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Evaluation of Mycorrhizae Symbiosis Efficiency with Barley (*Hordeum vulgare* L.) through ^{32}P Uptake under Soils Contaminated with Heavy Metals

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Abstract: This study designed to investigate more precise of mycorrhizal symbiosis in order to increasing mineral absorption by plant root system. Three pot experiments, radioactive with ^{32}P , non-radioactive and a trial with selected strain (from first and second trials) with heavy metals (Cd, Co and Pb) contaminated soil were set up for evaluation the efficacy of four mycorrhizae strains including *Glomus mosseae*, *G. etanicatum*, *G. intraradices*, mixed strains (combination of *G. mosseae*, *Gigarpora hartiga* and *G. fasciculatum*) in order to investigate the uptake, translocation and distribution of ^{32}P , P and also dry matter in barely in a glass house. Radioactive phosphorus (^{32}P) was used in this study. Results revealed that *G. mosseae* had the highest amount of P uptake in comparison with other strains. It indicates that differences exist among mycorrhizae strains towards ^{32}P uptake and its transportation to shoot. Increased strain count of *G. mosseae* was found in contaminated pots in trial with contaminated soil along with higher P concentration in root and shoot than non-inoculated plant roots.

Key words: Heavy metals, ^{32}P , mycorrhizae symbiosis, barely

INTRODUCTION

Arbuscular Mycorrhiza (AM) fungi can improve growth and nutrition of plants and have been shown to influence plant competition, plant community structure and biodiversity (Zhou *et al.*, 2001; O'Connor *et al.*, 2002; Callaway *et al.*, 2003; Klironomos, 2003). The function of all mycorrhizal systems depends on the efficiency of the fungal symbiont to uptake of inorganic and/or organic available nutrients in soil (Marschner and Dell, 1994). Arbuscular Mycorrhiza has importance due to its great capability to increase plant growth and yield under certain conditions. The major reason for this increase is the ability of plants in association with AM to uptake some nutrients such as phosphorus (P) efficiently (Podila and Douds, 2001). Extra-radical hyphae of the AM extend up to 8 cm beyond the root (Rhodes and Gerdemann, 1975) and act, in effect, as extensions of the root system in acquiring nutrients from the soil (Douds and Millner, 1999). P content also has an effect on physiological

parameters in plants (Paradi *et al.*, 2003). In the contaminated soil, P is a critical factor towards tolerance of plant against excess levels of heavy metals. Andrade *et al.* (2004) showed with enhancement of Pb concentration, symbiosis AM provided P for soybean. They concluded that the mycorrhizae effects on P uptake probably improved plant development and indirectly lessened the stress caused by excess Pb in the soil by maintaining higher P/Pb ratios on the shoots. There is support for the idea that AM may have role in P and dry matter distribution in plants. Arbuscular Mycorrhizal (AM) fungi provide an attractive system to advance plant-based environmental clean-up. During symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up heavy metals from an enlarged soil volume (Gohre and Paszkowski, 2006; Daei *et al.*, 2009). The present study was undertaken to evaluate the response of barely, planted in heavy metal contaminated soil, towards inoculation with

selected AM fungi strains with the objective to identify the most efficient strain in terms of establishing symbiosis, increasing P uptake and biomass allocation and P concentration in plant shoots and roots.

MATERIALS AND METHODS

Three pot experiments were executed under greenhouse conditions at Agriculture, Medicine and Industrial Research School, Institute of Nuclear Science and Technology, Karaj, Iran in 2006-2007.

Mycorrhizae strains production: The inoculums of arbuscular mycorrhizal species including *Glomus mosseae*, *G. etanicatum*, *G. intraradices*, mixed strains (combination of *G. mosseae*, *Gigarpora hartiga* and *G. fasciculatum*) were produced over a four-month period on sorghum plants under greenhouse conditions using sterilized sands (Miransari *et al.*, 2007, 2008). The mixture of sorghum roots and sand were used as the inoculum.

