American-Eurasian J. Agric. & Environ. Sci., 11 (3): 346-352, 2011
ISSN 1818-6769

Effect of Heavy Metals and Arbuscular Mycorrhizal Fungal on Growth and Nutrients (N, P, K, Zn, Cu and Fe) Accumulation of Alfalfa (*Medicago sativa* L.)

[1]Faezeh Zaefarian, [2]Mohammad Rezvani, [3]Farhad Rejali,
[4]Mohammad Reza Ardakani and [5]Ghorban Noormohammadi

[1]Department of Agronomy and Plant Breeding, Faculty of Crop Sciences,
Sari Agricultural Sciences and Natural Resources university, Mazandaran, Iran
[2]Assistant Professor of Agroecology,
Islamic Azad University, Qaemshahr Branch, Iran
[3]Department of Soil Biology, Soil and Water Research Institute, Tehran, Iran
[4]Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources,
Islamic Azad University, Karaj Branch, Iran
[5]Department of Agronomy and Plant Breeding, Faculty of Agricultural and Natural Resources,
Islamic Azad University, Science and research Branch, Tehran, Iran

Abstract: Two pot culture experiments were carried out to study the effect of arbuscular mycorrhizal inoculation on the growth and nutrients (N, P, K, Zn, Cu and Fe) uptake of alfalfa (*Medicago sativa* L.). In first pot experiment, efficiency of four mycorrhizal strains: *Glomus mosseae*; *G. etanicatum*; *G. intraradices* and mixed strains (combination of *G. mosseae, Gigaspora hartiga* and *G. fasciculatum*), on the uptake of nutrients was investigated. Results showed that *G. mosseae* had the highest efficiency of uptake, translocation and distribution of P, N and dry matter in alfalfa in comparison with the other strains. It can confirm existence of differences in ability of mycorrhizal strains in uptake and transport P into the shoot. A second experiment with heavy metal contaminated soil (Cd, Co, Pb and combinations) and inoculation to *G. mosseae* was executed. In this trial, uptake of nutrients was depended on kind and mixture of metals.

Key words: Alfalfa · Heavy metals · Mycorrhizae · Nutrients uptake

INTRODUCTION

Large areas of soil are being contaminated by heavy metals, such as Cu, Zn, Co, Cr, Ni, Pb and Cd. Excessive heavy metals in the environment are known to be toxic to most organisms and their effects on organisms are being increasingly studied. Heavy metal effects on plants growth and causes structural damage and nutrients uptake [1]. Arbuscular mycorrhizae (AM) represent an almost ubiquitous relationship between soil microflora and plants. The fungal symbiont increases its host's uptake of nutrients and can improve its growth and resistance to environmental stresses [2].

Populations of AM are the key factor in soil development and successful plant establishment. Their presence may reduce stress caused by lack of nutrients or organic matter [3].

Most land plants are symbiotic with AM, which take up mineral nutrients from the soil and exchange them with plants for photosynthetically fixed carbon. Growth stimulation, better mineral nutrition and lower heavy metal uptake are among the benefits of mycorrhizal plants growing in soils with excessive levels of metals [4,5].

Heavy metals not only inhibit root growth but also can hamper many physiological processes and, in particular, the uptake of nutrients and it has been suggested that the nutrient status of the root may be a factor of vital importance for plant tolerance to changes in the environment [6].

Heavy metals may decrease available contents of soils mineral nutrients [7], by inhibiting the mineralization processes and the litter decomposition rate in ecosystems under metal pollution stress is generally found to be reduced [7,8].

Corresponding Author: Mohammad Rezvani, Department of Agronomy and Plant Breeding,
Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

Deficiency of nitrogen could have deleterious effects on the formation of heavy metal-complexing compounds and therefore on the tolerance to metals [9].

At different Zn levels, mycorrhizal colonization increases zinc absorption and accumulation in the roots. This may help to explain the alleviation of zinc toxicity at high concentrations [10].

In legume plants the importance of AMF symbiosis has been attributed to high P requirements on the nodulation and N_2 fixation process which requires enhanced P uptake [11].

The study described in this paper focused on the effect of the AM fungus *Glomus mosseae* on growth and nutrients uptake of alfalfa grown in a multi-metal-contaminated soil.

MATERIALS AND METHODS

Experiment 1: The first experiment was set up for evaluation of efficiency of Mycorrhizae-Alfalfa symbiosis with five treatments in a Completely Randomized Design (CRD) [*Glomus mosseae*, *G. etanicatum, G. intraradices*, mixed strains (equal combination of *G. mosseae, Gigaspora hartiga, G. fasciculatum*) and un-inoculated (control)] with four replicates. Before the main experiments, strains were produced with the pot culture method. Inoculums were consisting of mixture of soil, root segments, mycelium and spores.

