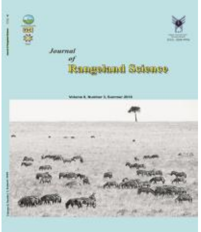
Contents available at ISC and SID

Journal homepage: www.rangeland.ir



Research and Full Length Article:

Effects of Drought Stress and Mycorrhiza on Viability and Vegetative Growth Characteristics of *Ziziphora clinopodioides* Lam.

Reyhaneh Azimi[A*], Golam Ali Heshmati[B], Mohammad Kia Kianian[C]
[A]Ph.D. Rangeland Sciences, Researcher of Arid Environments Research Centre. Mashhad, Iran *(Corresponding Author), Email: Reyhaneazimi36@yahoo.com
[B]Professor of Faculty of Range land and Watershed Management, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[C]Academic Member of Desert Studies Faculty, Semnan University, Iran

Received on: 10/10/2016
Accepted on: 21/10/2017

Abstract. In order to study the effects of drought stress and mycorrhiza fungi on establishment rate and some growth-morphological traits of *Ziziphora clinopodoides*, pot experiments were conducted as factorial design based on a completely randomized design at the research greenhouse of agricultural faculty, Ferdowsi University of Mashhad in 2014; pots were combined as four levels of drought stress including 100 (Control), 75, 50 and 25% of Field Capacity (FC) and three levels of mycorrhiza fungi including *Glomus intraradices*, *Glomus versiforme* and control. The results showed that drought stress of 25%FC decreased plants weight (0.75, 0.67 and 0.19g/pot), plant height (1.25, 1.18 and 0.7cm), establishment rate (67.50%, 57% and 14.25%) and plants root colonization by mycorrhiza fungi (50%, 35.5% and 0) by the application of *G. intraradices*, *G. versiforme* and control, respectively indicating significant effects of both *Mycorrhiza* species on drought resistance. Result indicated that the establishment rate was decreased significantly to 85%, 64% and 36% using *G. intraradices*, and *G. versiforme* and control at 50%FC, respectively. Similarly, the establishment rate was reduced in the inoculated plants with mycorrhiza treatments less than control (67.50%, 57% and 14.25% at 25%FC) so that symbiosis of Mycorrhiza fungi significantly improved *Z. clinopodoides* establishment rate under drought stress against the control. The effect of *G. intraradices* and *G. versiforme* on plants establishment rate (93% against 78.50% at 75%FC), plants height (5.93cm against 5.30cm at 75%FC) and dry weights (1.07g against 0.95g at 50%FC) was higher through drought stress. The *Glomus intraradices* reacted better to mild drought than *G. versiforme* and increased the establishment and morphological traits of plant. According to results, *G. intraradices* could be introduced as a biological fertilizer and a technique for retrofitting and increasing tolerance of *Z. clinopodoides* against drought stress.

Key words: Colonization, Drought, Biological fertilizer, Greenhouse

Introduction

Rangelands are considered as the most important and valuable natural resources in Iran. These ecosystems are more important in arid and semiarid climates, and preservation of its natural vegetation cover is more important. *Ziziphora clinopodioides* Lam is one of the most important medicinal plants of Lamiaceae family, which has nutritional and medicinal uses and its aroma is close to thyme. *Z. clinopodioides* has mint properties like being warm and dry, antibacterial, anti-cold and intestinal disinfectant. *Z. clinopodioides* is also used to treat gastrointestinal disorders such as diarrhea and heartbeat (Mamadalieva, 2017). Soil's poor nutrients and environmental stresses in many semiarid areas in the world induce restrictions on plants establishment (Azimi, 2013). The most important and vulnerable stage in biological improvement of rangelands is initial establishment of plant seedlings which may be mostly defeated in arid and semiarid areas due to environmental harsh conditions (Azimi *et al*., 2014b). Use of modern technologies and interactions between plants and other organisms may help in plant establishment in lands. Recently, *Vesicular arbuscular* mycorrhiza has been applied in many crops to cope with water scarcity and drought (Song, 2005). According to ecological and physiological researches, mycorrhiza symbiosis often leads to better water absorption from soil. *Mycorrhiza* fungi increases root uptake area so that host plants can absorb more water from soil (Auge, 2001). Cultivation of *Glomus* genus is recommended as arbuscular mycorrhiza fungi in desert soils because these genera have different kinds of survived reproductive organs which are compatible to unstable environment conditions. Also, some *Glomus* species such as *Glomus intraradices* and *Glomus mosseae* that are parts of vesicular-arbuscular mycorrhiza could be suitable for the reconstruction and rehabilitation of plant communities in harsh environmental conditions (Gonzalez-Chavez *et al.*, 2002). In a study, species of *Rhamnus lycioides, Retama sphaerocarpa* and *Olea europaea* Subsp. *sylvestris* L. were inoculated with mycorrhiza fungi from the *Glomus* genus in pots and then transferred to a semiarid area. The results of study suggested that Mycorrhiza fungi caused an increase in establishment and growth of these plants (Caravaca *et al.*, 2003). Busquets *et al.* (2010) in their studies found that *Glomus intraradices* and *Glomus mosseae* induced the increase of plant species establishments like *Anthyllis cytisoides* and *Spartium junceum* in semiarid areas as a result of improving nutrient absorption, growth and water relationships. Sometimes, mycorrhiza symbiosis protects plants against this stress by avoiding drought stress and it is attributed to the increased absorption area of phosphorus and other necessary nutrients for plants growth (Auge, 2001). Abdul-Naser (1998) in a study on *Lagenaria vulgaris* L. observed that plants inoculated with fungi (*Glomus intraradices*) tolerated drought better than Control. Although most studies have been conducted on crops in field conditions, further studies on the effect of Mycorrhiza on herbaceous medical plants are needed.