Experiment 1

Radioactive trial: The experiment 1 was ^{32}P radioactive trial, planted to evaluate the efficiency of Mycorrhizae-Barely symbiosis establishment. Trial was carried out in 2006 following Completely Randomized Design (CRD) with four replicates and five treatments (*Glomus mosseae*, *G. etanicatum*, *G. intraradices*, Mixed strains (combination of *G. mosseae*, *Gigarpora hartiga*, *G. fasciculatum*) and control).

A sample of silty clay soil from the surface of soil horizon (0-20 cm) was used. The soil was air dried, sieved and filled in pots of 30 cm height and 30 cm diameter (10 kg soil for each pot). Mycorrhizal treatments exerted with application of 50 g inoculums prepared in sandy substrate. Infection with each strain implemented individually for impeding the combination of strains. Inoculums were mixed with 5 cm upper surface of pot soil. After seeds germination, plants were thinned to maintain a plant density of 5 plants per pot under controlled conditions. During the trial, tap water was used to irrigate plants.

Application of ^{32}P : At the maximum vegetation growth stage, nearly 85 days after planting ^{32}P treatment was applied. Three mCi ^{32}P prepared in the form of orthophosphoric acid was diluted and 1 mL of solution was used for treating each pot. Radioactivity of each treated pot was 58.72 nCi. Irrigation was applied immediately following treatment application for even distribution of solution throughout soil horizon within each pot.

Harvest and measurements: In the early stage of flowering (135 days after planting), plants were harvested and stems and leaves were separated. Samples were dried in oven at 70°C for 48 h, weighed and ground. Materials were sieved to achieve homogeneous samples.

^{32}P activity in plant samples was counted by β counter (Multi low level counter FHT770-Eberline Company) for 1000 sec. As, calibration of β counter applied in the trial was performed according to efficiency of standardized ^{32}P source on the basis of gas proportional system, the value of E was considered 0.36. Amount of activity in each sample (1 g dry matter) was expressed by Bq (Bequerel).

Specific activity of sample was calculated by radioactivity of ^{32}P per unit weight /total amount of phosphorous (both active and stable isotopes) (IAEA, 1990). Specific activity of labelled fertilizer was 217.09 Bq g⁻¹.

Experiment 2

Non-radioactive trial: The experiment 2 was conducted to evaluate the ability of mycorrhizal strains for capturing soil phosphorous and estimating the mycorrhizal colonization in semi-natural condition without any application of ^{32}P or another form of phosphorus fertilizers. This experiment was managed in exactly the same way from sowing till harvest as experiment 1.

Harvest and measurements: Plants were uprooted from each pot and then they were separated to shoot and roots. Aboveground materials were washed with distilled water. Plant materials were placed in the oven at 70°C for 48 h. P concentration of shoot was measured by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). Roots were washed with tap water and sub samples were taken for mycorrhizal colonization evaluation. The colonization of each plant was evaluated on 51 cm root samples. Mycorrhizal root colonization (percentages of root length) was estimated by the Grid-Line Intersect Method (Giovannetti and Mosse, 1980) after clearing the root systems with 25 gL⁻¹ KOH and staining with trypan blue (Phillips and Hayman, 1970).

Experiment 3

Effect of heavy metals on P uptake and partitioning: The third experiment was set up in a 2×8 factorial completely randomised design, with four replications. The first factor was inoculation with *G. mosseae* (I) and non-inoculation (IO). The second factor included seven levels of contaminations Co, Cd, Pb, Co×Cd, Cd×Pb, Pb×Co and Pb×Co×Cd) plus control treatment (C) without any contaminants. A silty clay Soil used was same as for experiment 1 and 2 having total Co content =

51.91 mg kg⁻¹ dried soil, total Cd content = 8.5 mg kg⁻¹ dried soil and total Pb content = 436 mg kg⁻¹ dried soil. The heavy metal salts used were CoSO₄ for Co, CdCl₂ for Cd and Pb (NO₃)₂ for Pb.

The soil contamination was performed before planting by adding the calculated amounts of salt form of heavy metals dissolved in distilled water and mixing throughout the soil profile. They were allowed to stabilise for 15 days. Then, 50 g of *G. mosseae* inoculum was mixed with 5 cm upper surface of soil. After germination, plants were thinned to maintain a plant density of 5 plants per pot. During trial tap water was used as source of irrigation.