A sample of soil with clay 35%, silt 40%, sand 25%, pH 7.91 and organic mater 1.48% was used. Soil was air-dried and then passed through 2 mm sieve and large stones and plant root debris were removed and then, were filled in pots (10 kg soil for each pot). Mycorrhizae were applied as 50 g of inoculums mixed with 5 cm of upper surface of pot soil. Alfalfa (*Medicago sativa* L.) seeds were treated with *Sinorhizobium meliloti* (prepared in the Soil and Water Research Institute, Tehran, Iran) before planting. After germination, plants were thinned to maintain a plant density of 5 plants per pot and watered with tap water as required.

In the early stage of flowering (135 days after planting), plants were harvested and were separated into stems and leaves. Samples dried in oven at 70°C for 48 hours and then weighed and ground.

Nutrients (P, Zn, Fe and K) and heavy metals concentration of samples was measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). Plant material was analysed for N by Kjeldahl digestion.

Experiment 2: The second experiment was set up in a 2×8 factorial completely randomised design, with four replicates. The first factor was inoculation (I) with *G. mosseae* or un-inoculation (I0). The second factor had seven levels of contaminants: (Co, Cd, Pb, CoCd, CdPb, PbCo and PbCoCd) plus an uncontaminated control treatment (C). Same previous experiment, a sample of soil (clay 35%, silt 40% and sand 25%) were used. 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 included $CoSO_4$, $CdCl_2$ and $Pb(NO_3)_2$.

Soil was contaminated before planting by adding the calculated amounts of heavy metal salts in distilled water and mixed throughout the soil profile. They were allowed to stabilise for 15 days. Then, 50 g *G. mosseae* inoculum was mixed with 5 cm of upper surface of soil and Alfalfa seeds planted as before. After germination, plants were thinned to maintain a plant density of 5 plants per pot. During the trial, tap water was used as source of irrigation.

Plants were cut from soil surface in early flowering stage. Roots were extracted from pots. Aboveground materials separated into the stems and leaves were washed by distilled water. Plant material was dried at 70°C for 48 hours.

Heavy metals and nutrients (P, K, Cu, Zn and Fe) quantified by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Variant-Liberty 150AX Turbo). N concentration determined by Kjeldahl digestion.

Statistical Analysis: Statistical analysis of data was performed using SAS program (version 9.1). The data were analyzed by one-way analysis of variance (ANOVA) and comparisons between means were 15 performed using Duncan's multiple range test at the significance level of $P<0.05$.

RESULTS AND DISCUSSION

Exp. 1: Leaf biomass varied greatly among the mycorrhizal strains (Table 1). The *G. mosseae* had significantly more leaf biomass (Table 1) ($P<0.05$). Significant differences were detected for mycorrhizal treatments based on stem weight. The *G. mosseae* substantially increased stem biomass accumulation of plants grown (Table 1) ($P<0.05$).

Aboveground P was influenced by mycorrhizal strains and *G. mosseae* significantly increased P than other strains (Table 1) ($P<0.05$).

Table 1: Mean comparison of effect of different mycorrhizal strains on biomass and nutrients uptake of alfalfa (exp.1).

Treatments	Leaf biomass(g)	Stem biomass (g)	Shoot P (mgkg^{-1})	Shoot N (%)	K (mgkg^{-1})	Zn (mgkg^{-1})	Fe (mgkg^{-1})
Control	15.86 ^{b}	7.26 ^{b}	1243.04 ^{c}	3.05 ^{b}	1701.66 ^{b}	23.60 ^{e}	712.72 ^{a}
G. etunicatum	16.14 ^{ab}	8.57 ^{b}	1287.52 ^{c}	2.92 ^{b}	1530.91 ^{d}	37.70 ^{b}	507.10 ^{b}
G. intraradices	15.75 ^{b}	8.73 ^{b}	1287.52 ^{b}	2.99 ^{b}	1515.35 ^{d}	29.52 ^{d}	284.98 ^{e}
mixed strains	16.21 ^{ab}	8.33 ^{b}	1284.01 ^{b}	3.21 ^{a}	1648.06 ^{c}	33.09 ^{c}	369.79 ^{c}
G. mosseae	17.6 ^{a}	11.09 ^{a}	1451.95 ^{a}	3.02 ^{b}	1787.65 ^{a}	45.88 ^{a}	316.98 ^{d}

Mean values within the same column, followed by different letters are significantly different using Duncan's multiple range test (P<0.05).

