Investigation the Effect of Arbuscular Mycorrhizal Fungi on the Tolerance of Maize Plant to Heavy Metals Stress

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Abstract: An experiment was conducted in greenhouse to determine the influence of two species for mycorrhizal fungi *Glomus mosseae* and *Acaulospora laevis* in the presence of two heavy metals (HM), Cupper (Cu) and Cadmium (Cd) in three concentrations on maize plants to improve tolerance of plants to HM stress. Mycorrhizal root colonization (MRC), spore densities, plant growth parameters and plant HM uptake were taken as indexes to determined plant tolerance to HM. The MRC% was stimulated in Cu in both mycorrhizal fungi and spore density of *G. mosseae* was increased in presence of Cd. The greater values for almost plant growth parameters were found in treatment with *A. laevis* then *G. mosseae* comparing with untreated plants. The HM stress was caused increased in root/shoot ratio and plant proline content, but the inoculation with mycorrhizal fungi was caused decreased in that values. On contrast the plant soluble protein was decreased by increasing in HM concentrations, while that effect was removed in mycorrhizal fungi plants. The plant roots ability to absorbent HM were increased when inoculation with *A. laevis* greater than those in *G. mosseae*. The plant (*Zea mays* L.) was uptake Cu in various concentrations in solution more than Cd in the presence or absent of AMF. The inoculation plant by *A. laevis* was increased the accumulate HM in plant shoot tissues greater than those in *G. mosseae.*

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) of the phylum *Glomeromycota* **(Schüßler et al., 2001)** are a natural constituent of the soil for most ecosystems. They interact with the roots of more than 80% of terrestrial plants and can be considered functional extensions of plant roots considerably enlarging the soil volume for nutrient uptake **(Harrison, 1999; Almagrabi and Abdelmoneim, 2012),** enhance drought tolerance **(Ruiz-Lozano et al., 2001; Kaya et al., 2003; Abdelmoneim et al., 2013)** and reduce pathogenic infections **(Newsham et al., 1995; Abdalla and Abdel-Fattah, 2000).** Also AMF enhance of heavy metal (HM) uptake and tolerance depends on both plants and soil factors including soil microbes **(Li et al., 1991; Jakobsen et al., 2002; Fernando et al., 2011). Bradley et al. (1981)** firstly reported that ericoidous mycorrhizae reduced the uptake of Cu and Zn by *calluna valgaris*, many researchers have been increasingly interested in interactions between AMF and HM. **Xiong (1993)** found decrease Cd concentrations in plant tissues in AMF plants. **Jentschke et al. (1998)** showed that the total Pb in roots of *Picea abies* was reduced by mycorrhizal infection. **Hildebrandt et al. (1999)** reported that mycorrhizae improved the plants of *Viola calaminaria* tolerance to metal Zn and Pb stress in polluted soils. But **Joner and Leyval (1997)** found that mycorrhizae enhanced total uptake of Cd of *Trifolium subterraneum*. **Ahonen- Jonnarth and Finlay (2001)** also found that mycorrhizae enhanced total uptake of Cd and Ni of the host plant *Pinus sylvestris*. **Huang et al. (2002)** also found that the accumulations of Pb, Cu, Zn, and Cd in mycorrhizal plants of maize were 10%, 18% and 29% lower than that in non-mycorrhizal ones respectively. AMF have evolved a property of Zn tolerance and that they might play an important role in the bioremediation of the contaminated site **(Shetty et al., 1995; Gaur and Adholeya, 2004; Khan, 2005).** The inoculation by AMF can improve plant performance under HM stress, which is may be the results of a combination of antioxidant enzyme, lipid peroxidation and soluble amino acid profiles changes caused by the intimate relationship between AMF and host plant **(Andrade et al., 2009; Punamiya et al., 2010; Achakzai et al., 2012).** AMF have ability to protect plants against HM toxicity by mediating the interaction between metals and plant roots. They can bind HM in their cell wall, compartmentalize them in the vacuole or chelate them into the cytoplasm restricting the influx of HM into the plant **(Leyval et al., 1997).**