Harvest and chemical analysis of plant samples: Plants were cut from soil surface in early flowering stage. Roots were extracted from pot. Aboveground materials were separated into the stems and leaves and washed by distilled water. Total plant materials were put in an oven at 70° C for 48 h. Dried plant samples were ground.

Ground samples were digested in 10 mL nitric acid according to the microwave technique until clear and

diluted to 25 mL with deionised water. For heavy metals analysis and phosphorous, Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo) was used.

Statistical analysis: Data were analyzed using SAS (SAS Institute Inc., 1988). Analysis of variance was used to examine different experimental factors and their interactions. Treatment means also were compared (Steel and Torrie, 1988).

RESULTS

Experiment 1

Leaf, stem, spike and shoot biomass: In radioactive trial, there were significant differences among strains in leaves, stems and shoot biomass. Barely inoculation with *G. mosseae* caused the highest amount of biomass accumulation in stem, spike and shoot (Fig. 1B-D). But control, mixed strain and *G. mosseae* produced similar leaf biomass (Fig. 1A).

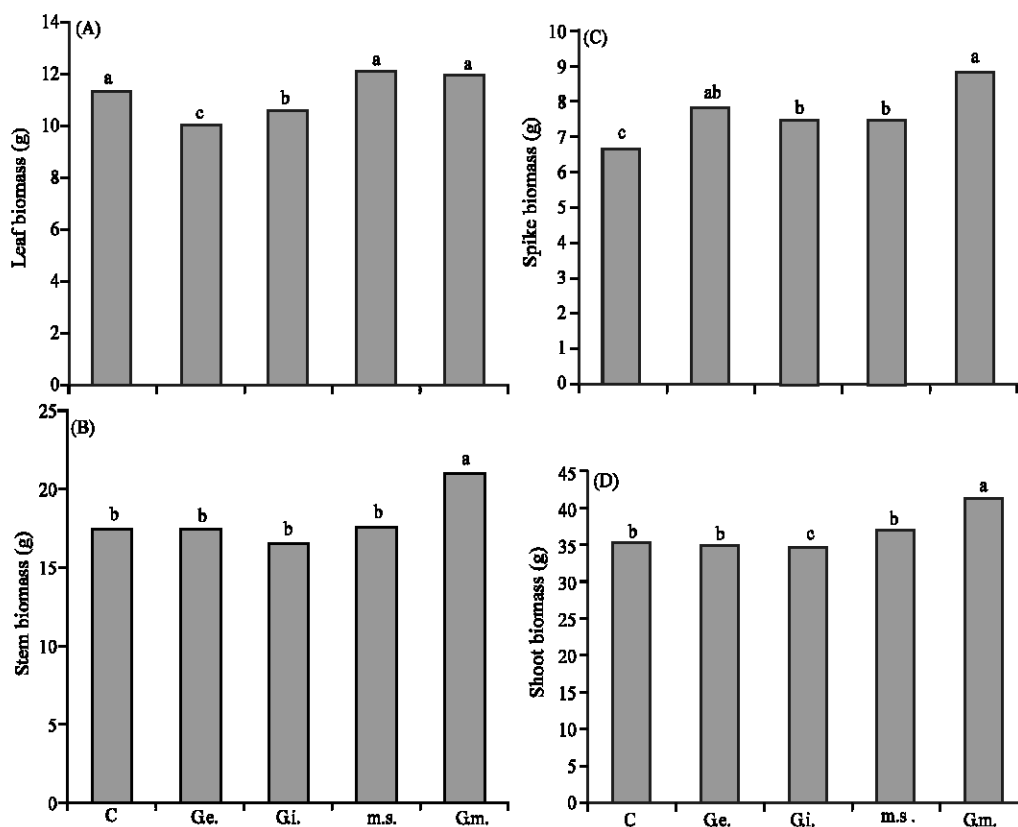


Fig. 1: Mean comparisons for effect of different stains on leaf biomass, stem biomass, spike biomass and shoot biomass by Duncan's multiple range test ($p < 0.05$) (radioactive trial). C: Control, G.e: *G. etanicatum*, G.i.: *G. intraradices*, G.m.: *G. mosseae* and m.s.: Mixed strain. Same letters indicate non-significant differences between interactions

