Study on the growth of (*Achillea millefolium* L.) medicinal plant by soil inoculation of mountainous area with selected mycorrhizal fungi

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Abstract

In recent years, the use of soil microbial potential including arbuscular mycorrhizal fungi is widely considered to enhance the better growth and nutrition of plants. This research was conducted in order to study the impact of mycorrhizal fungi on the increase of yarrow production and the plant establishment affected by the symbiotic relationships between fungi and yarrow plant, based on factorial experiment as a randomized complete block (RCB) design with 4 treatments. Inoculated treatments included non-inoculated condition as control mode, inoculated with G. fasciculatum, G. mosseae and G. intraradice fungi. At the end of growth season, some growth parameters such as colonization, plant establishment, essential oil percentage and also morphological properties including fresh and dry weight of shoot and root and the root volume were measured. Results showed that the inoculation of Achillea millefolium L. with arbuscular mycorrhizal fungi have a significant effect on the measured parameters. This means that the inoculation with mycorrhizal fungi leads to increasing the plant establishment and essential oil percentage, fresh and dry weight of shoots and roots, and also the root volume compared to the control mode. It is noteworthy that among the three treatments done with fungi, the treatment of G. fasciculatum showed the most increase in the above parameters.

Keywords: *Achillea millefolium* L.; arbuscular mycorrhizal fungi; inoculated treatments; growth parameters

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Introduction

The importance of medicinal plants in the preservation and stability of ecosystem, economic development at the macro level, and drug security is completely obvious (Chen et al., 2003). One of the most valuable medicinal plants in the world is *Achillea millefolium* L.

A member of the Asteraceae family, yarrow (*Achillea millefolium* L.), is a small perennial herb with 50 cm height. It has alternate, oblong-lanceolate leaves, small white flowers, and a slender cropping rootstock throwing numerous roots. Its fruits are shining with no pappus (Akram, 2013). *Achillea millefolium* L. is found on baulks, dry pastures, and roadsides. Several effects such as antibacterial (Candan et al., 2003; Stojanovic et al., 2005), antispasmodic (Moradi et al., 2013), anti-inflammatory (Benedek et al., 2007), antihypertensive, anti-hyperlipidemia (Asgary et al., 2000), and antitumor (Tozyo et al., 1994;

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Csupor-Loffler et al., 2009) have been reported for Achillea. It is cultivated in Iran and widely used in traditional medicine for gastrointestinal disorders (Nemeth and Bernath, 2008).

Compared to species collected from nature, domesticated medicinal plants are qualitatively on a lower level (Schippmann et al., 2006; Hamilton, 2013) because the pharmaceutical properties of these plants are mainly due to the presence of secondary metabolites whose concentration increases in the natural growing environment, especially in certain conditions of stress and competition with neighboring species and such conditions cannot be obtained except in the natural arena (Uniyal et al., 2000). But since the majority of studies on plants were done under conditions of farm, research about the effects of mycorrhiza on plants is inevitable due to the positive effects of mycorrhiza on the quantity and quality of plants.

Arbuscular mycorrhizal fungi (AMF) as symbionts with more than 80% of terrestrial plant species are distributed among a variety of environments (Brundrett, 2002; Smith and Read, 2008; Ardakani et al., 2017; Shahabivand et al., 2018).

Numerous studies have shown that AMF decreases the adverse effects of nutrient poverty, drought, and salinity stress (Sasanelli et al., 2009; Thrall et al., 2009) and increases the plant growth, nutrient uptake such as nitrogen and phosphorus, and plant resistance to stresses (Franken, 2012; Nikkah Naeeni et al., 2017). Therefore, this study aimed at investigating the potential of mycorrhizal fungi to be used as a compassable and symbiotic fungus with nature in order to increase the production and initial establishment of *Achillea millefolium* L.