Mycorrhiza fungi are more efficient in plants with branchless roots like *Z. clinopodioides*. Use of mycorrhiza fungi in such plants leads to increase the adsorption area and plant resistance to water-shortage condition (Bagheri *et al.*, 2011). In Iran, this species grows in mountainous areas, debris and rocky slopes and steppes at elevations ranging from 800 to 3700 m asl (Ghaharaman, 2000). Persistent droughts have affected large areas of the country in recent years. So, present study aimed to investigate the effects of drought stress and Mycorrhiza fungi on the establishment rate and some growth-morphological traits of *Ziziphora clinopodioides*.

Materials and Methods

Experiment outline

The experiment was conducted through greenhouse conditions at agricultural faculty of Mashhad′s Ferdowsi University, Iran in 2014 using a factorial experiment based on a completely randomized design with four replications. The first factor was drought stress that includes four levels of field capacity moisture (100, 75, 50 and 25%). The second factor was mycorrhiza fungi at three levels (*Glomus versiforme* ، *Glomus intraradices* and without mycorrhiza fungi). The seeds were sown in pots at 2/4/2014 and the samples were harvested at 10/9/2014.

After seed cultivation of *Z. clinopodoides* (15 seeds at each pot) and their inoculation with two mycorrhiza species (*Glomus versiforme* and *G. intraradices*), all pots (48 pots) were irrigated equally. *Mycorrhiza* inoculation substance was added to pot's soil with ratio of 1:10. It was continued up to a month so that the pots were irrigated enough daily or every two days (to extent not being flood irrigated) until the seeds were germinated and established in soil. If the stress is exerted immediately after sowing, plants may be died out; so, they were left to be compatible with conditions and get ready for receiving stress treatments. Every gram of mycorrhizal soil had at least 50 alive spores provided from Touran Biotechnology Company, Shahroud, Iran. Inoculation substance was consisted of soil, spore, plants roots and mycorrhiza fungi hypha. A month after plant growth and before exerting moisture treatments, plants were thinned and only seven healthy seedlings were remained with the same sizes in the pots.

Pots irrigation was done at four levels (100, 75, 50 and 25% of FC). Field capacity refers to moisture between saturation and wilting point levels in which micro and moderate pores of soil were filled with water. Under field conditions, 1 to 3 days after irrigation, water existing in macro pores left and air replaced it but micro pores were still full of water and it can be used.

Through successive irrigation and measuring soil moisture, it was concluded that at 100, 75, 50 and 0 % of field capacity, irrigation volume was 460 ml, 345 ml, 230 ml and 115ml, respectively for every 2 days. In such a case, the soil moisture percentage was 35%, 17%, 10% and 5%, respectively.

Data collection and analysis

The first count of established plants was done at seeds production stage and before falling leaves (10th of September). In addition to counting the established plants, their height also was measured. For doing this, 7 plants of each treatment were selected and removed thoroughly from soil. After measuring the length of stems, samples were placed in paper pockets separately and after drying in shade, they were dried out in oven at 70°C for 48 hours. The dry weight of stems, roots and leaves were measured and then, they were recorded. In order to determine the root colonization percent of *Z. clinopodoides* by mycorrhiza fungi, a part of plant fresh roots with weight of 0.2 g was randomly selected, washed and cut in 1 cm pieces and then, the resultant samples were transferred to glasses containing 10% of KOH solution and kept at room temperature in order to be prepared. Then, roots were washed and put in 0.1 M HCl solution for 2 minutes to neutralize alkaline environment. The Phillips & Himan modified method (1980) was used to stain the roots. Then, the colonization percent of Mycorrhiza fungi by roots was determined according to Giovannetti and Mosse (1980). Database was created in Excel software. In order to study the effects of treatments, two-way analysis of variance was used in which the simple and interaction effects of two factors including moisture level and mycorrhiza inoculation were evaluated. The Duncan mean comparisons were done at confidence level of 95%. All analyses were done using software SPSS ver.*18*.

Results

Result of variance analysis of root colonization and plants establishment is presented in Table 1. The main effects of inoculation with two mycorrhiza fungi were significant for both root colonization percent and *Z. clinopodoides* establishment ($p \leq 0.01$). Moreover, the main effects of drought stress for both traits were also significant ($p \leq 0.01$). The mycorrhiza fungi and drought stress interaction effects were also significant on colonization percent ($p \leq 0.01$) and plants establishment ($p \leq 0.05$) (Table 1).