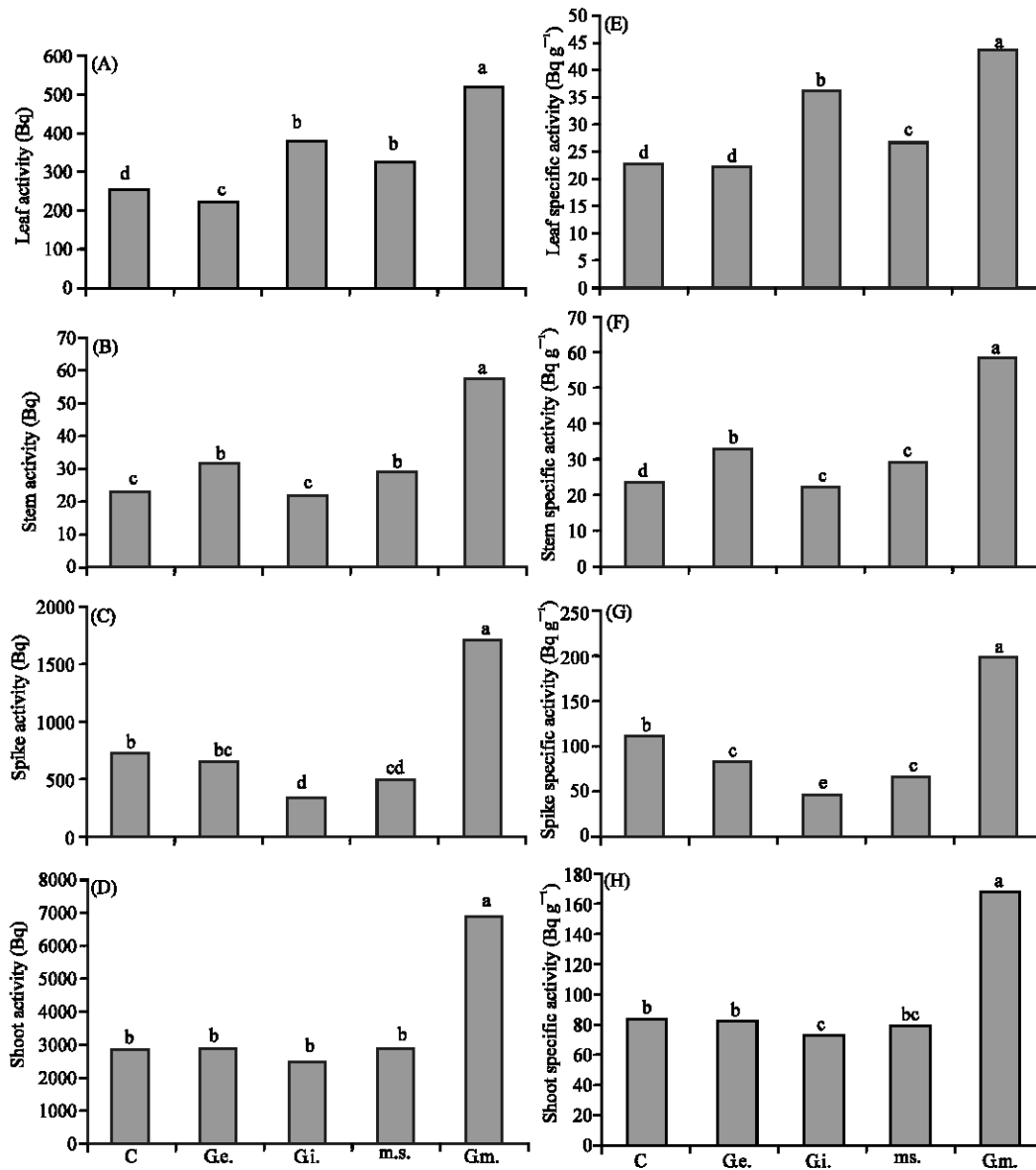


Fig. 2: Mean comparisons for effect of different strains on leaf activity, stem activity, spike activity, shoot activity, leaf specific activity, stem specific activity, spike specific activity and shoot specific activity by Duncan's multiple range tests ($p < 0.05$) (radioactive trial). C: Control, G.e: *G. etunicatum*, G.i.: *G. intraradices*, G.m.: *G. mosseae* and m.s.: Mixed strain. Same letters indicate non-significant differences between interactions