Materials and Methods

Area of study

The experiments were performed in Roudbarak in 2015 located about 65 km northeast of the Mehdishahr city. Roudbarak is a mountainous area with cool spring and summer, cold autumn, and snowy winter with the average temperature, humidity, and rainfall of 8.7° C, 62%, and 162 mm, respectively, as recorded in 2015. Physical and chemical properties of soil vary with soil depth and these characteristics affect the distribution of VAM fungi. Spore population may vary with changing clay, pH, and soil phosphorus. Therefore, the physical and chemical properties of soil under cultivation were evaluated. Soil texture was determined by the hydrometer method (Bouyoucos, 1962). For each of the soil samples, saturated mud was prepared and pH and EC (Electrical conductivity) of soil were measured using pH meter and conductometer, respectively (Sparks et al., 1996). Flame photometer was employed to measure sodium and potassium of the soil solution. In addition, Olsen and Kjeldahl methods were used to assay the soil absorbable phosphorus and nitrogen, respectively (Kjeldahl, 1883; Olsen et al., 1954). The results are presented in Table 1.

Planting

First, the terraces with 1 m width and 3 m length at a distance of 50 cm from each other were prepared for the cultivation of seedlings and after that their soil was plowed to a depth of 30 cm and then was flattened before seedlings were planted. All purchased seedlings were the same, juicy and had some green leaves and stems with length of 15-25 cm and were inside the washed sand. The

Table 1
Physical and chemical properties of soil under cultivation

Properties Values	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	EC (dS/m)	N (%)	P (mg/kg)	K (mg/kg)	Na (meq/l)
Acceptable values	-	40	30	30	6-7	< 4	0.10-0.15	25	350	-
Obtained values for tested soil samples	30-40	27	46	27	7.7	4.582	0.083	32	280	30

used mycorrhizal fungi species were prepared from Zistfanavar Turan Company. Mycopersica contains fungal spores, hyphae, and inoculated root fragments that are available in the consumer market. The number of spores per gram of soil for all three species of mycorrhizal fungi was 120. The method of using Mycopersica was as follows: 20 g of each type of fungus individually were placed on seedling root per bush. At the time of planting, the seedlings were inoculated with three species of mycorrhizal (Glomus fasciculatum, Glomus mosseae, and Glomus intraradice) in separate treatments. In this way, for each bush 20 g soil of the mycorrhiza was placed on seedling roots. The first irrigation was carried out after planting, and the next irrigations and the weed control were done every 10 days until the end of the growing season and every 15 days, respectively. The irrigation of terraces was performed separately. Harvesting was done at the end of vegetative growth and at the beginning of reproductive growth.

Harvesting plant and evaluation of properties

At the end of vegetative growth, plant shoots that had exited from the soil surface were cut and transferred to the laboratory and their fresh weights were measured using a digital scale. In order to extract and measure the essence, some of the harvested aerial parts were gradually dried for 3 weeks at room temperature with air conditioning. Mean weight of samples was 50 g; the extraction of essence was conducted through the method of distillation with water using a Clevenger apparatus and after weighing, the weight percentage was calculated. The remaining aerial parts were used to measure the dry weight of these parts. For this purpose, samples were put in the oven at 70° C for 48 h, and then each was weighed with the precision of 0.01 g. Evaluation of the seedling establishment percent at harvest was performed by counting the established seedlings.

Roots were washed after separation from the soil in order to remove the additional soil and their fresh weight was measured using a digital scale after dewatering. Some of the fine roots, after crushing to pieces of 1 cm, were transferred to bottles containing fixative solution to stain and study colonization. Roots were stained with the method described by Philips and Hayman (1970) using 5% trypan blue solution in lactophenol. Colonization percent of the stained roots was determined with the method proposed by Biermann and Linderman (1981). Another root sample was immersed in the distilled water inside a graduated cylinder with the volume of 1 L. after washing and removing excess water. Then, the volume of roots was determined in cubic centimeters (cm^3) by measuring the difference between the initial volume of water and the its final volume after immersing the roots. Also, to measure the dry weight of roots, the remaining roots were placed in an oven at 70° C for 72 h. Then, each of them was weighed separately. At harvest time, the samples were selected randomly and by eliminating marginal effect in each terrace their quantitative and qualitative properties were also investigated.

Statistical Analysis

Factorial experiment in a randomized block design including 4 treatments and 4 replications was used to analyze data. Simple variance was used to assess the impact of applied mycorrhizal treatments. All statistical analyses were performed using SPSS. Excel software was also used for diagramming. Also, Duncan's multiple range test method with difference at the 5% level was used for the comparison of means.

Results

To evaluate the impact of fungi on qualitative and quantitative performance of *Achillea millefolium* L., the effect of mycorrhizal symbiosis on some properties of this plant such as percentage of root colonization and establishment, the essence percentage, and morphological traits were investigated.