Achakzai et al. (2012) investigate the effect of AMF inoculation on the growth of maize and phytoextraction of HM (Ni; Pb; Cu) from a soil contaminated with crude oil. He found the inoculation by AMF promoted the vegetative growth attributes in all treatments, also promoted the hyperextraction of HM from AMF soils. Plants colonized by AMF also have greater ability to absorb nutrients like P, N, K, Ca, Mg, and water which results in better survival under stressed conditions **(Auge and Stodola, 1990).** AMF have been shown to interact with different groups of soil bacteria and modify the rhizosphere microbial community**. Albertsen et al. (2006)** showed that both bacterial and saprotrophic fungal biomass increased in the presence of some AMF species in a root free sand environment. The present study was mainly aimed to evaluate the effects of AMF on the growth of maize plants under HM stress. As well as estimated HM uptake into maize plant root and shoot tissue in the presence or absence of AMF species to assess the improve tolerance of maize plants to HM stress.

2. Materials and Methods

2.1. Preparation of fungal inocula

Arbuscular mycorrhizal fungi (AMF) isolates were isolated from the rhizosphere of *Zea mays* L. growing in soils at province Khulais, western of Saudi Arabia (latitude 22°, longitude 39°). About 500 intact spores were isolated using the wet sieving and decanting method Schenck (1982), and then propagated on *Cynodon dactylon* for three months in greenhouse conditions. Colonized root fragments, mycelium and dried sand soil mixture containing spores were used as AMF inoculum. When AMF colonization on *C. dactylon* roots reach to 90%, the AMF spore density was estimated (150±25 spores$10g^{-1}$ of air dried soil). AMF spores were identified morphologically according to the current taxonomic criteria **Schenck and Perez (1990).** In the present study, AM fungal inoculum consisted of several species, the dominant species was *Glomus mosseae* (Nicolson and Gerdeman) Gerdeman and Trappe (recoded by rate 57.5% from all collected samples), and the common species was *Acaulospora lavevis* Gerdeman and Trappe (recorded by rate 31% from all collected samples), which they used as AMF inocula.

2.2. Experimental design

There were three copper (Cu) levels (0. 5, 1.0, and 1.5 mg Cul^{-1}), three cadmium (Cd) levels (0.1, 0.5 and 1.0 mg Cdl^{-1}) and a common control (0 mg l^{-1}), and all of these were either with or without inoculation by AMF. For mycorrhizal treatments 50 g from inocula was mixed with the clean sand, while for nonmycorrhizal treatments 10 ml filtered extract through a 0.45 ml filter and 50 g sterilized soil (170 °C for 2 h in an oven) were added to the clean sand in order to keep similar condition except mycorrhizal fungi. Pots were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. The seeds (*Zea mays* L.) were sterilized before sowing using 3% H_2O_2 for 10 min and were subsequently washed several times with distilled water. Six seeds were transplanted into plastic pots (25 cm diameter and 30cm depth) each pot was filled with 1.5 Kg sterilized growth media (Peat moss soil: pH 5.4 the soil had 33.4±3.2 mg kg^{-1} extractable N, 6.2±0.45 mg kg^{-1} extractable P, and 44.6±5.6 mg kg^{-1} extractable K), after emergence three seedlings were left in each pot. After plants of maize had grown for 7 days, 200 ml 30% Hoagland nutrient solution without $CuSO_4.5H_2O$ was added weekly to keep normal growth. The different concentrations of HM were added to each corresponding pot three times every 10 days. Seedlings were grown in the greenhouse with 12 h light per day at 25-35°C. Water lost was replaced daily by top watering with tap water, and to maintain the moisture of the soil at about 60% (w/w) until the end of the experiment (45days).

2.3. Analytical methods

2.3.1. Determination mycorrhizal fungi root colonization% and spores densities

The plant roots were separated, washed and stored in the storage vial with formalineacetic acidealcohol (FAA) solution until staining according to the methodology described by Phillips and Hayman (1970). The stained roots placed on the glass slides for microscopic observations under 100×magnifications (*Leica* DM550Q, USA). The calculation of AMF colonization was estimated for each sample by examination about one hundred pieces of roots (1 cm long), and expressed as the following formula, and the AMF spores densities were calculated according to **Schenck (1982).**

$$\text{AMF colonization (\%)} = \frac{\text{Number of mycorrhizal root pieces}}{\text{Total number of observed root pieces}} \times 100\%$$