Leaf, stem, spike and shoot activity: Results of this experiment revealed that impact of different strains of mycorrhizae on activity of leaves, stems and shoot was significant. Over all, Leaf, stem, spike and shoot activity was higher due to *G. mosseae* as compared to other strains (Fig. 2A-D).

Among the other strains, *G. etunicatum* had the lowest leaf activity even lower than control (Fig. 2A). Control treatment and *G. intraradices* had the lowest stem activity (Fig. 2B). Applied mycorrhizal strains

(except *G. mosseae*) couldn't allocate higher ^{32}P to spike when compared with control. Also, shoot activity in control was similar to other strains (except *G. mosseae*) (Fig. 2D).

Leaf, stem, spike and shoot specific activity: Among the applied strains of mycorrhizae, *G. mosseae* produced maximum specific activity in stem, leaf and shoot (Fig. 2E-H). This trait showed that stem, leaf, spike and shoot had higher rate of labelled phosphorous.

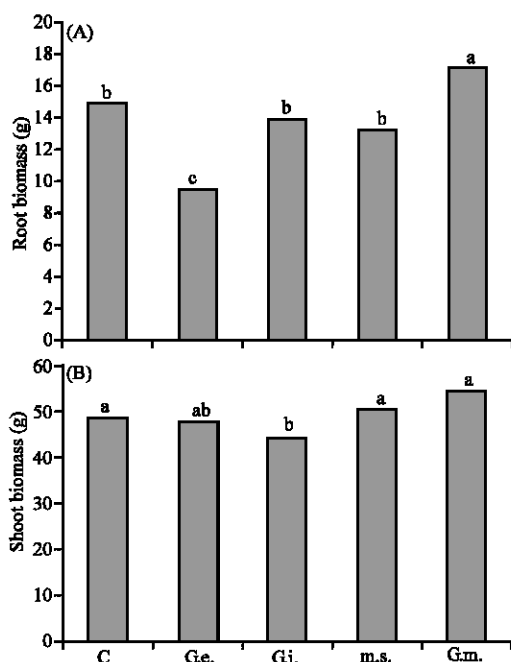


Fig. 3: Mean comparisons of effect of different stains on root biomass and shoot biomass of barely by Duncan's multiple range tests ($p < 0.05$) (non-active trial). C: Control, G.e: *G. etanicatorum*, G.i.: *G. intraradices*, G.m.: *G. mosseae* and m.s.: Mixed strain. Same letters indicate non-significant differences between interactions

Experiment 2

Leaf, stems, spike and shoot biomass: Results of experiment 1 confirmed better ability of *G. mosseae* in biomass production of leaf, stem and shoot then other applied inoculums (Fig. 1A, B, 3A, B).

P concentration in shoot: Amount of P in the barely shoot imposed by all of applied strains and *G. mosseae* had been higher than other strains (Fig. 4). In non-radioactive trial *G. mosseae* produced maximum P concentration in shoot, like active trial.

Mycorrhizal colonization index: Results of mycorrhizal colonization revealed differences amongst inoculums for roots colonization of barely. *G. intraradices* had the highest ability in establishing colonization in barely root (Fig. 5), but *G. mosseae* allocated the highest biomass and ^{32}P to root, stem, spike and shoot, but was weaker than *G. intraradices* in root colonization.