Percentage of root colonization for *Achillea millefolium* L. with treatment of mycorrhiza

As shown in Fig. I, the study of colonization suggests that *Achillea millefolium* L.

has the ability for symbiosis with mycorrhizal fungi and the highest percentage of root colonization (71%) was obtained for the plants inoculated with G. fasciculatum which was significantly higher than that obtained for the Glomus mosseae statistically.

Effect of mycorrhizal symbiosis on the establishment of *Achillea millefolium* L.

The establishment percentage of seedlings inoculated with G. fasciculatum and G. intraradice was evaluated at the end of the growth season. As shown in Fig. II, results revealed that the establishment percentage of inoculated plants was significantly higher than that obtained for control mode. So the symbiosis of *Achillea millefolium* L. with these two fungi not only leads to the seedling establishment, but also will increase the seedling establishment compared to the control mode about 25% and 10%, respectively.

Effect of mycorrhizal symbiosis on the essence percentage of *Achillea millefolium* L.

According to Fig. III, the results relating to the impact of arbuscular mycorrhizal fungi on the percentage and performance of essence indicates that although there was no significant difference between the results of three treatments, Glomus fasciculatum had a significant increase as compared to the control mode.

Effect of the treatments of mycorrhiza on the morphological properties of *Achillea millefolium* L.

Shoot weight is dependent on the plant inherent characteristics and the physical and chemical conditions of soil. Figs. IV (A) and (B) indicate that the inoculation of *Achillea millefolium* L. with Glomus fasciculatum causes a significant increase in the fresh and dry weight of shoot equal to 37 and 11 g, respectively compared to the control mode. In addition, in Fig. IV (A), it can be seen that the fresh weight of shoot inoculated with Glomus mosseae hasd a significant increase compared to the control mode.

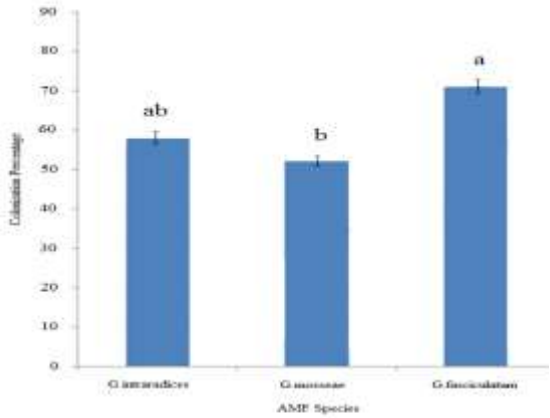

Fig. I. Percentage root colonization for *Achillea millefolium* L. at different treatments

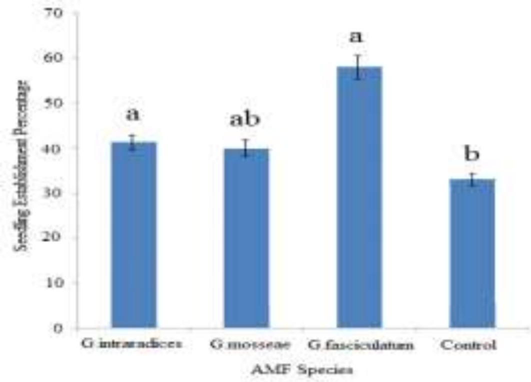

Fig. II. Establishment percentage for *Achillea millefolium* L. at different treatments

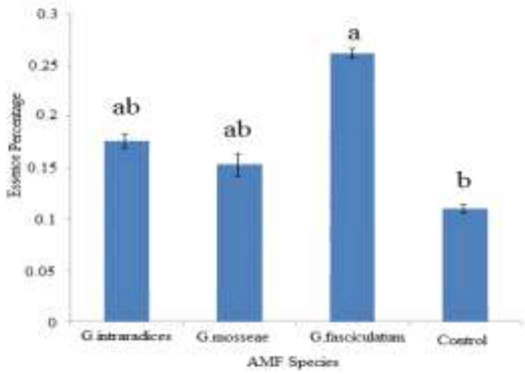

Fig. III. Essence percentage for *Achillea millefolium* L. at different treatments

In the case of fresh and dry weight of root, the results summarized in Figs. IV (C) and (D) show that the highest fresh and dry weights were obtained in the treatment with G. fasciculatum, 33.15 and 5.51 g, respectively which has the weight increase equal to 6.5 and 3 g compared to the control mode, respectively.

