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The effects of two arbuscular mycorrhizal fungi on some physical properties of a sandy loam soil and nutrients uptake by spring barley

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Abstract

The coarse-textured soils have mainly macrospores, therefore, water and nutrients holding capacity of these soils is considerably low. Although the effects of mycorrhizal fungi on physical properties and nutrients uptake has been studied in fine- textured soils but the effect of these fungi on physical properties and nutrients uptake in coarse-textured soils has not been studied. A completely randomized block experimental design was conducted with two species of arbuscular mycorrhizal fungi including *Glomus intraradices* (GI), *Glomus etunicatum* (GE) and a non-mycorrhizal (control) undergrowth of spring barley with four replications in a sterilized sandy loam soil under greenhouse conditions. The results showed that GI and GE fungi significantly ($P < 0.01$) increased the mean weight diameter of aggregates by 113.6 and 201.8%, mesopores by 20.8 and 27.8% and microspores by 5 and 14.1%, total porosity by 2.2 and 2.6%, available water capacity by 13.3 and 27.1%, while decreased macropores by 9.5 and 17.3% and saturated hydraulic conductivity (K_s) by 68.8 and 88.2% relative to the control, respectively. Furthermore, the percentages of increase were 45.9 and 164 for potassium and 53.5 and 135.1 for phosphorus in GI and GE relative to the control, respectively. According to the results of this study, mycorrhizal symbiosis improved physical quality and nutrients uptake of the alkaline coarse-textured soil.

Key words: Mycorrhizal symbiosis; Coarse-textured soils; Aggregate stability; Pore size distribution, Phosphorus uptake.

Introduction

Soil aggregation is of great importance in agriculture due to its positive effect on soil physical properties and plant growth. Soil aggregate stability is one of the most important properties controlling plant growth. Soil structure has a prevailing role in soil infiltration and biogeochemistry processes (Rillig, 2004). Improved soil structure means increased water retention, nutrient uptake, drainage, aeration and root growth. Coarse-textured soils have plentiful macropores that lead to loss of water and nutrient elements (Hillel, 1998; Asghari et al. 2011). Arbuscular mycorrhizal fungi (AMF) are widely present in agricultural soils. Studies conducted in different ecological conditions revealed that mycorrhiza plays a significant role in the formation of stable aggregates (Bearden and Petersen, 2000; Rillig, 2004). The hyphae of mycorrhiza along with mycorrhizal polysaccharide binds soil particles and results in the formation of stable aggregates (Rillig and Mummey, 2006). Caravaca et al. (2002) reported that adding organic fertilizers activates the arbuscular mycorrhiza which produces insoluble glycoprotein called glomalin. Bethlenfalvay et al. (1999) found a positive correlation between water-stable soil aggregates and arbuscular mycorrhiza soil mycelium development. As an interface between lithosphere and atmosphere, the soil controls the earth water budget via its physical properties which determine the runoff and infiltration fractions. Water quality is strongly influenced by infiltration through the soil as well. These properties result from the equilibrium between constituents, soil life, and external factors, which vary on different time and space scales. Characterizing and predicting soil physical properties and their changes with time as a function of these factors are essential. Therefore, integrated approaches aiming better understanding the interactions between physical and biological processes in the soil and with the aboveground system are encouraged (Young and Crawford, 2004). Roots are known to modify the soil porosity and aggregation via direct entanglement of particles, the creation of bio-pores or secretion of glue-substances