Table 1. Variance analysis of mycorrhiza fungi and drought stress effects on plant establishment and colonization percent of *Z. clinopodioides* seedlings

Sources of variation	df	MS	
		Colonization (%)	Establishment (%)
Mycorrhiza fungi (M)	2	22221.1**	6135.2**
Drought stress (D)	3	1377.2 **	4654.2**
M × D	6	346.9 **	313.2*
Error	36	9.00	65.20

*and ** mean significant at 5% and 1% probability levels, respectively

Results for morphological traits of *Z. clinopodoides* inoculated with two mycorrhiza fungi at 4 drought stress treatments are presented in Table 2. Results showed significant main effects of mycorrhiza fungi and drought stress for all traits ($p \leq 0.01$) and mycorrhiza fungi and drought stress interaction effects for all traits except root to aerial parts weight ratio ($p \leq 0.01$) (Table 2).

Table 2. Variance analysis results (mean of squares) of some morphological traits for *Z. clinopodoides* inoculated with two mycorrhiza fungi

Sources of variations	df	MS						
		Root weight	Stem weight	Leaf weight	Aerial weight	Plant whole weight	Root/aerial weight ratio	Plant height
Mycorrhiza Fungi (M)	2	0.73**	0.09**	0.084**	0.33**	2.004**	0.65	31.99**
Drought stress (D)	3	0.42**	0.10**	0.14**	0.47**	1.75**	3.90**	47.52**
M× D	6	0.20**	0.005**	0.004**	0.01**	0.03**	0.79	3.17**
Error	36	0.002	0.001	0.001	0.002	0.003	0.50	0.12

**, * and ns symbols mean significant at 0.01, 0.05 and non- significant respectively

The means comparison of root colonization percent for two Mycorrhiza Fungi species showed that *G. intraradices* had significantly higher colonization percent than that for *G. versiforme* (Table 3). The Mycorrhiza fungi symbiosis percent was decreased by increasing drought stress in both mycorrhiza species (Table 3). The root colonization percent of *Z. clinopodoides* without any stress was 8% higher for *G. intraradices* than that for *G. versiforme* (Table 3).

The effects of Mycorrhiza fungi species on plant establishment were significant. Result showed that *G. intraradices* increased plant establishment more than that for *G. versiforme* fungi (Table 3). Results indicated that in both fungi treatments, the means of seedling establishment were decreased significantly (about 50%) by increasing drought stress. Generally, both Mycorrhiza fungi increased plant establishment under drought stress higher than that for Control (Table 3). Under severe drought stress (25%FC), the rate of plants establishment with *G. intraradices* was higher (11%) than that for *G. versiforme* (Table 3).

About Mycorrhiza fungi effects on root dry weight, results showed that both mycorrhiza species (*G. intraradices* and *G. versiforme*) increased root dry weight in comparison with Control (Table 3). Also, results showed that root dry weight was increased by *G. intraradices* at drought stresses (100, 75, 50 and 25%FC) as 23, 48, 49 and 45%, respectively and those for *G. versiforme* were 15, 36, 45 and 40%, respectively against Control (Table 3). Stem dry weights inoculated with *G. intraradices* had higher values than that for *G. versiforme* and the control.

The stem dry weight decreased in all treatments while drought stress was exerted but in seedlings inoculated with *G. intraradices,* stem dry weight increased a little. The stem dry weights of mycorrhiza treatments were higher than that for control and the highest stem dry weights were observed at seedlings inoculated with *G. intraradices* (Table 3). Similarly, for leaf dry weight, both mycorrhiza species under drought stress caused a significant increase in leaf weight but the highest value was observed in seedlings inoculated with *G. intraradices* (Table 3). For aerial weight, both mycorrhiza fungi led to an increase in dry weight of aerial parts in comparison with control. It was observed that aerial parts dry weights values of plants inoculated with *G. intraradices* were higher than those inoculated by *G. versiforme* and control (Table 3). According to results, efficiency of *G. intraradices* in vegetative growth was higher than *G. versiforme* under drought stress (Table 3).

For whole plant dry weight, inoculation of *Z. clinopodoides* with both mycorrhiza fungi led to an increase in whole plant weight against control (Table 3). In normal irrigation, both mycorrhiza fungi increased whole plant dry weight 1.5 times than that for control. The whole plant dry weight decreased in all treatments by increasing drought stress (Table 3). For plants height under normal irrigation, mycorrhiza species increased plants height 1.5 times than that for control (Table 3). According to results, there was a significant difference between *G. intraradices* and *G. versiforme* and both of them had significant differences with control ($p \leq 0.01$) (Table 3). Finally, for root to aerial dry weight ratio, inoculation effect of two mycorrhiza species was significant ($p \leq 0.01$) (Table 2). Comparing drought stress treatments, the trend of this trait was reverse and higher ratio was obtained under severe drought stress (25%FC) indicating that in severe drought stress, weight of root was increased and weight of aerial parts was decreased (Table 3).

