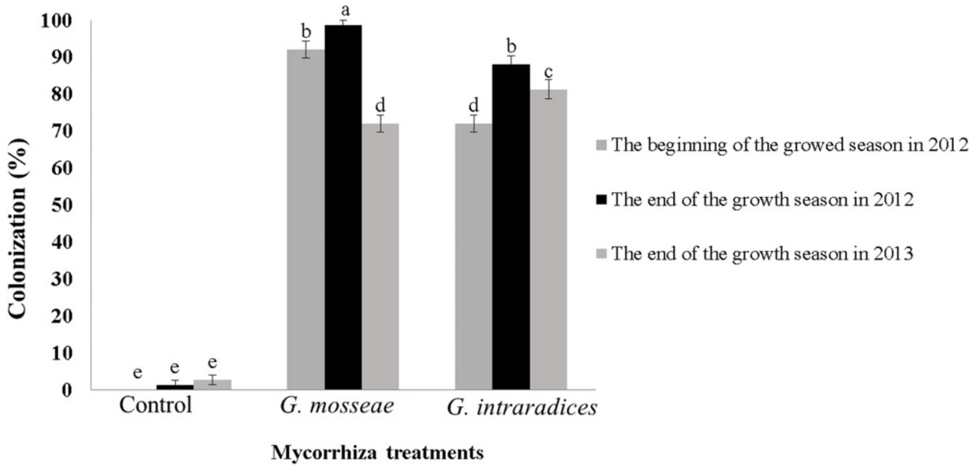

Figure 2. The root colonization percentage of *Ziziphora* after treatments with *G. mosseae* and *G. intraradices* in 2012 and 2013.

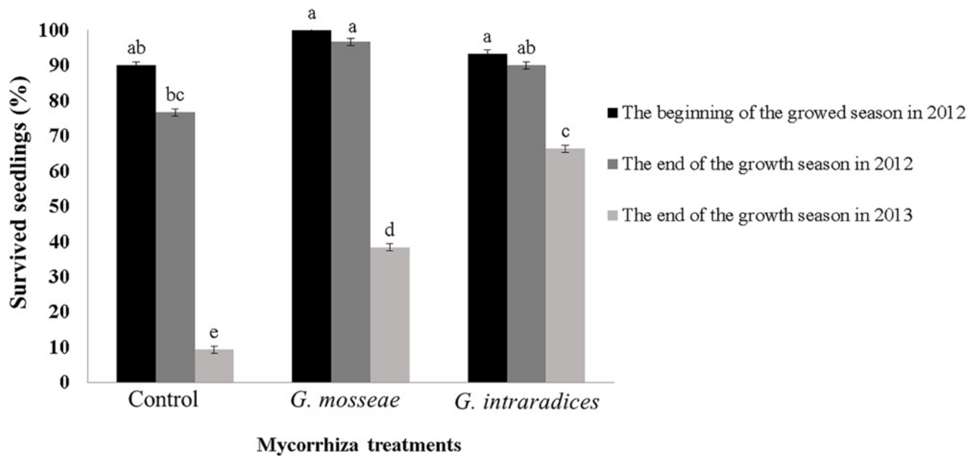

Figure 3. The percentage of survived seedlings of *Ziziphora* in 2012 and 2013 with/without treatments with *G. mosseae* and *G. intraradices*.

Table 3. The variance effect of the two studied mycorrhizal fungi on morphological traits of *Ziziphora*.

Sources of variations	Degree of freedom	Root dry weight	Stem dry weight	Leaf dry weight	Aerial part weight	Whole plant dry weight	Root dry weight to aerial part weight ratio	Height	Root length	Root length to aerial part length ratio
Replications	2	0.01	0.002	0.001	0.003	0.02	0.12	0.64	0.13	0.5
Mycorrhizal treatments	2	0.3**	0.06**	0.14**	0.38**	1.35**	1.23**	17.98**	26.08**	32.19**
Main error	4	0.009	0.002	0.0001	0.001	0.007	0.03	0.31	2.07	0.33
Time	1	0.02^{ns}	0.02*	0.15**	0.28 ^{ns}	0.47**	2.64**	4.83**	74.34**	0.47^{ns}
Time × Mycorrhizal treatments	6	0.0005^{ns}	0.03**	0.2**	0.08**	0.07^{ns}	1.08**	1.1^{ns}	31.28**	5.38**
Minor error	12	0.02	0.005	6.66	0.005	0.03	0.15	0.65	2.7	0.67

*,**and ns are: mean significant at 0.01, 0.05 and not significant, respectively.