sticking particles together as reviewed by Angers and Caron (1998). Similar to plant roots, AMFs are a very important components of the soil system. They influence soil aggregation by binding and enmeshing soil particles into larger aggregates. They also secrete a glycoprotein called glomalin that act as a glue-substance (Rillig and Mummey, 2006). Feeney et al. (2006) suggested that soil structure and water repellency can be influenced by root and microbial activity extremely quickly. Their investigation showed that the number of aggregates > 2000 μm and their water repellency both significantly increased over a 30 days period; this was attributed to increased fungal activity, particularly in the rhizosphere. Martin et al. (2012) reported that total porosity significantly increased in *Glomus mosseae* treatment relative to the control in a sandy loam soil. Arbuscular mycorrhizal fungi promote aggregate stabilization through action of their extraradical hyphae (Thomas et al. 1993). Even AMF hyphae alone in the absence of other soil biota are sufficient to increase soil aggregation (Rillig et al. 2010). Celik et al. (2004) reported that mycorrhizal symbiosis increased mean weight diameter of aggregates (18%), total porosity (8.3%) and available water capacity (34.2%) in a clay loam soil. Arbuscular mycorrhizal fungi produce an extensive network of hypha and high amount of organic matter. The glomalin content, which is a glycoprotein of organic matter, is a source of C for other microorganisms. The production of organic matter protects soil structure through improving soil aggregation (Miller and Jastrow, 2000; Tisdall, 1994).

To measure the plant response to AMF infection, mycorrhizal fungi need to be eliminated from the control plants. This can be achieved by the use of certain crop rotations or various soil sterilization techniques (Bever, 2002). It has been reported that AMF inoculation significantly enhanced the growth of barley in the soils with low available phosphorus (P) (Clarke and Mosse, 1981), while such improvement in barley growth was not significant in an irradiated soil containing moderate amounts of available P (Zarea et al. 2009).

From a functional point of view, mycorrhizae are characterized by the transfer of limiting nutrients, in particular phosphorus and nitrogen, from the fungal hyphae to the plant. In exchange for this improved nutrient uptake, plants deliver carbon compounds to the symbiotic fungi, consuming up to, 20% of the plant photosynthate in case of AM (Harrison, 2005). Mycorrhizal associations increase the absorptive surface area of the plant due to extra-matrical fungal hyphae exploring rhizospheres beyond the root hair zone, which in turn enhance water and mineral uptake. The protection and enhanced capability of greater uptake of minerals result in greater biomass production, a pre-requisite for successful remediation.

Despite the literature just cited, the effects of mycorrhizal symbiosis on the soil pores size distribution (according to the recent classification of SSSA, 1997) have not been thoroughly studied. Improving effects of AMF on the structural stability and nutrient uptake in the coarse-textured and alkaline soils still need further investigation. The objectives of this study were to investigate the effects of two AM fungal species on the physical properties of an alkaline sandy loam soil including pores size distribution (macro, meso, micro), mean weight diameter of aggregates (MWD), total porosity (f), available water capacity (AWC), saturated hydraulic conductivity (K_s) and also P and K uptake in the spring barley.

Materials and Methods

Preparing fungal inoculates

Two species of arbuscular mycorrhizal fungi as *Glomus intraradices* (GI), *Glomus etunicatum* (GE) (provided by Soil Biology Laboratory of Tabriz University, Iran) were propagated with maize plants in 5 Kg pots containing sterile mixture of sand / soil (5/1). The pots were irrigated with half strength Rorison's nutrient solution (twice a week) and tap water to bring the soil moisture to field capacity (FC) (Merryweather and Fitter, 1991) and then kept in growth room with 28/20 ± 2°C day/night temperatures and 16 h photoperiod. After four months, the aerial parts of plants were cut and pot materials containing soil, mycorrhizal roots, hyphae and spores were thoroughly mixed and used as fungal inoculums. Root colonization percentage (Giovanetti and Mosse, 1980) and number of spores per 10 g soil (Gerdemann and Nicolson, 1963) were assessed to determine inoculum potential. Both inoculums had an average of 70 to 75 root colonization percentage and nearly 280 spores per 10 g soil. AMF spores were isolated by wet sieving, decanting and subsequent centrifugation using 50% sucrose solution (Gerdemann and Nicolson, 1963). The isolated spores were washed with water to remove all the adhering sucrose solution and counted under a dissecting microscope at 30× magnification. Based on the fungal inoculums potential, 100 g inoculums were added to each pot containing 8 kg soil. The same amount of sterilized inoculum (mixture of sand + soil + spores + hyphae + root) was added to the control pots.