Table 3. Means comparison of some morphological traits for *Z. clinopodoides* inoculated with mycorrhiza fungi affected by drought stress

Mycorrhiza Fungi	Drought stress (FC %)	Colonization (%)	Establishment (%)	Root weight (g/pot)	Stem weight (g/pot)	Leaf weight (g/pot)	Aerial weight (g/pot)	Plant whole weight (g/pot)	Root/aerial weight ratio	Plant height (cm)
G. intraradices	100	85.0 a	100.00 a	0.92 a	0.40 a	0.39 a	0.78 a	1.69 a	1.19 d	6.65 a
	75	76.5 b	93.00 ab	0.84 b	0.28 b	0.29 b	0.56 b	1.40 b	1.49 cd	5.93 b
	50	63.0 d	85.75 bc	0.72 c	0.19 c	0.08 e	0.35 e	1.07 d	2.11 bc	3.93 d
	25	50.0 f	67.50 de	0.55d	0.12 d	0.12 d	0.20 f	0.75 f	2.74 ab	1.25 e
G. versiforme	100	77.0 b	96.50 ab	0.84 b	0.29 b	0.30 b	0.60 b	1.44 b	1.40cd	6.25ab
	75	86.0 c	78.50 cd	0.80 b	0.25 b	0.23 c	0.48 c	0.48 c	1.69 bc	5.30 c
	50	54.5 e	64.00 e	0.68 c	0.16 c	0.12 e	0.27 f	0.95 e	2.54abc	3.93 d
	25	35.5 g	57.00 e	0.50de	0.09de	0.07 e	0.17 f	0.67 g	3.01 a	1.18ef
Control	100	-	78.50 cd	0.69 c	0.20 c	0.21 c	0.41 d	1.10 d	1.69bcd	4.30 d
	75	-	64.00 e	0.44 e	0.10de	0.09 e	0.19 f	0.63 g	2.33 bc	1.48 e
	50	-	36.00 f	0.23 f	0.06ef	0.04 f	0.08 g	0.30 h	3.08 a	0.96 e
	25	-	14.25 g	0.10 g	0.04 f	0.03 f	0.09 g	0.19 j	2.01 bc	0.70 f

Data with the same letters at each column are not significantly different based on Duncan (5% probability level) test

Discussion

Mycorrhiza colonization was increased significantly with inoculation of two mycorrhiza species. Under drought stress, root colonization percent of *Z. clinopodoides* inoculated with *G. intraradices* was higher than that inoculated with *G. versiforme* which is indicative of different abilities of various fungal species at establishing symbiotic association with host plants. Jacobsen *et al.* (1992) also stated that abilities of various fungal species in contaminating host plants are different particularly under drought stresses. Root colonization percent of plants was decreased by increasing drought stress.

This decrease in colonization was due to water shortage in the studied pots because environmental factors strongly affect colonization (Van der and Sanders, 2002). Wu and Xia (2006) found that drought stress significantly decreased the mycorrhiza colonization of *Glomus versiforme* on *Citrus tangerine*. They suggested that arid and semiarid environments had adverse effects on mycorrhiza fungi developments in host plants.

Treatments with Mycorrhiza had more seedling establishment in pots under drought stress against with no mycorrhiza treatments because of more available moisture, phosphorous, nitrogen and organic matter prepared by mycorrhiza fungi (Porras *et al.*, 2009 and Smith *et al.*, 2009). Plant establishment was decreased in all treatments under drought stress. This decrease can be stated as the irrigation decline. Plants inoculated with mycorrhiza fungi showed more establishments as compared with control. Between two mycorrhiza species, plants inoculated with *G. intraradices* showed less dying out against *G. versiforme*. The establishment percent of plants inoculated with *G. intraradices* was higher than those inoculated with *G. versiforme* and control. It was observed that *Z. clinopodoides* survival inoculated with *G. intraradices* had been increased at field after two years of establishment compared to other treatments (Azimi, 2013). The effect of *G. intraradices* on plant establishment was increased by increment at drought stress and environmental harsh conditions. Also, drying out of plants inoculated by fungus was less than other treatments. The differences in mycorrhiza species effects might be due to this fact that there are differences among mycorrhiza fungi species. Some may have many advantages in a normal condition but under other undesired conditions, some may not be effective, particularly when dealing with stress factors such as drought, ion toxicity and soil high or low temperatures (Vosatka and Dad, 1998). At present study, *G. intraradices* likely improved water use efficiency and increased establishment percent of *Z. clinopodoides* in greenhouse. The results of present study showed that mycorrhiza fungi had desired effects on some morphological traits and growth of *Z. clinopodoides*. Under drought stress conditions, some morphological traits and growth of *Z. clinopodoides* were decreased compared to normal irrigation that was probably due to irrigation decline but in spite of this fact, mycorrhiza treatments significantly affected some morphological traits and growth of *Z. clinopodioides* seedlings. Between mycorrhiza treatments under drought stress, *G. intraradices* had higher effects on some morphological traits.