2.3.2. Plant growth parameters and biochemical

The harvested plant (shoots and roots) after 45 days were rinsed with tap water and then with distilled water. The plant height; shoot and root weight; root length and root/shoot ratio were estimated for all treatments. The chlorophyll concentration was measured on the second fully expanded leaf using CL-01chlorophyll content meter (Hansatech Instruments, USA). Soluble protein content and was determined by extraction method as described by **Zhang (1990).** Free proline content was

determined according to **Gilmour et al. (2000).** The youngest leaf was collected from the plant sampled and samples were stored at -4° C prior to analysis. Each sample with a weight of 1g was homogenized with a chilled mortar and pestle in 10ml of 100mg ml^{-1} trichloroacetic acid buffer (pH 8.0). Homogenates of samples were centrifuged at 4000 rpm for 10 min. Top aqueous layer was then transferred into 5ml tubes which were incubated in a boiling water bath and quickly placed in an iced-water bath for 5 min, then centrifuged again. Two ml thiobarbituric acid reagent was added to 2 ml of extracted supernatant. The supernatant was spectrophotometrically determined by using an UV-visible spectrophotometer (Thermo Electron, Model Bio Mate 3, Massachusetts, USA) at wavelength 520 nm. Proline concentration was determined using calibration curve and expressed as µg proline g^{-1}FW.

2.3.3. Determination plant heavy metals uptake

After 45 days the plants (shoots and roots) were first rinsed with tap water and then with distilled water, dried at 70°C for 48 h. The two HM (Cu and Cd) concentrations in plant tissues were determined by the extraction method according to **Cao (1996)** using an atomic absorption spectrophotometer (PYE UNICAM SP9, England).

2.4. Data analysis

Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

3. Results

No mycorrhizae were found in all the treatments without mycorrhizal inoculation because the filtered extract of AMF in this treatment was sterilized in oven. The AMF root colonization% (infection rate) in both species *Glomus mosseae* and *Acaulospora laevis* was stimulated in two concentration 0.5 mg Cu l^{-1} by rate 55% and 1.0 mg Cu l^{-1} by rate 53% to *G. mosseae* and 65%; 63% for *A. laevis* comparing with control (0 mg Cu l^{-1}) 52% and 60% respectively with two Cu concentrations. As well as in Cd the stimulated effect was found only in low level (0.1 mg Cd l^{-1}) by rate 56% for *G. mosseae* and 67% to *A. laevis* comparing with control. When Cu concentrations in solution were higher than 1.0 mg Cu l^{-1} the AMF infection rates were slowly decreased in both species *A. laevis* and *G. mosseae* (40%, 35% respectively). While in Cd concentrations in solution were higher than 0.1 mg Cd l^{-1} the AMF infection rates were sharply decreased in *A. laevis* (25%) more than *G. mosseae* (35%) Fig. 1. The spore density to both AMF species were greatly affect with the increase of Cu concentrations in solution comparing with control (0 mg Cu l^{-1}). The sporulation ability for *G. mosseae* and *A. laevis* was stimulated in concentration 0.5 mg Cu l^{-1} by rates 450 and 490 spores$100g^{-1}$ dry soil respectively comparing with other levels including control. While the moderate concentration of Cu (1.0 mg Cu l^{-1}) had gave negatively effect on sporulation of *A. laevis* (400 spores $100g^{-1}$ dry soil), and still had little stimulant effect on other species *G. mosseae* (390 spores $100g^{-1}$ dry soil) comparing with control (420; 370 spores$100g^{-1}$ dry soil respectively). The third concentration of Cu (1.5 mg Cu l^{-1}) was caused sharply decreased in number of spores density by rates 210 spores $100g^{-1}$ dry soil to *G. mosseae* and 180 spores $100g^{-1}$ dry soil for *A. laevis* Fig. 2. In case of Cd the spore density of *G. mosseae* was stimulated in different concentration of Cd in solution (420, 410 and 380 spores to 0.1, 0.5 and 1.0 mg Cd l^{-1} respectively) comparing with control (370 spores in 0mg Cd l^{-1}). In contrast *A. laevis* was recorded a regular change by increase in spores density values (480 and 450 spores $100g^{-1}$ dry soil) by increase of Cd concentration in solution (0.1 and 0.5 mg Cd l^{-1}), except the concentration 1.0 mg Cd l^{-1} was caused a slightly inhibition effect in *A. laevis* spore forming (400 spores $100g^{-1}$ dry soil) comparing with control (420 spores $100g^{-1}$ dry soil) Fig. 2.