Measuring soil physical and chemical properties

A sandy loam soil with low available phosphorus content (5.4 mg kg^{-1}) and relatively low acidity (pH = 7.81) was taken from 0 - 30 cm layer of a bare land in Agricultural Research Station of Tabriz University, Iran. Some physical and chemical properties of the soil (Table 1) were determined according to the procedures described by Klute (1986) and Page (1985). The soil samples were air-dried and passed through a 4.75 mm sieve and then autoclaved for 2 h at 1 atmosphere pressure and 121°C.

Growing plant

The sterilized soil was filled in sterilized plastic pots with 19.75 cm diameter and 20 cm height according to the soil bulk density (1.28 g cm^{-3}) at field conditions. Six surface sterilized seeds of spring barley were grown in pots then thinned to three plants after 1 week. Each pot received 100 g mycorrhizal fungal inoculum. The control pots (non mycorrhizal) received 100 g of sterilized inoculum. Pots were kept in growth room with (27 ± 2/18 ± 4)°C day/night temperatures and 11 h photoperiod. Soil water contents of the pots were maintained at 0.7 FC. Field capacity moisture was formerly determined in the examined soil at h = 10 kPa using pressure plate apparatus. All pots received 1.51 g nitrogen as urea.

Measuring of parameters

At the end of the experiment after harvesting the plants, disturbed and undisturbed soil samples were taken from the centre of each pot at the depths of 10 - 15 cm. Undisturbed soil samples were taken by steel cylinders (5 cm diameter and 5 cm height) and used for determining pore size distribution, total porosity (f), available water capacity (AWC) and saturated hydraulic conductivity (K_s). Bulk density (D_b) was calculated from cylinder volume and oven dry (105°C) soil mass. Soil particle density (D_P) was measured by the pycnometer method (Klute, 1986). Total porosity (f) was calculated using D_b and D_P according to the following equation (Danielson and Sutherland, 1986):

$$f = 1 - \frac{Db}{Dp}$$

Saturated hydraulic conductivity was determined by the constant head method (Klute and Dirksen, 1986). Soil water content at 0, 40, 100 and 15000 cm matric suctions were measured by the hanging water column and pressure plates methods (Gardner, 1986). Available water capacity (AWC) calculated from the difference in soil water content at 100 cm (Field capacity, FC) and 15000 cm (Permanent wilting point, PWP) suctions (Bauer and Black 1992).

$$AWC = FC - PWP$$

Pore size distribution according to SSSA (1997) classification [macropores (> 75 µm), mesopores (75 – 30 µm) and micropores (< 30 µm)] was calculated using soil water retention data and capillary rise equation (Danielson and Sutherland, 1986). The modified wet sieving method (Yoder, 1936) was used to determine the mean weight diameter (MWD) of aggregates. Sieves had a pore size of 2, 1, 0.5, 0.25 and 0.106 mm. After the soil samples were passed through a 4.75 mm sieve, approximately 50 g of the soil was put on the first sieve of the set and gently moistened to avoid a sudden rupture of aggregates. Once the soil had been moistened, the set was sieved in distilled water at 30 oscillations per minute. With 5 min of oscillation, the soil remaining on each sieve was dried, and then sand and aggregates were separated. The MWD of soil aggregates was calculated according to the Van Bavel (1950) equation as follows:

$$MWD = \sum_{i=1}^{n} Xi\ Wi$$

Where MWD is the mean weight diameter of water stable aggregates, *Xi* is the mean diameter of each size fraction (mm) and *Wi* is the proportion of the total sample mass in the corresponding size fraction after the mass of sands deducted (upon dispersion and passing through the same sieve).