Table 4. Reciprocal effects of mycorrhiza fungi on mean growth and aerial biomass of *Ziziphora* in 2012 and 2013.

	2012			2013		
Source variance	Control	*Glomus mosseae*	*Glomus intraradices*	Control	*Glomus mosseae*	*Glomus intraradices*
Stem dry weight (g)	$0.23^{c} \pm 0.03$	$0.41^{a} \pm 0.03$	$0.29^{bc} \pm 0.03$	$0.1^{d} \pm 0.03$	$0.25^{c} \pm 0.03$	$0.39^{ab} \pm 0.03$
Leaf dry weight (g)	$0.22^{d} \pm 0.01$	$0.54^{a} \pm 0.01$	$0.5^{b} \pm 0.01$	$0.08^{e} \pm 0.01$	$0.24^{d} \pm 0.01$	$0.38^{c} \pm 0.01$
Aerial part dry weight (g)	$0.45^{c} \pm 0.04$	$0.95^{a} \pm 0.04$	$0.79^{b} \pm 0.04$	$0.18^{d} \pm 0.04$	$0.48^{c} \pm 0.04$	$0.77^{b} \pm 0.04$
Root dry weight to aerial part dry weight ratio	$1.26^{bc} \pm 0.18$	$0.98^{c} \pm 0.18$	$1.3^{bc} \pm 0.18$	$2.85^{a} \pm 0.18$	$1.79^{b} \pm 0.18$	$1.15^{c} \pm 0.18$
Root length (cm)	$1.65^{a} \pm 0.83$	$16.56^{a} \pm 0.83$	$16.89^{a} \pm 0.83$	$16.8^{a} \pm 0.83$	$12.81^{b} \pm 0.83$	$8.12^{c} \pm 0.83$
Root length to aerial part length ratio	$5.16^{b} \pm 0.42$	$2.45^{cd} \pm 0.42$	$2.85^{c} \pm 0.42$	$7.4^{a} \pm 0.42$	$2.57^{cd} \pm 0.42$	$1.4^{d} \pm 0.42$

In each row, mean values with the same letters do not differ substantially based on the Duncan multiple range test at the 0.05 confidence level.

Nevertheless, the establishment percentage of the inoculated seedlings with both fungi was shown to have 30% increase, and even higher at the end of the second year by 35%. Compared to the *G. mosseae* results, plants inoculated with *G. intraradices* survived more at the end of the second year by 28%.

Effects of mycorrhizal treatments on the growth and morphological traits of Ziziphora

Table 3 shows in both years, the plant inoculation with the mycorrhizal fungi significantly affected the dry weights of the whole plant, root, stem, leaf, leaf number, height, and the ratio of the root dry weight to the dry weight of the aerial part. These findings confirm that mycorrhizal fungi favorably influenced some morphological traits and growth of *Ziziphora* organs. Although at the end of the second year, some morphological traits and the growth of *Ziziphora* organs decreased in comparison with the first year, mycorrhizal treatments still traced a significant change on morphological traits and the growth of *Ziziphora* organs. This was more evident for the treatment with *G. intraradices* fungus.

Stem dry weight

In the first year, only *G. mosseae* caused a significant increase ($p \leq .01$) in stem dry weight. In 2013, although the measured stem dry weight showed decreases in all treatments, it was measured to be more than the control. In this year, the stem dry weight of the inoculated *Ziziphora* with *G. intraradices* had higher values than the other treatment (Table 4).

Root dry weight

Compared to the control, the inoculation with both *G. intraradices* and *G. mosseae* increased the root dry weight of *Ziziphora* by 1.5 times in both years ($p \leq .01$) (Table 5). The difference in the root dry weight between 2012 and 2013 was not significant (Table 3).