Data presented in Table (1) showed that the effect of inoculation by using *G. mosseae* and *A. laevis* on growth parameters of maize plant under stress of Cu and Cd in different concentration. In general all plant growth parameters, that determined in this study were decreased by increasing concentrations of both HM in the absent of AMF inoculation. In the presence of inoculation by AMF were recorded a stimulant effect in all plant growth parameter in presence of HM in different concentrations.

The greater values for plant height were recorded when plant inoculation with *A. laevis* in the presence of 0.50 mg Cu l^{-1} and 0.10 mg Cd l^{-1} comparing with non-inoculated plants with AMF at the same previous Cd concentrations.While the greater value for plant root length in the presence of HM were recorded at 0.5 mg Cu l^{-1} with inoculation by *G. mosseae*, and *A. laevis* in Cd concentrations 0.10 mg Cd l^{-1} comparing with non-mycorrhizal plant at the same HM and their concentration. The great values for plant shoot length; root biomass and shoot biomass were recorded in plants for *A. laevis* in the presence of 0.50 mg Cu l^{-1}. Also in Cd concentration 0.10 mg Cd l^{-1} comparing with non-inoculated plants with AMF. The root/shoot ratio was affected by present or absent HM as well as mycorrhizal inoculation. Heavy metals stress was caused increased in values of root/shoot ratio. On contrast

the inoculation by the two species of AMF were caused decreased in values of root/shoot ratio.

The effect of *G. mosseae* and *A. laevis* on physiological parameters of maize plant under stress of HM in greenhouse conditions was presented in Table (2). The higher values of plant soluble protein (mg g^{-1}) were found in the treatment, which inoculated by *A. laevis*, then *G. mosseae* comparing with control in the absent of two HM. As well as plant chlorophyll content, this was found in a higher value in the presence of inoculation with *G. mosseae* followed by *A. laevis* comparing with control free from HM. The beast results for chlorophyll content in the presence of HM were observed in plants inoculation by *A. laevis*. While the plant soluble protein was decreased by increasing in values of Cu and Cd concentration. The plant inoculation by AMF in the presence of HM in different concentration was decreased the HM stress comparing with non-inoculated plant. The infection with AMF causing decreased in plant proline values in the presence or absent of HM comparing with control (untreated plants).

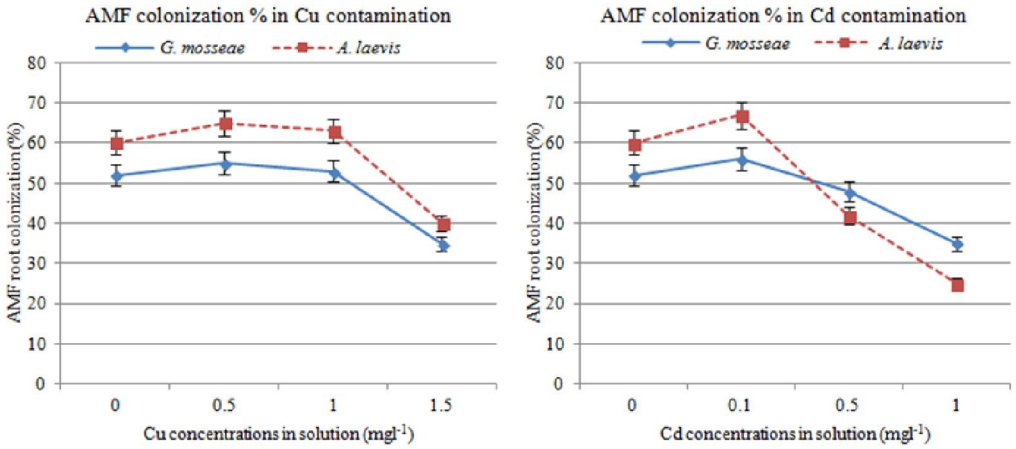

Fig. 1: Effect of different three concentrations of two heavy metals (Cu and Cd) on colonization rate of *Glomus mosseae* and *Acaulospora laevis* on maize plant (*Zea mays* L.) roots after 45days from infection.