Plant roots were collected after harvesting spring barley for measuring mycorrhizal root colonization percentage (RCP). A fraction of the roots (0.5 g fresh weight) were carefully washed and cut into one cm long segments. Then, root samples were preserved in 50 % ethanol, cleared in 10% (w/v) KOH at 90℃ in a water bath for 30 min. The prepared solution was acidified with 1% HCL for 3 min and stained using 0.5% Trypan Blue in lactoglycerol, roots and left in clear lactoglycerol overnight (Rufykiri *et al*., 2000). The RCP was evaluated by the grid line intersect method, dispersing the stained roots above a grid of square drawn on a petri dish and observing under a dissecting microscope at 40× magnification, with a dissecting microscope, scan only the gridlines and record the total number of root intersections with the grid as well as the number of intersects with colonized roots (Norris *et al*., 1992). Vertical and horizontal gridlines were scanned under a dissecting microscope. The presence of colonization was recorded at each point where the roots intersect a line. The percentage of AM colonization was calculated using the formula (Wu *et al*., 2008):

$$\text{Percentage colonization} = \frac{\textit{Number of root segments colonized}}{\textit{Total number of root segments observed}} \times 100$$

To determine nutrients content, oven - dried samples were ashed in a muffle furnace at 500 °C for 4 h and then dissolved in 10 ml of 1M HCl. Concentration of P was determined spectrophotometrically by ammonium vanadate molybdate (yellow colour method) and K was determined by flame photometry (Anonymous, 1980). Then P and K uptake by plant was calculated for each pot.

Statistical Analysis

A completely randomized block design was conducted with two species of arbuscular mycorrhizal fungi, GI and GE and non-mycorrhizal (control) treatments in a sandy loam soil at 4 replicates. The analysis of variance and mean comparison by Duncan's Multiple Range Test were carried out using MSTATC software.

Results

Some physical and chemical properties of the examined soil are shown in Table 1. Due to the low organic carbon and clay content, the soil had low water aggregate stability and mean weight diameter of aggregates (MWD = 0.2 mm). Also, the available water capacity of this soil was very low due to high percentage of sand (72%). Alkaline reaction (pH = 7.81) of the soil may decrease uptake of some nutrient elements such as P, K and Fe by plants. No significant difference was found between measured parameters in bare soil (Table 1) and non-mycorrhizal (control) (Figure 2, 3, 4, 5, 6, 7 and 8) treatments.

Table 1. Some physical and chemical properties of the bare soil

D_b ($g\ cm^{-3}$)	Clay (%)	Sand (%)	Silt (%)	Texture class	AWC (% w/w)	MWD (mm)	K_S (cm/min)	O C (%)	K ($mg\ kg^{-1}$)	P ($mg\ kg^{-1}$)	EC ($dS\ m^{-1}$)	pH -
1.28	15	72	13	sandy loam	8.9	0.2	6.9	0.42	250	5.4	0.68	7.81

Db: bulk density; AWC: available water capacity; MWD: mean weight diameter; K_S: saturated hydraulic conductivity; O C: organic carbon; EC: electrical conductivity.

According to Table 2, mycorrhizal fungi significantly ($P < 0.01$) affected all measured physical parameters of the examined coarse-textured soil.

Table 2. Analysis of variance (F value) for measured physical parameters

Source	Df	RCP	MWD	f	AWC	K_S	Pores		
							macro	meso	micro
Block	3	3.12^{ns}	0.49^{ns}	1.25^{ns}	1.60^{ns}	0.23^{ns}	2.29^{ns}	1.48^{ns}	3.49^{ns}
AMF	2	1505.96^{**}	94.56^{**}	140.49^{**}	142.19^{**}	166.29^{**}	1892.75^{**}	416.10^{**}	475.68^{**}
Error	6	-	-	-	-	-	-	-	-
CV (%)	-	4.55	10.14	0.23	2	15.09	0.44	1.22	0.62

Df: degree of freedom; RCP: root colonization percentage; MWD: mean weight diameter of aggregates; f: total porosity; AWC: available water capacity; KS: saturated hydraulic conductivity; AMF: Arbuscular mycorrhizal fungi; CV: coefficient of variation; $^{n.s}$: not significant; **: ($P < 0.01$).