Table 5. Mean comparison of the growth and aerial part biomass of *Ziziphora* under mycorrhizal treatments, and the control.

Time	Morphological traits	Control	*Glomus mosseae*	*Glomus intraradices*
2012	Root dry weight (g)	$0.56^{b} \pm 0.07$	$0.93^{a} \pm 0.07$	$0.99^{a} \pm 0.07$
	Whole plant dry weight (g)	$1.01^{b} \pm 0.1$	$1.87^{a} \pm 0.1$	$1.77^{a} \pm 0.07$
	Height (cm)	$3.5^{b} \pm 0.56$	$5.9^{a} \pm 0.56$	$6.7^{a} \pm 0.56$
2013	Root dry weight (g)	$0.51^{b} \pm 0.05$	$0.85^{a} \pm 0.05$	$0.9^{a} \pm 0.05$
	Whole plant dry weight (g)	$0.68^{b} \pm 0.05$	$1.33^{a} \pm 0.05$	$1.68^{a} \pm 0.05$
	Height (cm)	$2.25^{b} \pm 0.2$	$4.99^{a} \pm 0.2$	$5.83^{a} \pm 0.2$

In each row, mean values with the same letters do not differ substantially based on the Duncan multiple range test at the 0.05 confidence level.

Leaf dry weight

Mycorrhizal *G. mosseae* and *G. intraradices,* respectively, increased the leaf dry weight by 32 and 28% in the first year, and 30 and 16% in the second year, compared to the control (Table 4). Overall, the 2013 mean leaf dry weight was 19% less than the one in 2012.

Aerial part dry weight

The observation in both years suggests that the inoculation increased the aerial part dry weight of Ziziphora. For the first year, the increments were 50% and 33% using *G. mosseae* and *G. intraradices* (Table 4); and in 2013, they were recorded 61% and 31% for the corresponding fungi, respectively.

Whole plant dry weight

Like the other weights, the inoculation of *Ziziphora* with both fungi enhanced the whole plant dry weight by about 2.5 times as compared with the control in both years (Table 4). The yearly whole plant dry weight had a decline by about 32% in the second year. Mean comparison of growth and aerial part biomass of *Ziziphora* in two years is brought in Table 6.

Plant height

The height of the treated plants did have a notable difference for the treatments with *G. intraradices* and *G. mosseae* in the second year, and the differences were considerably compared to the control in both years ($p \leq .01$, Table 4). The studied mycorrhizal fungi increased the heights of *Ziziphora* plants two-fold of the control in 2012–2013.

The ratio of root dry weight to aerial part dry weight

Mycorrhizal treatments of *G. intraradices* and *G. mosseae* did have effect on this ratio in 2013 compared with the control, but there was no major difference in their influences on the ratio in the same year ($p \leq .05$, Table 4). In contrast, the treatments could impose a minor change in the ratio in 2012.

Root length

The effects of time and mycorrhizal treatments on the root length of *Ziziphora* were significant ($p \leq .01$) (Table 3) when compared with the no mycorrhizal treatments. Both *G. intraradices* and *G. mosseae* fungi were very much effective in the second year with the lower mean root lengths

Table 6. Mean comparison of growth and aerial part biomass of *Ziziphora* in two years.

Source of variance	2012	2013
Whole plant dry weight (g)	$1.55^{a} \pm 0.04$	$1.23^{b} \pm 0.04$
Height (cm)	$5.4^{a} \pm 0.22$	$4.36^{b} \pm 0.22$

In each row, mean values with the same letters do not differ substantially based on the Duncan multiple range test at the 0.05 confidence level.

of inoculated species with fungi, when compared with the control ($p \leq .01$) (Table 3). The root length decreased in the second year in comparison with the first year of the study (Table 4).

The ratio of the root length to the aerial part length

Compared to the control, the ratio of the root length to the aerial part length in the first year was significantly improved when the plants had undergone the treatments with the fungi (Table 3).