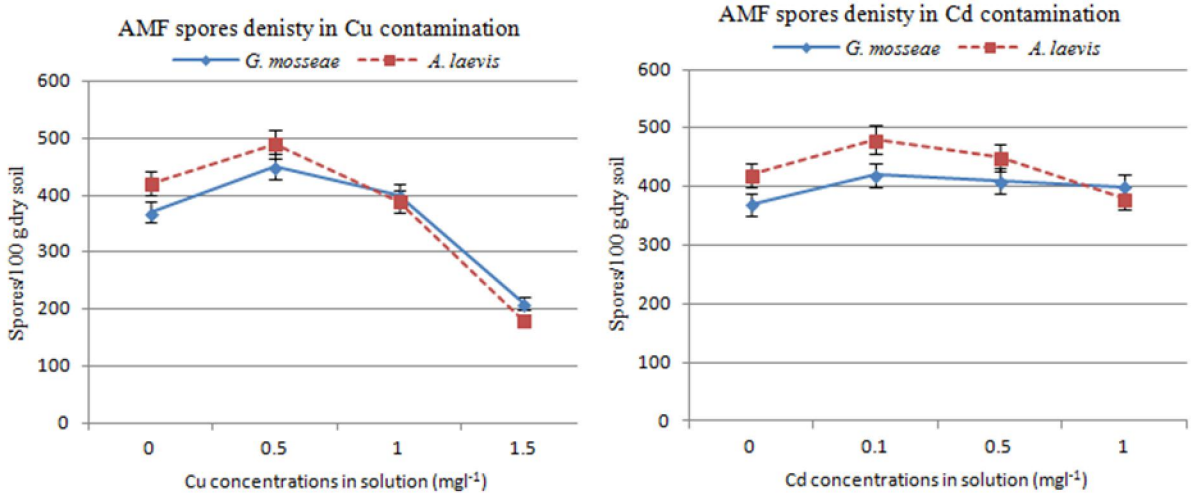

Fig. 2: Influence of three concentration of Cu and Cd on spore densities of two AMF species (*Glomus mosseae* and *Acaulospora laevis*) under maize plants (*Zea mays* L.) after 45days from soil infestation

Table 1: Effect of inoculation by two AMF species (*Glomus mosseae*, *Acaulospora laevis*) under stress of two heavy metal (Cu and Cd) in four concentration on maize plant (*Zea mays* L.) growth parameters