Tables 3 also shows arbuscular mycorrhizal fungi (AMF) significantly affected the phosphorus (P) and potassium (K) uptake of spring barley aerial parts.

Table 3. Analysis of variance (F value) for P and K uptake by spring barley

Source	Df	Aerial Parts	
		K	P
Block	3	0.089^{ns}	0.078^{ns}
AMF	2	239.37^{**}	75.46^{**}
Error	6	-	-
CV (%)	-	6.43	9.62

AMF: arbuscular mycorrhizal fungi;Df: degree of freedom; CV: coefficient of variation; P: phosphorus; K: potassium $^{n.s}$: not significant; **: ($P < 0.01$).

Root Colonization

Fig. 1 shows the status of *Glomus intraradice's* vesicles (a) and *Glomus etunicatum's* spores (b) on spring barley*'s* roots. Figure 2 shows that the rate of RCP in G. *etunicatum* (GE) was 26.6 % more than the G. *intraradices* (GI).

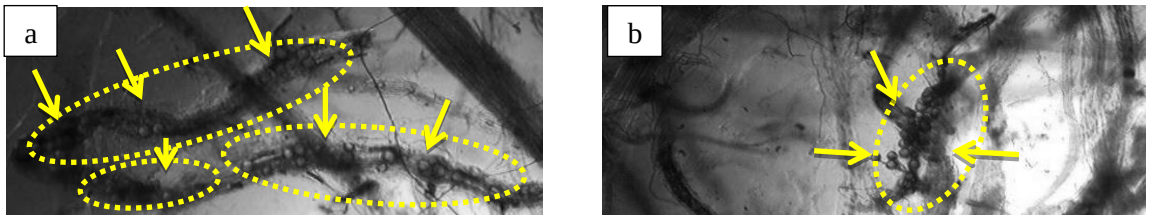

Figure 1. *Glomus intraradices's* vesicles (a) and *Glomus etunicatum 's* spores (b) on spring barley*'s* roots.

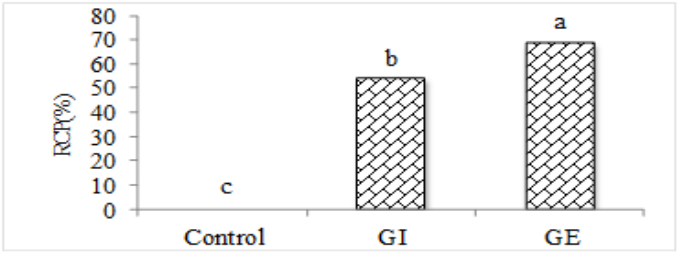

Figure 2. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on the root colonization percentage (RCP). Dissimilar letters indicate significant differences at (P < 0.01). (Duncan's multiple range test).

Mean weight diameter (MWD) of aggregates

According to fig. 3, mycorrhizal symbiosis significantly (P < 0.01) increased MWD of aggregates 201.8 and 113.6 % in GE and GI treatments compared with the control, respectively.

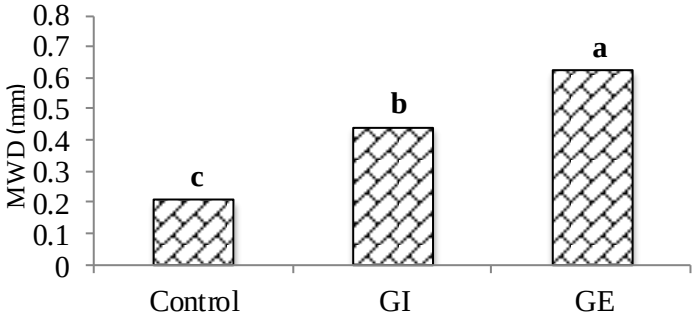

Figure 3. Effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on the mean weight diameter (MWD)) of aggregates. Dissimilar letters indicate significant differences at (P < 0.01). (Duncan's multiple range test).