In a study by Busquets *et al.* (2010), two plant species (*Anthyllis cytisoides* and *Spartium junceum)* were inoculated with *Glomus intraradices* and *Glomus mosseae* and then received a short drought stress. The establishment and morphological traits of plants before and after drought stress showed that there was no significant difference between mycorrhiza treatments in establishment and morphological traits before drought stress but after doing stress, there was a significant effect of mycorrhiza treatments on whole plant and leaf dry weight and relative water content.

More effect on relative water content and increase in whole plant and leaf dry weight of species was observed at *Glomus intraradices* inoculation than that of *Glomus mosseae*. The stem dry weight of treatments under drought stress was more in plants inoculated with Mycorrhiza fungi compared to control. The inoculation of *Citrus aurantifolia* roots with *Glomus intraradices* and *Glomus mosseae* significantly increased leaf and aerial parts dry weights compared with Control (Wu and Xia, 2006). In another study, it was observed that plants inoculated with *G. intraradices* had more aerial dry weight than control (Azimi *et al.*, 2014a). According to results of means comparison, stem dry weights of *Z. clinopodoides* seedlings under drought stress were lower than normal condition (no stress) because the plants were subjective to water shortage (Bahrami Sirmandi *et al.*, 2011). It may be due to water shortage at any stage of plant growth, reduced absorption, transport and consumption of nutrients (Hu & Schmidhalter, 2005). Also, dehydration and temperature rise reduces photosynthesis and stomatal conductance in early stages of stress which may result in a reduction in carbon storage and dry matter (Yordanov *et al.*, 2003).

In present study, leaf dry weights of mycorrhiza treatments were higher than that of control. The leaf dry weight decreased under drought stress because under such circumstance, plants were faced to water shortage stress. Naturally under drought stress, plant decreases its photosynthetic area through a decrease in leaf area and number; then, plant photosynthetic capacity decreases following a decrease in leaf area; this event leads to more leaf loses and decrease in photosynthetic area (Amiri Dehabadi *et al.*, 2012).

Misra and Sriacastiva (2002) also reported that water stress caused a significant decrease in leaf area of mint (*Mentha piperita* L.). Under drought stress, the leaf area and number of *Thymus mastichina* and *T. granatensis* decreased in order to prevent from high transpiration (Mota *et al.*, 2008). The leaf dry weight of plants inoculated with Mycorrhiza *Glomus intraradices* was more than that of *G. versiforme* and control. This result was in agreement with observations of Busquets *et al.* (2010). They stated that two mycorrhiza fungi (*Glomus intraradices* and *G. versiforme)* led to an increase in leaf dry weight following drought stress, but the increase in leaf dry weights of plants inoculated with *Glomus intraradices* was more than those inoculated with *G. versiforme*. According to the results of present study, aerial dry weight of mycorrhiza treatments was more than Control. Azimi *et al.* (2015) concluded that plants inoculated with mycorrhiza had more aerial dry weight than control treatment because Mycorrhiza fungi are considered as a stimulus for increasing photosynthetic activity (Demir, 2004). Another reason for this effect is an increase in total sugar content and plant hormone levels such as Cytokinin and Gybberllin (Demir, 2004).

The aerial dry weight decreased due to water shortage stress. The reasons for yield loss in treatments may be attributed to less vegetative growth followed by limited photosynthetic area and therefore lower dry matter production under drought stress (Amiri Dehabadi *et al.*, 2012). Alkira *et al.* (1993) also observed that drought stress in mint decreased plant height, stem, root and leaf dry weights. The root dry weights at all mycorrhiza treatments were higher than control. Also in other studies, the root inoculation with *Glomus intraradices* and *Glomus mosseae* increased root dry weight significantly compared with control (Wu and Xia, 2004). The increase in root dry weight of *Z. clinopodoides* in comparison to control is probably due to an increase in oxine level within symbiotic plants with mycorrhiza fungi. The produced oxine by fungal partners may cause the increase in root dry weight and stimulation of symbiosis in plants (Rahmatzade and

Khara, 2008). Results of Whitmore and Whalley (2009) showed that this may be due to an increase in mechanical strength of soil or a reduction in hydraulic conductivity of roots (Ladjal *et al.,* 2005). The results reported by Abdelmoneim *et al.* (2014) showed that all plant treatment in presence of *G. mossease* caused the increase in all plant growth parameters due to more fungus mycelia, which it extends the root surface area and improves uptake of water and nutrients by roots (same as Bethlenfalvay *et al.,* 1988). The effects of *G. mosseae* on plant water status have been associated by the enhanced nutrition of host plant, especially phosphorus (P) (Giovannetti and Mosse, 1980; Graham and Syvertson, 1984; Almagrabi and Abdelmoneim, 2012).