Experiment 3

P concentration in shoot: Results showed that the highest concentration of P in shoot was due to ICoCd

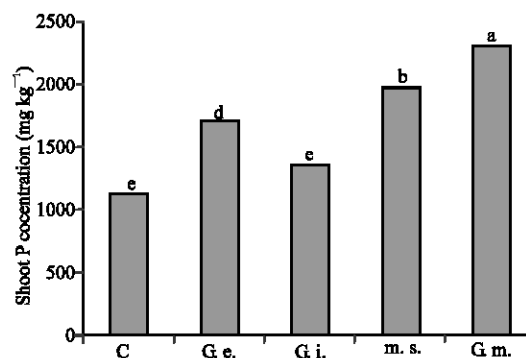


Fig. 4: Mean comparisons of effect of different stains on shoot phosphorus concentration of barely by Duncan's multiple range tests ($p < 0.05$) (non-active trial). C: Control, G.e: *G. etanicatorum*, G.i.: *G. intraradices*, G.m.: *G. mosseae* and m.s.: mixed strain. Same letters indicate non-significant differences between interactions

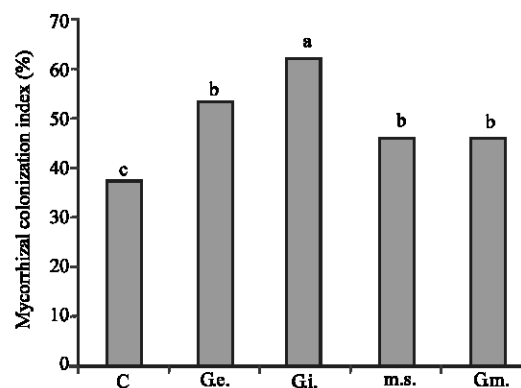


Fig. 5: Mean comparisons for effect of different stains on mycorrhizal colonization by Duncan's multiple range tests ($p < 0.05$) (non-active trial). C: Control, G.e: *G. etanicatorum*, G.i.: *G. intraradices*, G.m.: *G. mosseae* and m.s.: Mixed strain. Same letters indicate non-significant differences between interactions

treatment. In all, barely-*G. mosseae* symbiosis was desirable than non-inoculated ones and could take up and translocation of more P than none inoculated plants (Fig. 6A).

P concentration in root: Inoculated treatment (except ICoCd) had more concentration of P in comparison with non-inoculated treatments (Fig. 6B). In the contaminated soil trial, in all pots that were polluted by CoCd, PbCd and Pb, Co, Cd, Cd concentration of root, in non-inoculated plants by *G. mosseae* was more than inoculated ones. It is consider that in each Cd contaminated pot, there was enhanced P concentration in root.

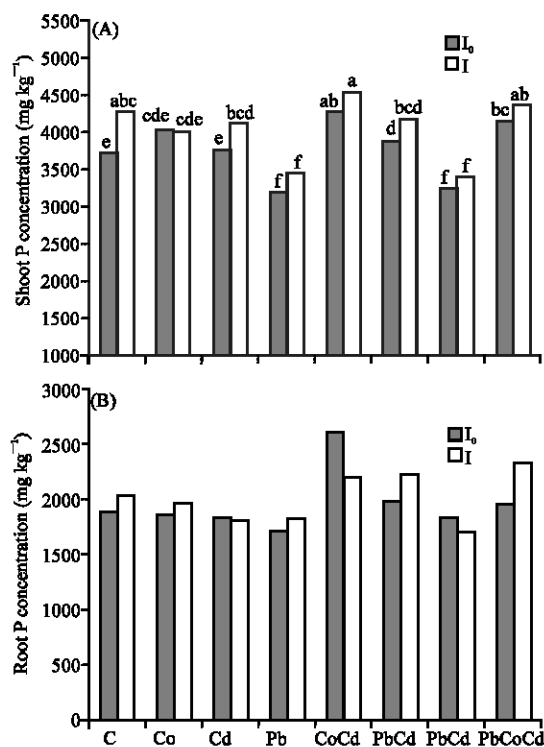


Fig. 6: Means comparison of interaction effect between *G. mosseae* and applied contaminants on shoot and root phosphorus concentration by Duncan's multiple range tests ($p < 0.05$). I₀: Non-inoculated, I: Inoculated plants with *G. mosseae*. Same letters indicate non-significant differences between interactions