Total porosity

Fig. 4 shows that total porosity of soil significantly (P < 0.01) increased in both GE and GI mycorrhizal fungi compared with the control; the percentage of increase was 2.6 and 2.2 in GE and GI, respectively. However, significant difference was not found between two fungi.

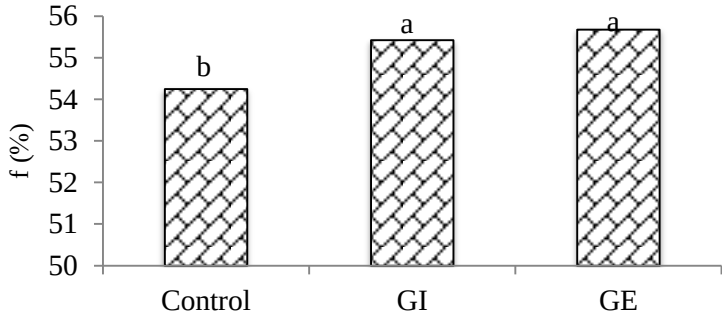

Figure 4. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on soil total porosity (f). Dissimilar letters indicate significant differences at (P < 0.01). (Duncan's multiple range test).

Pores size distribution

According to Fig. 5, both mycorrhizal fungi, GE and GI significantly ($P < 0.01$) decreased macropores 9.5 and 17.3% and increased mesopores 20.8 and 27.8% and micropores 5 and 14.1 % compared with the control, respectively. The GE fungus was more effective than GI fungus in increasing micropores and mesopores and decreasing macropores.

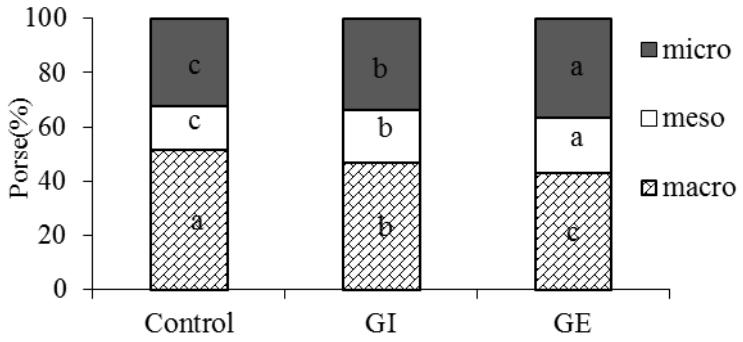

Figure 5. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on the soil pore size distribution. Dissimilar letters indicate significant differences at ($P < 0.01$). (Duncan's multiple range test).

Available water capacity

According to Fig. 6, both mycorrhizal fungi, GE and GI significantly ($P < 0.01$) increased AWC in the sandy loam soil with high sand and low organic carbon contents (Table 1). The increase of AWC was 27.1 and 13.3 % in GE and GI fungi relative to the control, respectively. The increasing effect of GE on AWC was 12.2 % more than GI due to the higher increase of MWD by GE relative to GI.

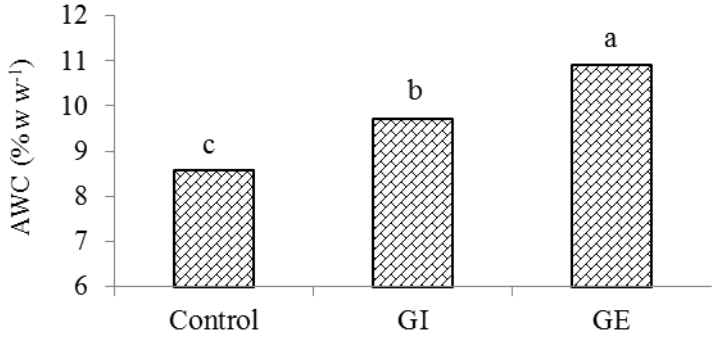

Figure 6. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on the soil available water capacity (AWC). Dissimilar letters indicate significant differences at ($P < 0.01$). (Duncan's multiple range test).