Under drought stress, the whole plant dry weight decreased due to water shortage. Misra and Sriacastiva (2002) observed that water stress in mint (*Mentha piperita* L.) led to a significant decrease in leaf area, whole wet and dry weights and amount of chlorophyll and essence yield. The inoculation of *Z. clinopodoides* with *G. intraradices* and *G. versiforme* under drought stress caused an increase in whole plant dry weight compared to Control. Van de Staaij *et al.* (2001) also showed that plant hormones such as oxine and cytokinin released by mycorrhiza fungi may be effective in the increase of plant growth. The inoculation of *Z. clinopodoides* with *G. intraradices* and *G. versiforme* under stress or normal conditions caused an increase in plant height in comparison with control. In a study, differences at heights of *Anthyllis cytisoides* and *Spartium junceum* were not significant between mycorrhiza *G. intraradices* and *Glomus mosseae* but the difference between two treatments and the control was significant (Busquets *et al.,* 2010). The heights of *Z. clinopodoides* seedlings decreased under drought stress due to water shortage. Decrease in long term growth of aerial parts in areas subjected to drought and water stress is generally associated with a decrease in oxine level and prevention of hormone polar transfer in these tissues (Rahmatzade and Khara, 2008).

Conclusion

It was concluding that both studied mycorrhiza species could colonize the roots of plants and improve their establishment under severe drought stress. There was a positive relationship between colonization percent, some morphological traits and establishments and survivals of plant. The *Glomus intraradices* reacted better to short drought than *G. versiforme* and increased establishment and morphological traits of plant. According to results, it was observed that mycorrhiza colonization increased growth and drought resistance in *Ziziphora clinopodioides* Lam. It is recommended to get more conclusive results and the experiments should be tested at field conditions and on more *Ziziphora* species**.**

References

Abdelmoneim, T.S., Moussa, T. AA, Almaghrabi, O.A., Alzahrani, S.H. and Abdelbagi, I., 2014. Increasing Plant Tolerance to Drought Stress by Inoculation with Arbuscular Mycorrhiza Fungi. *Life Science Jour.*, 11(1): 10-17.

Abdul-Naser, A., 1998. Effects of inoculation *Glomus intraradices* on growth, nutrient uptake and metabolic activities of squash plants under drought stress condiction. Annuals of Agricultural Science Cairo, 1: 119-133.

Alkira, B.H., Simon, J.E., Palevitch, D. and Putievsky, E., 1993. Water management for Midwestern peppermint (*Mentha piperita* L.) growing in highly organic soils. Indiana, USA. *Acta Horticultureae*, 344: 544-556.

Almagrabi, O.A. and Abdelmoneim, T.S., 2012. Using of Arbuscular mycorrhiza fungi to reduce the deficiency effect of phosphorous fertilization on maize plants (*Zea mays* L.). *Life Science Jour*., 9 (4):1648-1654.

Amiri Dehabadi, A., Rezvani Moghaddam, P. and Ehyaee, H. R., 2012. The effect of drought on yield and some morphological traits of three medical plants dill (*Anethum graveolens*), coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare*) under greenhouse conditions. *Iran Agricultural Research,* 10 (1): 116-124. (In Persian).

Auge, R.M., 2001. Water relation drought and *vesicular arbuscular* mycorrhiza symbiosis. *Mycorrhiza*, 11: 3-42.

Azimi., R., 2013. Investigating effects of mycorrhiza inoculation on the establishment and growth characteristics *Bromus kopetdaghensis*, *Medicago sativa, Thymus vulgaris* and *Ziziphora clinopodioides* in rangeland of Bahar Kish Quchan. Thesis in M.Sc., Ferdowsi Mashhad University. 167 p. (In Persian).

Azimi, R., Jankju, M. and Asghari, H., 2014a. Effect of mycorrhiza inoculation on seedlings establishment and morphological parameters of alfalfa (*Medicago sativa* L.) in rangeland of Bahar Kish Quchan. *Agroecology*, 5(4): 424-432. (In Persian).

Azimi, R., Jankju, M. and Asghari, H., 2014b. Effects of mycorrhiza Symbiosis on Initial Establishment and Morphological Traits of Thyme (*Thymus vulgaris*) Under Natural Conditions. *Iranian Jour. Field Crops Research*, 11(4): 666-676. (In Persian).

Azimi, R., Jankju, M. and Asghari, H., 2015. Effect of mycorrhiza inoculation on seedlings establishment and morphological parameters of *Bromus kopetdaghensis* L. under field conditions. *Jour. Range and Watershed Management (Iranian Jour. Natural Resources)*, 67(4): 559-570. (In Persian).

Bagheri, V., Shamshiri, M., Shirani, H. and Rusta, H., 2011. The effect of Mycorrhiza arbuscular and drought stress on growth, water relationship, the accumulation of proline and soluble sugar in seedlings of two basic variety of domestic pistachio (*Pistacia vera* L.). *Iranian Jour. Horticultural Sciences*, 42 (4): 365-377 (In Persian).

Bahrami Sirmandi, S., Azimi, M., Naghizade, M., Hasanzade, R., Arman, M. and Farahnak, A., 2011. Mycorhiza Symbiosis. Behta Research Press, Esfahan. (In Persian).