DISCUSSION

Existence of radioactivity in plant dry weight indirectly is an indication of efficiency of mycorrhizal symbiosis (Ardakani *et al.*, 2004). Since, amount of ^{32}P applied, distribution and application period were similar for all pots, observed differences in radioactive amounts in plant dry weight express the rate of symbiosis efficiency. The role of mycorrhiza in this respect deserves high attention because of extension of fungal mycelia promoting absorption of more elements along with ^{32}P . According to our findings, *G. mosseae* had more efficiency in uptake of P and ^{32}P than other strains and non-inoculated plants. Also, this strain had preference in P and labelled P uptake and their translocation to the leaf, stem and spike. Results showed that there were relationships between uptake and sequestration of P, ^{32}P and biomass production by *G. mosseae*, thereby, suggesting that it can be an effective strain for P uptake and accumulation. But there was no correlation between these traits and mycorrhizal colonization index.

Arbuscular mycorrhizal can dramatically increase absorption of mineral nutrition, particularly immobile nutrients by host plant from the soil (Safir *et al.*, 1971). There are indirect evidences that show mycorrhizal roots are more efficient in nutrient uptake than non-colonized roots. This evidence originates from the fact that mycorrhizal plants are frequently not only larger but also contain higher concentration of P in their tissues than non-colonized control plants (Smith and Read, 1997). P is a vital element in photosynthesis that has important role in energy transportation as energy carrier in photosynthesis and biological systems. Presence of mycorrhizal symbiosis in terrestrial ecosystems has effect on organic and inorganic plant nutrition, water relation and carbon cycle in plants (Entry *et al.*, 2002).

Use of mycorrhizae can increase plant biomass by enhancement of water and nutrients absorption and increasing the photosynthesis activity of plants. Use of mycorrhizal fungus can lead to higher biomass production and its larger size can be considered as a competitive advantage against non-inoculated/non-mycorrhizal plants. Therefore, increment of ability and efficiency of barely-*G. mosseae* association in uptake and allocation of P can be useful for obtaining more growth and biomass production following improvement of CO_2 assimilation activity. The roles of AM symbiosis are characterized by (1) an increased heavy metals phytoextraction via mycorrhizospheric Enhanced Uptake at low soil-heavy metals concentrations and (2) a reduced heavy metals bioavailability via AM fungal Metal-Binding processes at high soil-heavy metals levels, hence resulting in increased plant biomass and enhanced plant tolerance through heavy metals stress-avoidance (Audet and Charest, 2007).

High concentration of P in barley shoot in exp. 1, enhancement of ^{32}P and specific activity of plant arise on effect of *G. mosseae* on promotion of barely root. Mycorrhizal hyphae increase capability of root system for exploring the higher volume of soil for obtaining water and nutrient. This unique ability is exhibited by production of extra radicle hyphae that grow several centimetres in soil far away roots. As, AM increased root absorption surface by hyphae production, it leads to improvement of water and nutrients uptake by plants. About 80% of P uptake by plants is estimated to be exerted by AM (Marschner and Dell, 1994).

Generally, in contaminated soil inoculation with *G. mosseae* increased uptake and movement of P to the shoot and also roots. It is considered that *G. mosseae* was an effective strain in this trials, which may be useful for barely in heavy metal stressful condition by more uptake of P for tolerance and detoxification contaminants.

Sequestration of P can help detoxification of heavy metals to plant in stressful land. These advantageous

supplies by molecules of phytates that neutralise excess metals, or P can provide metabolic energy indirectly as ATP for possible compartmentalisation within the cell vacuoles (Davies *et al.*, 1991). Also, results of Meharg *et al.* (1994) suggested that tolerant plants of *Hocus lanatus* L. to heavy metals are related on mycorrhizae symbiosis for P uptake and sequestration. Metals may be sequestered in the hyphae and not translocated to the plants. Sequestration of metals by polyphosphate in the fungus is important in reduction of movement to plant (Turnau *et al.*, 1993). In conclusion, it is possible efficiency of mycorrhizal plants in uptake and sequestration of heavy metals and also tolerance to the metals, particularly to multi-metals contaminated soils or exceeded content of metals depend on contribution of P uptake of mycorrhizal plants roots.

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