Treatment		Plant growth parameters					
AMF species	Heavy metal Con.(mgl^{-1})	Plant height (cm)	Root length (cm)	Shoot length (cm)	Root biomass (g pot^{-1})	Shoot biomass (g pot^{-1})	Root/shoot ratio
- In the presence of Cupper (Cu)							
Glomus mosseae	0.00	34.43 ± 1.8**	14.31 ± 0.8*	20.03 ± 0.9*	6.60 ± 0.6*	10.21 ± 0.6*	54.05 ± 8.1*
	0.50	33.80 ± 1.5*	14.60 ± 1.2*	18.36 ± 0.8	8.55 ± 0.5	12.20 ± 1.3*	70.08 ± 4.2*
	1.00	30.48 ± 0.6**	14.32 ± 0.8*	16.08 ± 0.0	8.58 ± 0.3*	13.05 ± 0.8*	65.74 ± 6.2*
	1.50	28.15 ± 0.9*	13.30 ± 0.7*	14.74 ± 0.1	7.95 ± 1.3	09.78 ± 1.2*	81.28 ± 10.*
Acaulospora laevis	0.00	45.49 ± 1.3**	13.52 ± 0.6	31.19 ± 0.7**	7.77 ± 0.9	14.8 ± 0.3**	52.50 ± 1.5*
	0.50	47.57 ± 2.1**	13.83 ± 1.5	34.70 ± 0.3**	8.60 ± 1.6*	15.11 ± 1.2**	56.91 ± 7.2*
	1.00	33.80 ± 1.5**	12.34 ± 2.7	21.30 ± 0.1**	8.00 ± 0.6*	11.50 ± 0.6**	69.56 ± 4.1*
	1.50	29.91 ± 1.2**	11.76 ± 3.5*	18.10 ± 0.5**	7.50 ± 0.0	10.75 ± 1.5**	69.76 ± 5.7
Non-inoculation	0.00	30.60 ± 0.6	12.23 ± 1.4	17.30 ± 0.7	4.60 ± 0.4	9.43 ± 1.2	69.98 ± 4.1
	0.50	29.45 ± 0.8	11.76 ± 3.8	17.34 ± 0.3	5.32 ± 0.3	8.66 ± 0.4	61.43 ± 3.1
	1.00	25.40 ± 4.7	11.10 ± 1.6	14.10 ± 0.2	5.55 ± 0.5	7.22 ± 1.3	76.86 ± 1.5
	1.50	22.90 ± 7.4	09.50 ± 2.2	13.10 ± 0.3	5.10 ± 0.2	7.13 ± 0.4	71.52± 1.2
-In the presence of Cadmium (Cd)							
Glomus mosseae	0.00	34.43 ± 1.8*	14.31 ± 0.8*	20.02 ± 0.1	6.60 ± 0.6*	10.21 ± 0.6*	54.05 ± 8.1*
	0.10	38.10 ± 4.7**	14.60 ± 0.7	23.10 ± 0.4*	9.10 ± 0.4*	11.60 ± 1.2*	64.64 ± 8.6*
	0.50	30.12 ± 3.6*	12.80 ± 0.6	17.22 ± 0.1	7.84 ± 0.6	10.20 ± 0.5	76.86 ± 12.2
	1.00	27.60 ± 4.5*	12.56 ± 0.1	15.0 ± 0.40*	6.81 ± 0.3	9.83 ± 0.44	69.27 ± 7.2
Acaulospora laevis	0.00	45.49 ± 1.3**	13.52 ± 0.6	31.19 ± 0.7**	7.77 ± 0.9*	14.80 ± 0.3**	52.50 ± 1.5*
	0.10	46.80 ± 1.1**	16.63 ± 1.4*	30.2 ± 0.1**	8.52 ± 0.4*	15.7 ± 1.23*	54.26 ± 1.9
	0.50	39.60 ±4.9**	14.33 ± 1.9*	25.1 ± 0.1**	7.50 ± 1.2	10.4 ± 1.50*	72.11 ± 1.7
	1.00	33.20 ± 1.3**	13.57 ± 2.8*	19.03 ± 0.6*	6.72 ± 2.1	9.82 ± 0.65	68.43 ± 1.4
Non-inoculation	0.00	30.60 ± 0.6	12.23 ± 1.4	17.30 ± 0.7	4.60 ± 0.4	9.43 ± 1.23	69.98 ± 4.1
	0.10	28.15 ± 0.9	12.52 ± 0.6	15.30 ± 0.3	4.70 ± 0.0	9.00 ± 1.20	52.22 ± 6.3
	0.50	25.64 ± 3.0	11.30 ± 0.5	14.10 ± 0.3	5.80 ± 1.7	8.23 ± 0.65	70.47 ± 1.5
	1.00	23.50 ± 2.2	11.66 ± 0.8	11.32 ± 0.5	5.60 ± 3.2	8.01 ± 0.24	69.91 ± 2.6

- Mean of three replicates and ± is standard error (n=3) - * Significant and ** highly significant level at 5%

The influence of inoculation and non-inoculation plants with AMF on uptake of the two studied HM in various concentrations in solution on root tissues was illustrated in Fig.(3). The plant roots ability to absorbent HM were increased when inoculation with *A. laevis* greater than those in *G. mosseae*. The effect of inoculation by *G. mosseae* or *A. laevis* on accumulation of Cu and Cd in various concentrations on *Zea mays* L. shoots tissues were illustrated in Fig. (4). Heavy metals uptake in plant shoots were less than which recorded in plant roots in the plant inoculated or non-inoculated by AMF. The inoculation plant by *A. laevis* was increased the shoot tissues absorbent HM greater than those in *G. mosseae*.