Bethlenfalvay, G.J., Brown, M.S., Ames, R.N. and Thomas, R.S., 1988. Effects of drought on host and endophyte development in mycorrhiza soybeans in relation to water use and phosphate uptake. *Physiol Plant*, 72:565–571.

Busquets, M., Calvet, C., Camprubí, A. and Estaún, V., 2010. Differential effects of two species of arbuscular mycorrhiza on the growth and water relations of *Spartium junceum* and *Anthyllis cytisoides*. *Symbiosis*, 52:95–101.

Caravaca, F., Berea, J., Palenzuela, J., Figueroa, D., Alguacil, M. and Roldan, A., 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or *Allochthonous arbuscular* mycorrhiza fungi. *Jour. Applied Soil Ecology*, 22(2): 103-111.

Demir, S., 2004. Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. Turk *Jour. Biology*, 28: 85-90.

Giovannetti, M. and Mosse, B., 1980. An evaluation of techniques for measuring *vesicular-arbuscular* mycorrhiza infection in roots. Journal of New Phytologist, 84: 489-500.

Gonzalez-Chavez, C., D'Haen, J., Vangronsveld, J. and Dodd, J. C., 2002. Copper adsorption and accumulation by the external mycelium of three arbuscular mycorrhiza fungi from polluted soils. *Jour. Plant and Soil*, 240(2): 287–297.

Graham, J.H. and Syvertson J.P., 1984. Influence of *vesicular arbuscular* mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol, 97:277–284.

Hu, Y. and Schmidhalter, U., 2005. Drought and salinity: A comparison of their effects on mineral nutrition of plants. *Jour. Plant Nutrition*, 168: 541-549.

Jacobsen, I., Abbott, L. K. and Robson, A., 1992. External hyphae of *Vesicular arbuscular* Mycorrhiza fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *Jour. New Phytologist*, 120: 371-380.

Ladjal, M., Huc, R. and Ducrey, M., 2005. Drought effects on hydraulic conductivity and xylem vulnerability to embolism in diverse species and provenances of Mediterranean cedars. *Jour. Tree Physiology*, 25: 1109 –1117.

Mamadalieva, Z. N., Akramov, D.Kh., Ovidi, E., Tiezzi, A., Nahar, L., Azimova, Sh.S. and Sarker, D. S., 2017. Aromatic Medicinal Plants of the Lamiaceae Family from Uzbekistan: thnopharmacology, Composition, and Biological Activities, Essential Oils. *Medicines*, 4 (8): 1-12.

Misra, A.O. and Sriacastiva, N.K., 2002. Influence of water stress on Japanese mint. *Jour. Herbs, Species and Medicinal Plants*, 7: 51-58.

Mota, J.F., Medina-Cazorla, J.M., Navarro, F.B., Pérez-García, F.J., Pérez-Latorre, A., Sánchez-Gómez, P., Torres, J.A., Benavente, A., Blanca, G., Gil, C., Lorite, J., and Merlo, M.E., 2008. Dolomite flora of the baetic ranges glades (South Spain). *Flora*, 203: 359–375.

Phillips, J. M. and Hayman, D. S., 1970. Improved procedure for clearing roots and staining parasites and *vesicular arbuscular* mycorrhiza fungi for rapid assessment of infection. *Jour. Transactions of the British Mycological Society*, 55: 158–161.

Porras-Soriano, A., Soriano-Martín, M.L., Porras-Piedra, A. and Azcón, R., 2009. Arbuscular mycorrhiza fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *Jour. Plant Physiology*, 166: 1350-1359.

Rahmatzade, S. and Khara, J., 2008. The effect of UV-C ray on growth and some morphological and physiological traits of symbiotic wheat seedlings with three Mycorhiza fungi species. *Iranian Jour. Field Crops Research*, 21 (1): 52-63. (In Persian).

Smith, F.A., Grace, E. J. and Smith, S. E., 2009. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhiza symbioses. *Jour. New Phytologist*, 182: 347–358.

Song, H., 2005. Effects of VAM on host plant in the condition of drought stress and its Mechanisms. Electronic *Jour. Biology*, 1(3): 44-48.

Van der Heijden, M.G.A. and Sanders, I., 2002. Mycorrhiza ecology. *Jour. Mycorrhiza Ecology*. Springer, Berlin, Heidelberg. 469 pp.

Van de Staaij, J.W.M., Rozema, J., Van Beem, A. and Aerts, R., 2001. Increased solar UV-B radiation may reduce infection by arbuscular mycorrhiza fungi (AMF) in dune grassland plants: evidence from five years of field exposure. *Jour. Plant Ecology*, 154: 171-177.

Vosatka, M. and Dodd, J.C., 1998. The role of different arbuscular mycorrhiza fungi in the growth of *Calamagrostis villosa* and *Deschampsia flexuosa* in experiments with simulated acid rain. *Jour. Plant and Soil*, 200: 251-263.

Whitmore, A.P. and Whalley, W.R., 2009. Physical effects of soil drying on roots and crop growth. *Jour. Experimental Botany*, 60(10): 2845-2857.