Saturated hydraulic conductivity

The results showed that both GE and GI fungi significantly ($P < 0.01$) decreased the soil saturated hydraulic conductivity (K_s) compared with the control (Fig. 7). This decrease was 88.2 and 68.8 % for GE and GI treatments, respectively. Also, the decreasing effect of mycorrhizal symbiosis on the K_s in GE was 62.3 % more than GI.

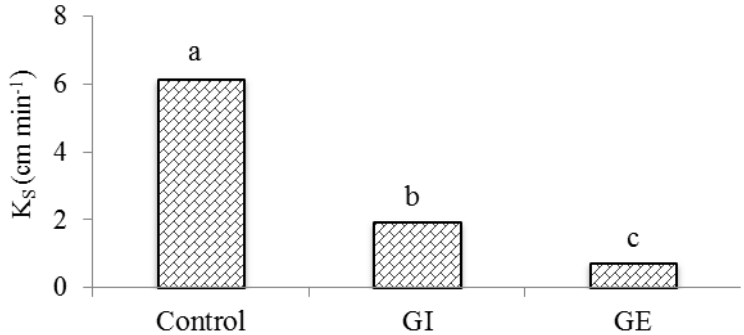

Figure 7. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on the soil saturated hydraulic conductivity (Ks). Dissimilar letters indicate significant differences at ($P < 0.01$). (Duncan's multiple range test).

Phosphorus and potassium uptake

Mycorrhizal symbiosis significantly ($P < 0.01$) increased phosphorus (P) and potassium (K) content in spring barley compared with the control in the soil (Table 1); the percentages of increase was 45.9 and 164 for K and 53.5 and 135.1 for P in GI and GE, respectively (Fig. 8). This is in accordance with the previous results obtained by Smith *et al*. (2011).

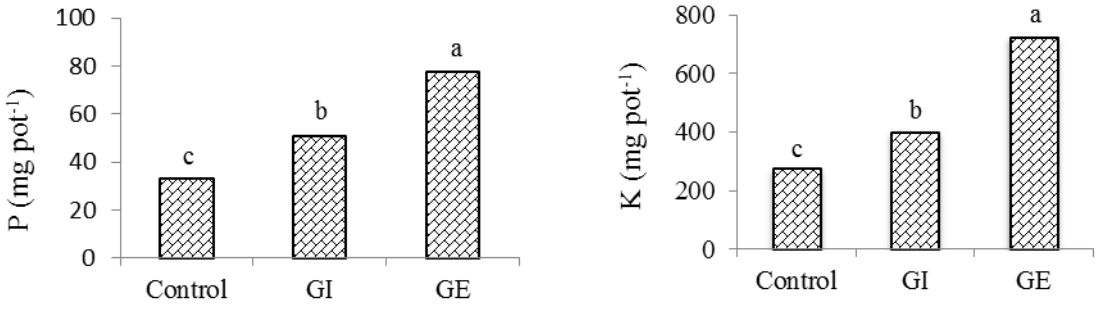

Figure 8. The effects of *Glomus etunicatum* (GE) and *Glomus intraradices* (GI) on P and K uptake by spring barley. Dissimilar letters indicate significant differences at ($P < 0.01$). (Duncan's multiple range test).

Discussion

The rate of RCP depends on the type of host plant, soil conditions and mycorrhizal fungi species. These findings are in accordance with the results of Sarikhani and Aliasgharzad (2012) who reported that the rate of root colonization in GE was 13 % more than the GI in potato's root.