Colonization effect on the establishment and survival of Ziziphora

Mycorrhizal colonization through the inoculation with both fungi increased significantly in the first year of the experiment. Under the normal environmental conditions in 2012, the root colonization percentage of *Ziziphora* by *G. mosseae* was more than that by *G. intraradices*; but in the second year, with a harsh environmental condition and less irrigation water, the root colonization percentage of *Ziziphora* by *G. intraradices* exceeded the percentage magnitude using *G. mosseae*. This indicates the difference in the ability of various species in having symbiosis associations with the host plant. Jakobsen, Abbott, and Robson (1992) referred to the different abilities of various fungal species, particularly under environmental stresses, in contaminating the host plant. Aarle, Olsson, and Soderstrom (2002) also observed that three *Glomus* species acted completely similar in attracting nutrients from the soil. When the soil pH was changed under different basal environments, the amount of intake differed subsequently. Decrease in the root colonization in 2013 was due to the higher temperature and the rain shortage in the area in this year. These environmental factors affected the colonization profoundly (Van der Heijden and Sanders 2002), and as a result, different mycorrhizal species could exhibit different patterns of root colonization at varied thermal ranges (Borges and Chaney 1989). Wu and Xia (2006a) found that drought stress substantially decreased the mycorrhizal colonization of *Citrus tangerine* using *Glomus versiforme*. These investigators (Borges and Chaney 1989) suggested that arid and semiarid areas had an adverse effect on the mycorrhizal development in a host plant. The number of arbuscular declined in the second year, 2013. As the arbuscular organs contributed to the nutrient exchange between the plant and fungus, the decrease in the number of such organs limited the nutrient flows during the growth stages (Rahmatzadeh and Khara 2008). Van de Staaij et al. (2001) stated that the decrease in the number of arbuscular organs negatively affected the colonization, although it did not affect the number of vesicular. Plenchette and Duponnois (2005) reported that the plant species *Atriplex nummularia* was symbiotic with mycorrhizal *G. intraradices* which improved the establishment and growth of the studied plant under a phosphorous shortage. Hypha and vesicles were clearly observed in the plant's root colonized with the mycorrhizal *G. intraradices*. With the absence of arbuscular, they (Plenchette and Duponnois 2005) concluded that arbusculars were not efficient in improving the growth and establishment of *Atriplex nummularia*. In this study, mycorrhizal treatments established the plant more than the setup with the non-mycorrhizal neighboring in both years, but in the first year, the improved establishment was a result of more available moisture, phosphorous, nitrogen, and organic matter by mycorrhizal fungi for *Ziziphora clinopodioides* (Porras-Soriano et al. 2009; Smith, Grace, and Smith 2009).

The lack of rainfall and high temperature in the second year specifically decreased the plant survival in the Ghouchan region, a semi-arid zone. Harrington (1991) attributed the decrease in the survival and high mortality to the limited available water resulted from very low rainfall in semi-arid areas. Busquets et al. (2010) stated that, for the same reason, mycorrhizal *G. intraradices* and *G. mosseae* raised the establishments of *Anthyllis cytisoides* and *Spartium junceum* in semi-arid areas. For a better establishment, *G. mosseae* was more beneficial in the first year, while *G. intraradices* was more effective in the second year. In the presence of the stressors such as drought, ion toxicity, and high temperatures fungi may not be as effective as they are in the normal environmental conditions (Vosatka and Dodd 1998). In the present study, however, it was likely mycorrhizal *G. intraradices* to cause the improvement in the water use efficiency and enhanced the establishment of *Ziziphora*.