Shoot nitrogen concentration was significantly more for plants inoculated with mixed strains. In the case of plants inoculated with *G. mosseae*, shoot N concentration was significantly lower than the control (Table 1) (*P<0.05*).

Different strains had significantly differences. The highest tissue K concentration was in *G. mosseae* (1787.65 mg kg^{-1}), followed by control (1701.66 mg kg^{+}) and mixed strains (1648.06 mgkg^{-1}) (Table 1) (*P<0.05*).

The mycorrhizal trains had significantly varied ability in Zn uptake Shoot Zn content was higher in *G. mosseae* than others (Table 1) (*P<0.05*).

Mycorrhization had not increased Fe content of shoot significantly. The control plants had higher Fe concentration than inoculated plants (Table 1) (*P<0.05*). *G. etunicatum* had the highest Fe content among the strains (Table 1) (*P<0.05*).

In the experiment 1, all strains of mycorrhizae produced more content of P, K, N, Zn, leaf, stem and shoot biomass than control plants (Table 1). Reviewed literatures show legumes have a relatively high P requirement for nodule development and nitrogen fixation, therefore normal levels of nodulation may depend on the presence of mycorrhizal fungi [12,13]. The extra P in mycorrhizal roots could be due either to better soil exploration by the extramatrical mycelium, or to the ability of the fungus to utilize or mobilize sources of soil P not available to plant roots. The primary mechanism by which mycorrhizal fungi improve P uptake is through more extensive soil exploration rather than a unique capacity to mobilize sources of P not available to plants [14,15].

Despite its importance as a nutrient, very little is known about AM uptake nitrogen and translocation it to the host plants. Mycorrhizal plants had more amounts of leaf area, leaf, shoot and root dry mater and N, K, P, Cu, Fe and Zn concentration than un-inoculated plants [16]. Tawaraya *et al.* [17] reported that welsh onion cultivars were highly colonized with *G. fasciculatum*. Shoot P uptake and shoot dry weight were different among cultivars and were increased by mycorrhizal colonization. Other investigation indicated mycorrhizal symbiosis enhanced P [18], N, K, Mg [19] and Zn [20] uptake. Gilmore [21] showed that arbuscular mycorrhizal fungus could increase host Zn content and Ross and Harper [22] demonstrated the same result for Cu. Mycorrhizal strains hadn't increased Fe concentration of shoot significantly. There are contrasting reports about effect of mycorrhizae on Fe content of plant. Some reports showed AM symbiosis decreased, increased or had no effect on shoot concentration of Fe [23].

Exp. 2: In without contamination pots, IC treatment had significantly shoot N content more than I0C. Shoot N content was higher in ICo, ICd and IPb than un-inoculated plants (I0Co, I0Cd and I0Pb) by *G. mosseae.* But, in the contaminated pots to PbCo and PbCoCd, un-inoculated plants had more N concentration than inoculated ones (Fig. 1A) (*P<0.05*).

Comparison of controls indicated IC produced the most amount of whole plant phosphorous. Inoculation of plants with *G. mosseae* fungi significantly increased P content of whole alfalfa plants at contaminated pots to Co, Pb and PbCd. In the rest of treatments, un-inoculated plants had more phosphorous than inoculated ones (Fig. 1B) (*P<0.05*).

Results revealed that in the dual inoculation of C, Co, Cd, Pb, CoCd and PbCd and *G. mosseae* fungi, Shoot K was higher. But, in the PbCo and PbCoCd polluted and un-inoculated pots shoot concentration of K was more than inoculated plant with *G. mosseae* (Fig. 1C) (*P<0.05*).

The results obtained proved that in the uncontaminated pots, un-inoculated plants more Cu concentration of shoot. In the heavy metals contaminated soil different behaviour respect to type and combinations on contaminants was observed. In the polluted pots to Cd, CoCd, PbCd and PbCoCd un-inoculated plants produced significantly more amounts of shoot Cu (Fig. 2A) (*P<0.05*).