Finally, data of Fig. IV (E) depicts the significant increase of root volume in the presence of Glomus fasciculatum and Glomus mosseae.

Discussion

In symbiosis of mycorrhizal fungi with the host plant, a part of carbon produced by photosynthesis becomes available for the fungus and instead, the hyphae extensive network of mycorrhizal fungi enhances the absorption and transfer of water, minerals, and numerous nutrients such as nitrogen, calcium, sulfur, potassium, copper, and zinc for the plant from the inaccessible areas for plant's root system and this symbiosis helps the plant for better growth. This means that both the host plants and soil benefit from making the connection with fungi (Ridsdale, 2012). Mycelium of arbuscular mycorrhizal fungi with more dispersion around the roots provides a wider absorbent surface for the transfer of soil nutrients to the plants root. Of other advantages of this useful relationship, one can note enhancing the production of plant growth hormones (such as cytokinin, auxin, etc.), increasing crop yields, improving plant resistance to root pathogens, helping the decrease of consuming the chemical fertilizer, and decreasing environmental stresses such as temperature, soil contamination by pesticides or heavy metals and salinity (Vadassery et al., 2008).

The present study provides clear evidence for the growth response of micro propagated *Achillea millefolium* L. to mycorrhizal symbiosis which is in agreement with the results obtained by other researchers so that all of the measured parameters (such as percentage of root colonization and establishment, the essence percentage, and morphological traits) showed a significant increase by mycorrhizal inoculation.

The increase in colonization percentage may depend on the fungal species and host plant. In this context it was found that isolations of one species collected from various areas may have different colonization percentages while their difference in morphological or physiological properties of host plant may in some cases not affect colonization percentage (Jarstfer et al., 1998). High potential of G. fasciculatum has also been reported for colonization of basil (Raei and Weisany, 2013).