Mycorrhizal treatment effect on the growth and morphological traits of Ziziphora

Despite the harsher environmental conditions in 2013, mycorrhizal treatments, in particular, *G. intraradices*, had desirable effects on the morphological traits and growth of *Ziziphora*. Similar results were presented by Busquets et al. (2010) who inoculated *Anthyllis cytisoides* and *Spartium junceum* with *G. mosseae* and *G. intraradices*, and studied their establishments and morphological traits before and after drought stress. Before the drought stress, no significant difference was found between mycorrhizal treatments in the establishment and morphological traits of the plants. After the stress, however, the mycorrhizal treatments had a significant effect on the leaf, the whole plant dry weight, and relative water content. As such, the effect of mycorrhizal *G. intraradices* on the water content of plant's organs was more sensible than *G. mosseae*. In the first year of the study, the stem dry weight of the inoculated plants with *G. mosseae* were more than those inoculated with *G. intraradices* and the control. In the second year, the stem dry weights of mycorrhizal treatments were also more than the control. Wu, Zou and Xia (2006b) reported similar findings for *Citrus aurantifolia*, inoculated with *G. Mosseae* and *G. intraradices*, which resulted in increasing the leaf and aerial part dry weights compared with the control. In the second year with harsher conditions, consuming the carbon by mycorrhizal fungi for growth and respiration decreased the stem dry weight of *Ziziphora* (Bahrami Sirmand et al. 2012). The trend of the higher leaf dry weights upon mycorrhizal treatments and less amount of the weight in the second year was repeated after the plant faced less rain. Naturally, under drought stress, plant decreases its photosynthetic area through the decrease in the leaf number and area, which speeds up further declines in the number of the leaves and photosynthesis (Amiri Dehabadi, Rezvani Moghaddam, and Ehyaee 2013). Misra and Sriacastiva (2002) also reported that drought stress caused a significant decrease in the leaf area of mint *Mentha piperita* L. Under drought stress, medicinal plants like *Thymus mastichina* and *T. granatensis* used more leaves with larger areas to prevent high evapotranspiration (Mota et al. 2008). Here, we found that at the end of the growth season of the second year, the leaf dry weight of plants inoculated with *G. intraradices* was more than that of plants inoculated with *G. mosseae* and the control. The results were in agreement with the results of the studies by Busquets et al. (2010) who reported a higher leaf dry weight of plants inoculated with *G. intraradices* compared with using *G. mosseae*. The aerial part dry weights were higher in mycorrhizal treatments than in the control in both years of the study, because the mycorrhizal fungi are considered as the stimulators for the increase in the photosynthetic activity (Demir 2004). Also, using such fungi increases the total sugar content and hormone level such as cytokinin and gibberellins in mycorrhizal plants. The increase in the levels of such hormones, particularly cytokinin, can cause an increase in the photosynthesis speed through transferring effective ions in opening the stomata and regulating the chlorophyl level, as well as in carbohydrate content of the plant. The reason for the decreased yields of treatments in the second year was probably the lower vegetative growth followed by limited photosynthetic area and, thereby, production of lower crop dry matter under drought stress (Amiri Dehabadi, Rezvani Moghaddam, and Ehyaee 2013). Plants close their stomata under drought stress in order to prevent the high transpiration. With a decrease in transpiration under such a condition, the water intake and transfer through roots decrease. This subsequently decreases the plant growth and yield (Levitt 1980; Amiri Dehabadi, Rezvani Moghaddam, and Ehyaee 2013). Alkira et al. (1993) also reported that drought stress decreased the plant height, stem, root and leaf dry weights in mint. The sensitivities of plant and fungal species towards each other, and their compatibilities with each other in establishing mycorrhizal symbiosis, are among the important factors affecting the nutrient intake and photosynthesis. They usually cause an increase in photosynthesis, which is followed by the need resultant from mycorrhizal formation in roots, and more sugar consumption. If the need in the plant is not satisfied sufficiently, it may lead to decreasing the host plant growth (Bahrami Sirmand et al. 2012). The root dry weight was more in mycorrhizal treatments with both fungi than the control in both years of the study (Wu and Xia 2004). The increase in root dry weight is due to the increase in auxin levels in symbiotic plants with mycorrhizal fungi. The produced auxin by fungal partners may cause the increase in root dry weight and symbiosis stimulation in plants (Rahmatzadeh and Khara 2008). Similarly, the increase in the whole plant dry weight in both years of the study originates from releasing the plant hormones (mainly auxin) by mycorrhizal fungi that help plant growth (Van de Staaij et al. 2001).

The whole plant dry weight decreased in the second year of the study due to water shortage and high temperature. Misra and Sriacastiva (2002) observed that less irrigation water led to a significant decrease in leaf area, the whole plant fresh and dry weights, the amount of chlorophyll and essence yield in mint (*Mentha piperita* L.). In another study, Busquets et al. (2010) reported that mycorrhizal treatments caused increase in the whole plant and leaf dry weights of plant species *Anthyllis cytisoides* and *Spartium junceum*, among which the increasing effect of *G. intraradices* on the measured parameters was more distinguishable.