Table 2: Effect of *Glomus mosseae* and *Acaulospora laevis* on maize plant (*Zea mays* L.) physiological parameters under stress of heavy metal (Cu and Cd) under greenhouse conditions

Treatment		Plant physiological parameters		
Heavy metal (mg l^{-1})	AMF species	Soluble protein (mg g^{-1})	Proline content (µg g^{-1}FW)	Chlorophyll content (Unit)
In the presence of Cupper (Cu)				
0.5 mg Cu l^{-1}	M_0	18.20 ± 0.28	30.07 ± 1.51	12.18 ±1.23
	M_1	19.73 ± 0.33*	16.74 ± 2.32	13.28 ± 1.07
	M_2	20.26 ± 0.44*	12.71 ± 0.47	14.27 ± 1.08
1.0 mg Cu l^{-1}	M_0	16.48 ± 0.43	32.11 ± 0.33*	10.82 ± 0.77
	M_1	21.30 ± 0.56*	17.83 ± 2.68	11.83 ± 0.73
	M_2	23.72 ± 0.56	16.34 ± 2.76	12.55 ± 1.22
1.5 mg Cu l^{-1}	M_0	15.70 ± 0.39*	36.33 ± 1.72	8.21 ± 1.64*
	M_1	18.33 ± 0.23*	20.44 ± 0.23	10.00 ± 1.10
	M_2	18.56 ± 0.35*	22.60 ± 0.86	11.23 ± 2.41
In the presence of Cadmium (Cd)				
0.1 mg Cd l^{-1}	M_0	19.35 ± 0.41	40.26 ± 0.92*	10.64 ± 0.63
	M_1	20.35 ± 0.44*	20.41 ± 1.23	12.31 ± 1.41
	M_2	23.54 ± 0.35*	23.50 ± 0.57	13.45 ± 0.70
0.5 mg Cd l^{-1}	M_0	14.55 ± 0.54*	48.11 ± 0.61*	09.32 ± 2.31
	M_1	16.36 ± 1.10*	22.86 ± 1.32	12.33 ± 0.44
	M_2	16.83 ± 1.35*	24.21 ± 3.45	11.66 ± 2.76
1.0 mg Cd l^{-1}	M_0	11.04 ± 0.11*	33.56 ± 1.66*	08.23 ± 0.14*
	M_1	12.65 ± 0.31*	23.60 ± 0.11	10.31 ± 0.11*
	M_2	13.00 ± 1.23*	24.87 ± 0.44	11.01 ± 0.65*
In the absent of both Cu and Cd				
Untreated	M_0	19.37 ± 0.38	30.63 ± 1.32	11.03 ± 6.00
	M_1	25.41 ± 0.13	18.56 ± 0.54	16.33 ± 3.10
	M_2	26.57 ± 0.41	12.84 ± 0.26	15.80 ± 1.95

-M_0: Plant untreated with AMF species; M_1: plants inculated with *G. mosseae* ; M_2: plant was inculated with *A. laevis*
- Mean of three replicates and ± is standard error (n=3) - * Significant level at 5%

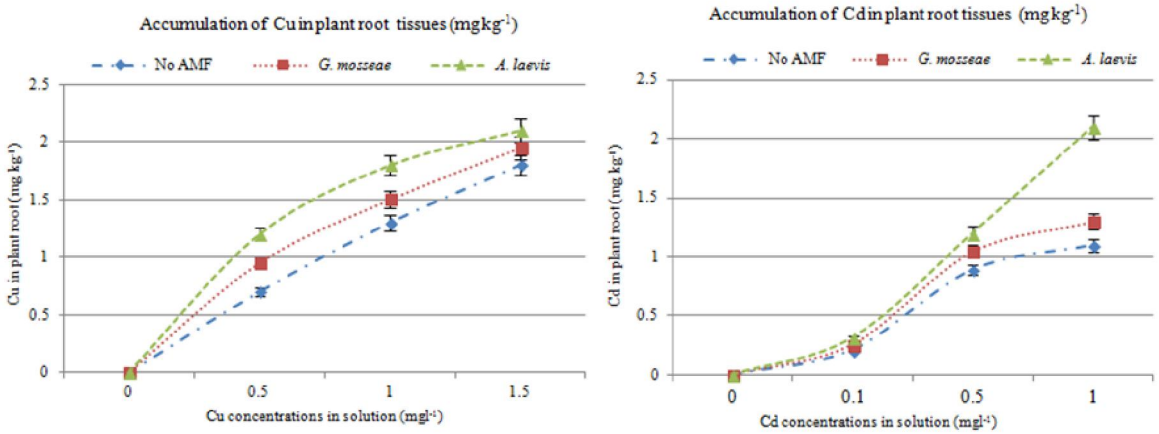

Fig. 3: Influence of inoculation and non-inoculation plants (*Zea mays* L.) by using AMF on uptake of two heavy metals (Cu, Cd) in various concentrations in solution on root tissues after 45 days from growth under greenhouse conditions