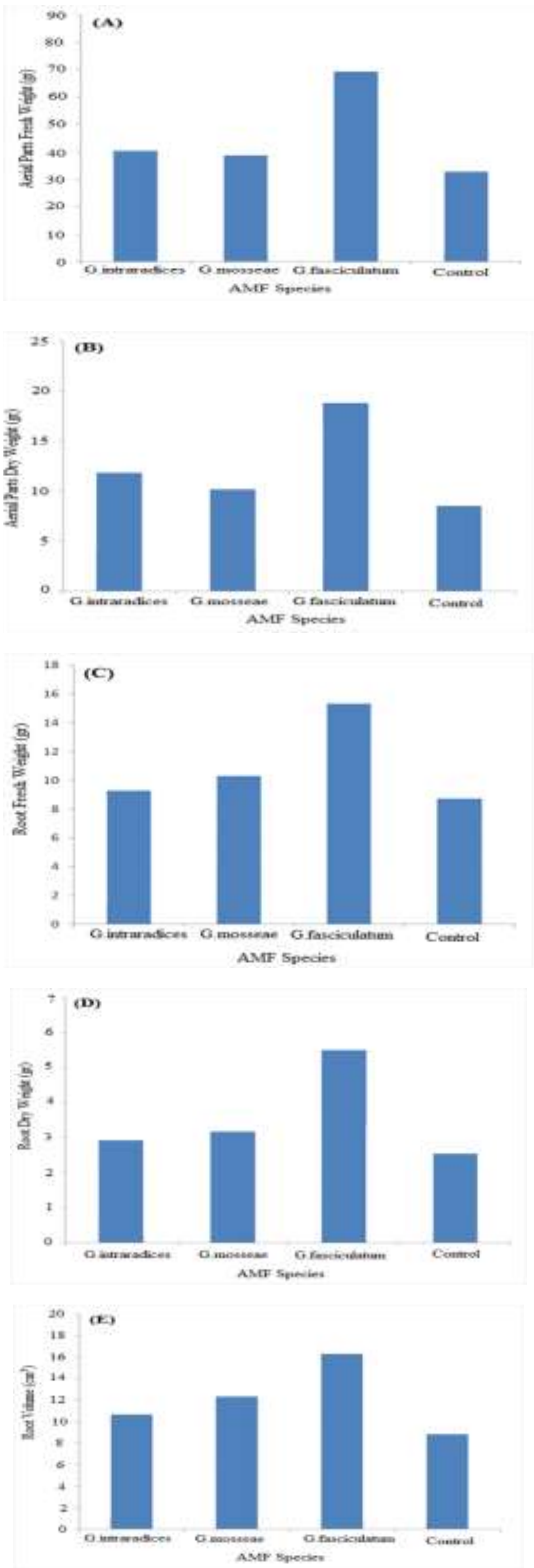

Fig. IV. a. aerial parts fresh weight; b. aerial parts dry weight; c. root fresh weight; d. root dry weight; e. root volume for *Achillea millefolium* L. at different treatments

Also, it has been found that mycorrhiza has positive effects on the establishment, survival, and growth of the tested species and improves vegetation restoration in natural condition (Azimi et al., 2016). Facilitation of seedlings establishment is probably due to providing more moisture, phosphorus, nitrogen, and mineral (Porras-Soriano et al., 2009) and improving soil structure (Rashid et al., 2016) in mycorrhizal inoculation.

In the case of the essence percentage, the observed increase may be due to the impact of arbuscular mycorrhizal fungi on the various nutrients uptake such as nitrogen, calcium, and potassium. Some studies showed that the increase in the essence of aerial parts of *Ocimum basilicum* L. var. *Genoves* inoculated with G. Gigaspora (Copetta et al., 2006) and oregano inoculated with G. mosseae (Khaosaad et al., 2006) was due to the improved phosphorus uptake in mycorrhizal plants. In addition, Kapoor et al. (2007) found that the fungi species of G. fasciculatum and G. macrocarpum increased phosphorus, manganese, and iron in the aerial parts of *Artemisia annua* L. medicinal plant and led to the increase in the essence content through developing the foliage.

The findings of this study indicated that the inoculation of the plants with arbuscular mycorrhizal fungi has a significant effect on the fresh and dry weight of shoots and roots and also the roots volume. The performance of this favorable impact is likely to be attributed to the idea that a part of rows enters the root system and leads to the decrease in Abscisic acid and increase in Cytokinin concentration. This causes enhanced root system and increased water and nutrients uptake (Dolatabadi et al., 2012). Other reported studies also explain the reasons of these positive effects as follows. This increase in shoot weight can be caused by the impact of arbuscular mycorrhizal fungi on the uptake of water and various nutrients such as nitrogen, calcium, sulfur, potassium, copper, and zinc (Querejeta et al., 2006; Dolatabadi et al., 2012). The positive effect of mycorrhiza on the root weight is mainly due to enhancing the production of plant hormones and increasing the enzymes activity (Sirrenberg et al., 2007; Vadassery et al., 2008) and consequently developing the root and improving the moisture and nutrients uptake which increase the chance of drought-resistant plants such as *Achillea millefolium* L. for avoidance of drought. External hyphae of mycorrhiza are the main factor in providing carbon for the soil (Alguacil et al., 2004; Swift, 2004; Barea et al., 2005). Finally, mycorrhizal fungi cause changes in the shape and volume of roots in two aspects: 1. change in plant nutritional status, 2. change in plant hormones level (Yao et al., 2005). Change in the branches and volume of roots is affected by the nutrients especially phosphorus (Lopez-Bucio et al., 2003). On the other hand, it is confirmed that the change in the number of root branches as a result of symbiosis of mycorrhizal fungi is due to the changes in the plant hormones including polyphenolic compounds that prevent oxidation of Oxine (Perez-Perez, 2007).

Conclusion

The present work was performed in order to study possibility replacement of biological fertilizers instead of chemical fertilizers and increasing the required yield in semi-arid areas. The results of this study showed that *Achillea millefolium* L. is able to coexist with mycorrhizal fungi and the plant inoculation with mycorrhiza could increase the growth and establishment performance of *Achillea millefolium* L. relative to the control conditions. Also among studied fungi (G. fasciculatum, G. Mosseae, and G. Intraradice), the highest rate of colonization, the greatest increase in the establishment percentage, essence percentage, and morphological properties were observed in the treatment with G. fasciculatum. Thus, the results of this study demonstrate the potential of G. fasciculatum mycorrhizal fungi for using as a symbiotic fungus in the increase in the production and initial establishment of *Achillea millefolium* L.

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