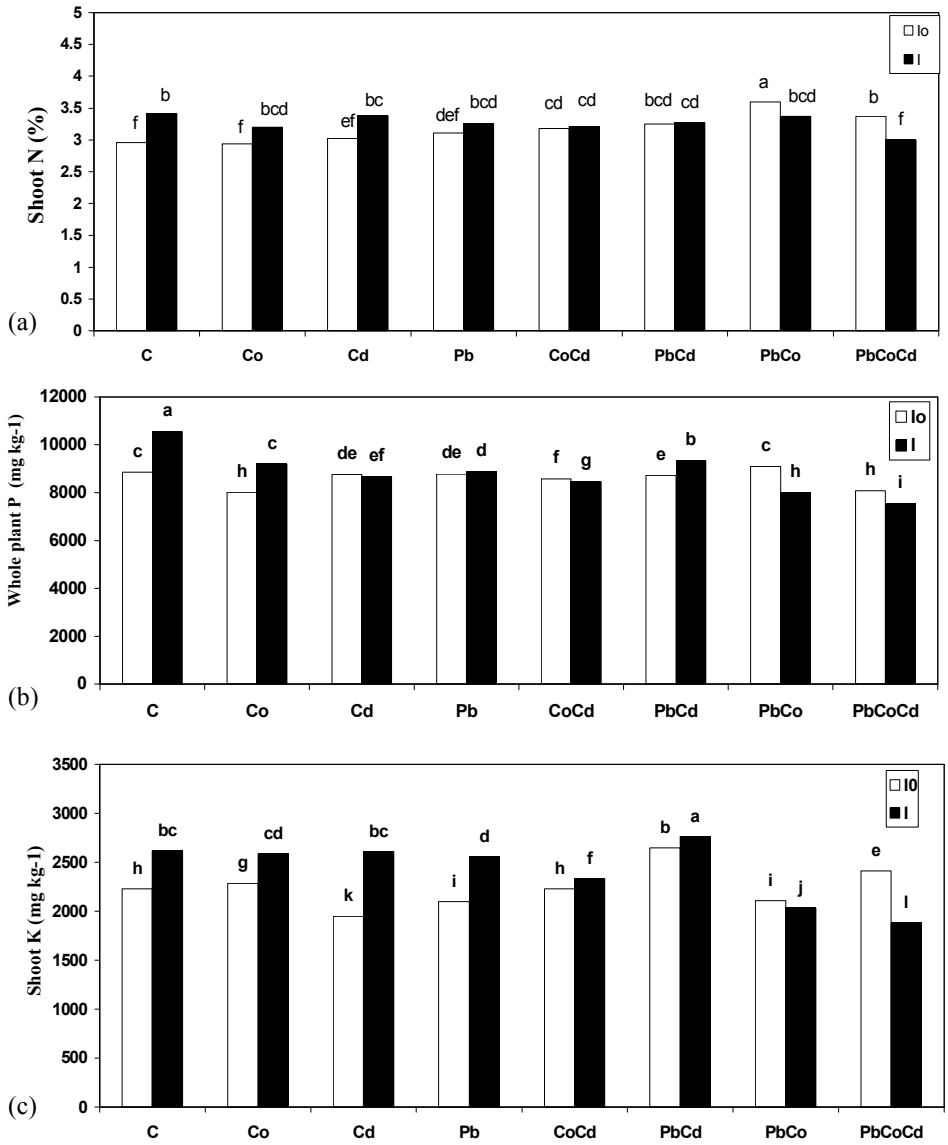

Fig. 1: Nutrients contents of different plant tissues as affected by heavy metals concentrations for control and mycorrhizal treatments. Mean values within the same column, followed by different letters are significantly different using Duncan's multiple range test (P<0.05). C: Control, I0: Un-inoculated plants, I: Inoculated plants with *G. mosseae*.

In the control treatment, there was IC<I0C. In the polluted pots to Co, Cd, Pb, CoCd, PbCd and PbCo, un-inoculated plants with *G. mosseae* showed a significant (*P<0.05*) increase in Fe content of shoot. At PbCoCd contaminated pots, *G. mosseae* significantly (*P<0.05*) enhanced, Fe concentration of shoot compared to controls (Fig. 2B).

In the control and Co, Cd and PbCo polluted pots non-inoculated plants had significantly more Zn concentration than inoculated plants. But, in Pb, CoCd, PbCd and PbCoCd inoculation with *G. mosseae* increased significantly Zn concentration of shoot (Fig. 2C) (*P<0.05*).