The inoculation of *Ziziphora* with *G. intraradices* and *G. mosseae* caused the increase in plant height in comparison with the control in both years of the study. In a study, the difference in the heights of *Anthyllis cytisoides* and *Spartium junceum* was not significant using *G. intraradices* and *G. mosseae*, but these two treatments differed significantly from the control (Busquets et al. 2010). We also found a significant difference in the heights of *Ziziphora* between the first and the second year. The decrease in the long growth of the plant aerial parts in 2013 with water shortage and drought stress was likely attributed to the decrease in auxin level and the prevention from polar transferring of the hormone in such tissues. Auxin was transferred from the tips of the aerial parts and young leaves to the lower tissues, and the established concentration gradient controlled many aspects of the growth and development (Rahmatzadeh and Khara 2008). The effects of the two mycorrhizal fungi on the ratio of the root weight to the aerial dry weight were significant in the first year of the study. Busquets et al. (2010) also reported the significant effects of *G. intraradices* and *G. mosseaeon* on the ratio of the root weight to the stem dry weight of the two plant species, *Anthyllis cytisoides* and *Spartium junceum*. The root to the aerial dry weight ratio was more in the control than the mycorrhizal treatments in the second year of the study in which the root to the aerial dry weight ratio increased due to the decrease in the aerial part dry weight in the control compared to the mycorrhizal treatments.

The difference in the root length was not significant between the treatments in the first year, but the root lengths after the mycorrhizal treatments were lower than the control in the second year. It is assumed that root branching increased with the colonization. For this reason, mycorrhizal fungi did not lead to any increase in the root length, but instead increased the root branching. Berta et al. (1990) showed that the branching of the secondary roots of leek (*Allium porrum*) increased following the colonization with mycorrhizal fungi genus *Glomus*. The change in the branching of the colonized roots by the endo mycorrhizal fungi can be associated with the improvement in the nutrients intake by the plant. The increase in the root branching leads to a more efficient root system and higher nutrients intake from the soil (Eissenstat et al. 2000). Symbiotic *Ziziphora* with mycorrhizal fungi also leads to lower investment and costs of *Ziziphora* root length increasing in comparison with the control under environmental harsh conditions, so that the root to the aerial part length ratio in *G. intraradices* and *G. mosseae* was less than the control. The difference in the root to the aerial part length ratio was not significant between the two years of the study.

Conclusions

Both mycorrhizal *G. intraradices* and *G. mosseae* fungi species were able to colonize with the roots of *Ziziphora* plant and improve its establishment and survival in a semiarid rangeland. There was a positive relationship between the colonization percentage, the establishments, and the survival of some morphological traits of the plants. *Glomus intraradices* responded better than *Glomus mosseae* to the short time drought stress and increased the plant survival and growth in the semiarid environment. The mycorrhizal fungus species selection determined the success of the increase in the establishment and restoration of the area, so that in the present study, *Glomus intraradices* was the most appropriate species in this regard compared to the other treatments. Mycorrhizal inoculation can, therefore, be used as a standard method in order to rehabilitate natural landscapes of semiarid areas, particularly in the degraded lands. In addition, proper mycorrhizal species are able to establish symbiotic associations with plants, even in few numbers, can affect the increase in plant establishment positively. In the present study, the plant establishment and some morphological traits increased with the increase in the colonization. It was shown that the plant growth decreased whether under stress at the second year or under normal condition of the first year with the decrease in root colonization. As the colonization of the plant root system by mycorrhizal species is one of the most important indices of mycorrhizal fungal activity, the decrease in or lack of *Arbuscular mycorrhizal* formation is associated with the decrease in hydrocarbons. On the other words, there was a positive relationship between the root colonization percentage and the increase in plant growth. The observed decrease in the growth of the root and the aerial parts of the plant in the second year can be considered a result of such a decrease in the root colonization and the mineral intake; the longest plants during the two years of the study were those inoculated with *G. intraradices*. The results of the experiments showed that the plant mycorrhizal inoculation can cause increase in the plant growth and yield in comparison with the control. Mycorrhizal species can change some effective factors in the plant growth and production and improve the plant yield.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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