Wu, Q. S. and Xia, R.X., 2004. Effects of mrbuscular mycorrhiza fungi on plant growth and osmotic adjustment matter content of trifoliate orange seedlings under water stress. *Jour. Plant Physiology and Molecular Biology*, 30: 583-588.

Wu, Q.S. and Xia, R.X., 2006. Arbuscular mycorrhiza fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Jour. Plant Physiology*, 163(4): 417-425.

Yordanov, I., Velikova, V. and Tsonev, T., 2003. Plant responses to drought and stress tolerance. *Jour, Plant Physiology*, 187-206.

بررسی اثر تنش خشکی و میکوریزا بر زنده‌مانی و خصوصیات رشد رویشی کاکوتی کوهی (*Ziziphora clinopodioides* Lam)

ریحانه عظیمی[الف*]، غلامعلی حشمتی[ب]، محمدکیا کیانیان[ج]

[الف*] دکتری علوم مرتع، پژوهشگر پژوهشکده محیط‌های خشک (نگارنده مسئول)، پست الکترونیک: Reyhaneazimi36@yahoo.com

[ب] استاد دانشکده مرتع و آبخیزداری، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

[ج] عضو هیات علمی دانشکده کویرشناسی دانشگاه سمنان

تاریخ دریافت: ۱۳۹۵/۰۷/۱۹

تاریخ پذیرش: ۱۳۹۶/۰۷/۲۹

چکیده. تنش کمبود آب یکی از مهم‌ترین عوامل محدودکننده استقرار، رشد و تولید گیاهان مرتعی-دارویی در مناطق خشک و نیمه خشک محسوب می‌شود. به منظور بررسی اثرات قارچ میکوریزا بر کاهش تنش خشکی و میزان استقرار و برخی خصوصیات مورفولوژیکی-رشدی کاکوتی کوهی (*Ziziphora clinopodioides*)، آزمایش گلدانی به صورت فاکتوریل در قالب طرح کاملا" تصادفی در گلخانه تحقیقاتی دانشکده کشاورزی دانشگاه فردوسی مشهد در سال ۱۳۹۱ انجام شد، که در آن چهار سطح تنش خشکی شامل ۱۰۰ (شاهد)، ۷۵ ، ۵۰ و ۲۵ درصد ظرفیت مزرعه و سه سطح میکوریزا (*Glomus versiforme*، *Glomus intraradices* و شاهد) به صورت فاکتوریل ترکیب شدند. نتایج نشان داد، که در تنش خشکی۲۵ درصد ظرفیت مزرعه ، وزن گیاه (۰/۷۵، ۰/۶۷ و ۰/۱۹ گرم در بوته)، ارتفاع (۱/۲۵، ۱/۱۸ و ۰/۷ سانتیمتر، استقرار گیاه (۶۷/۵۰، ۵۷ و ۱۴/۲۵ درصد) و میزان کلونیزاسیون ریشه گیاهان (۵۰، ۳۵/۵۰ و صفر درصد) توسط قارچ‌های میکوریزا *G. intraradices*، *G. versiforme* و شاهد) کاهش یافت که نشاندهنده تاثیر معنی دار قارچ‌ها در افزایش مقاومت به خشکی این گیاه می‌باشد. نتایج نشان داد، که در هر دو تیمار قارچی، میانگین‌های میزان استقرار نهال‌ها (۸۵ و ۶۴ و ۳۶ درصد) در تنش ۵۰ درصد ظرفیت مزرعه و (۶۷/۵۰، ۵۷ و ۱۴/۲۵ درصد) در ۲۵ درصد ظرفیت مزرعه به ترتیب توسط قارچ میکوریزا *G. intraradices*، *G. versiforme* و شاهد) بود، بطوری که همزیستی قارچ میکوریزی به طور چشمگیری موجب افزایش استقرار گیاه کاکوتی کوهی (*Z. clinopodioides*) تحت تنش خشکی در مقایسه با شاهد گردید. اثر قارچ‌های *G. intraradices* و *G. versiforme* بر روی میزان استقرار گیاهان (۹۳ درصد در مقایسه با ۸۷/۵۰) ، ارتفاع گیاهان (۵/۹۳ سانتیمتر در مقایسه با ۵/۳۰ سانتیمتر) در ۷۵ درصد ظرفیت مزرعه و وزن خشک (۱/۰۷ گرم در مقایسه با ۰/۹۵ گرم) در تنش ۵۰ درصد ظرفیت مزرعه بیشتر بود. گونه‌ی *G. intraradices* در مقایسه با گونه‌ی *G. versiforme*، به تنش خشکی کوتاه-مدت بهتر پاسخ داد و موجب افزایش خصوصیات رویشی و استقرار گیاه گردید. طبق نتایج، *G. intraradices* را می توان به عنوان یک کود زیستی در افزایش تولید علوفه و تکنیکی جهت مقاوم سازی و بالا بردن تحمل گیاه کاکوتی کوهی نسبت به تنش‌های خشکی پیشنهاد کرد.

کلمات کلیدی: کلونیزاسیون، خشکی، کود بیولوژیک، گلخانه