In the experiment 2, the interaction between heavy metals and nutrients uptake have been mainly varied in inoculated and un-inoculated plants. A vast amount of literature is available on the effects of mycorrhizae on plants under heavy metals stress. But, the effects of two or three heavy metals and AM were not investigated on plant

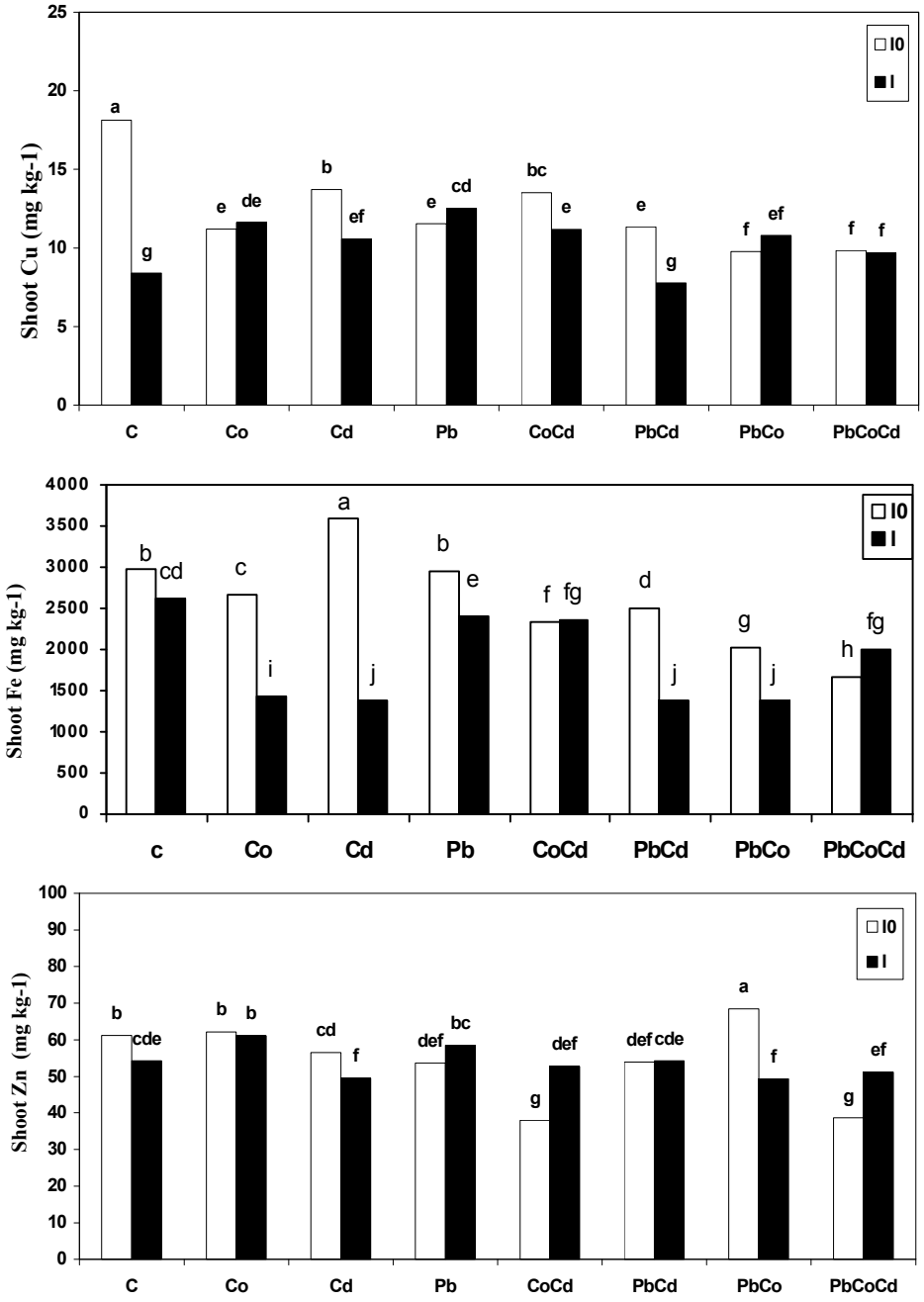

Fig. 2: Nutrients contents of different plant tissues as affected by heavy metals concentrations for control and mycorrhizal treatments. Mean values within the same column, followed by different letters are significantly different using Duncan's multiple range test (P<0.05). C: Control, I0: Un-inoculated plants, I: Inoculated plants with *G. mosseae*.

growth and nutrients take up and translocation of them into shoot. Only a few studies have demonstrated the effect of heavy metals on nutrients uptake by plants, because the interactions are complicated by the presence of mycorrhizal symbionts.

The effects of AM on the heavy metal uptake of mycorrhizal plants are metal specific and depend on metal concentration and availability, plant species, AM species, soil properties, or some other unidentified factors [24].

CONCLUSION

The presented results show that in the contaminated soil reaction of *G. mosseae* was different. For example: In the mono metal contaminated pots mycorrhizal plants had more N of shoot but in the PbCd, PbCo and PbCoCd contaminated pots un-inoculated plants had higher N than inoculated ones. Such results also obtained for other nutrients. The amount of nutrients uptake was affected on type and combination of heavy metals.

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