The hyphae of mycorrhizae along with mycorrhizal polysaccharide binds soil particles together and results in the formation of stable aggregates (Rillig and Mummey, 2006). Mean weight diameter (MWD) of aggregates in GE treatment was 41.3 percent more than GI treatment. This increase could be related to the high RCP of GE treatment. Caravaca et al. (2002) reported that the addition of organic fertilizers on the silt loam soil activated arbuscular mycorrhiza and consequently increased glomalin. Glomalin is a glycoprotein substance that can join soil particles together and result in the formation of stable aggregates (Marshnr and Dell, 1994). Bethlenfalvay et al. (1999) found a positive correlation between water-stable aggregates and the development of arbuscular mycorrhiza myceliums. Sutton & Sheppard (1976) found that aggregation of sand-dune soil by mycorrhiza treatment was 5 times greater than that of sandy soil particles without mycorrhiza treatment. Rezaul et al. (2012) reported that AMF increased mean weight diameter of aggregates 102 % in a sandy soil under growth of sorghum compared with the control.

Mycorrhizal symbiosis significantly ($P < 0.01$) decreased soil bulk density (data not shown) and consequently increased total porosity by the formation of large and stable aggregates (Fig. 3) in the coarse – textured soil. Celik et al. (2004) also found that the total porosity of the clay loam soil under the growth of wheat (*Triticum aestivum* L.), pepper (*Capsicum annuum* L.), maize (*Zea mays* L.) and wheat were sequentially planted increased about 24 percent compared with the control in the depth of 0 to 15 cm using arbuscular mycorrhizal fungi. Milleret et al. (2009) found that total porosity increased 10.9 percent in *Glomus intraradices* treatment compared with the non-mycorrhizal loam soil under the growth of leek. Martin et al. (2012) reported that *Glomus mosseae* significantly increased total porosity compared with the non-mycorrhizal soil treatment (14.81% vs 9.77%) in a sandy loam soil under the growth of *Plantago lanceolata*.

It seems that mycorrhizal symbiosis due to the formation of large aggregates (Figure 2) increased intera-aggregate pores (mesopores and micropores) and decreased inter-aggregate pores (macropores) (Hillel, 1998) in the coarse-textured soil. Celik et al. (2004) reported that macroporosity and microporosity increased in depth of 15 to 30 cm of the clay loam soil 33 and 12.8 % in mycorrhizal and compost treatment compared to the control.

It is inferred that mycorrhizal symbiosis increased AWC because of the formation of large and stable aggregates (Fig. 3) and consequently modifying pores size distribution (decreasing macropores and increasing micropores) (Fig. 5) in the examined coarse-textured soil. Celik et al. (2004) reported that AWC increased in the mycorrhizal and compost treatments in a clay loam soil under the growth of wheat (*Triticum aestivum* L.), pepper (*Capsicum annuum* L.), maize (*Zea mays* L.) 33.3 and 55.7 percent compared with the non-mycorrhizal treatment, respectively.

Apparently, GE and GI fungi by significantly decreasing macropores and increasing micropores (Fig. 5) reduced K_s in the examined coarse-textured soil. Therefore, it is expected that the application of mycorrhizal symbiosis can reduce the losses of water and nutrients in the sandy soils. Celik et al. (2004) found that saturated

hydraulic conductivity increased in mycorrhizal treatment compared with the control (1.52 cm h^{-1} vs 0.76 cm h^{-1}) in a clay loam soil. It seems that the effect of mycorrhizal symbiosis on Ks is different in coarse and fine textured soils.

Plant traits that can influence P uptake efficiency include rhizosphere acidification, root exudation of organic anions, root morphology, uptake kinetics and mycorrhizal association; mycorrhizal roots acquire P more efficiently than nonmycorrhizal roots, especially at low soil fertility levels (Covacevich et al. 2007). Arafat and Chaoxing (2011) indicated that *Glomous mosseae* significantly ($P < 0.05$) increased P and K concentration of leaves by 100 and 16.6 percent in tomato plant cultivated on the alkaline soil compared with non-mychorrizal treatment, respectively.

Conclusion

Results showed that application of both arbuscular mycorrhizal fungi as *G. etunicatum* (GE) and *G. intraradices* (GI) improved the physical quality of coarse – textured soil by increasing total porosity**,** mean weight diameter of aggregates, available water capacity and micropores and decreasing hydraulic conductivity and macropores. Of course, the efficiency of GE in improving the above cited soil parameters was more than GI fungus.

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