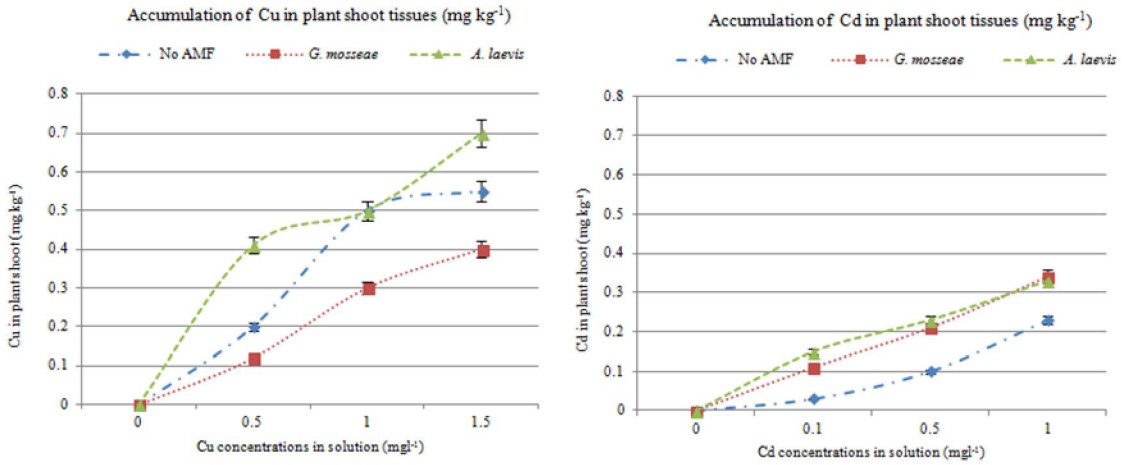

Fig. 4: Effect of inoculation by *Glomus mosseae* or *Acaulospora laevis* on accumulation of Cu and Cd in various concentrations in solution on *Zea mays* L. shoots tissues after 45 days of growth under greenhouse conditions

4. Discussion

The data reported in the present work clearly indicated that the symbiotic relationship between maize and AMF can be established under heavy metal stress conditions. The results showed that Cu and Cd are stimulated AM fungal colonization rate in the low heavy metal concentration in solution and reduced its value when heavy metal using in high concentration (under level of plant toxicity). These results are in agreement with **Gildon and Tinker (1983); Chao and Wang (1990); Weissenhorn et al. (1995); Chen et al. (2007) and Zhang et al. (2010)** they found mycorrhizal colonization or infection rate had been shown to be delayed, reduced, and even eliminated by high concentrations of Cu and Cd. Mycorrhizal colonization promoted plant biomass accumulation when compared to nonmycorrhizal plants in the presence of both HM concentrations in the solutions. These results are also in agreement with previous studies carried out with other plant species and metals **(Joner and Leyval, 2001, Liao et al., 2003; Chen et al., 2005; Chen et al., 2007; Gupta et al. 2009; Zhang et al., 2010 and Fernando et al. 2011)**, indicating the important contribution of AM fungal inoculation to plant growth under metal stress conditions. The root and shoot of mycorrhizal plants are usually higher than those of non-mycorrhizal plants. One of the reasons may be that plant roots are needs for the AMF to absorb and immobilized HM to less their toxic effects and improve the growth of the hosts. Plant under HM stress may also alter it was changed amino acid contents, especially proline. Accumulation of proline in plants under HM stress was considered to be a trait of adaptation process. One of the proposed roles of proline is to reduce free radicals levels and metal-induced proline accumulation in plant tissues. Also AM fungal inoculation can improve the antioxidant enzyme systems to alleviate destructive stress. As discussed above, similarly with **Sharma and Dietz (2006)** and disagreement with **Andrade et al. (2009),** reports on the effects of mycorrhizal symbiosis in proline content are scarce.

The inoculation of AMF decrease heavy metal toxicity to plants, it is therefore, due to the ability of AMF for immobilizing heavy metal, which can reduce the translocation of heavy metal from soil to plant roots or for the ability of AMF to bind HM in their cell wall, compartmentalize them in the vacuole or chelate them into the cytoplasm restricting the influx of HM into the plant. These results are in agreement with many previous studs including **Huang et al., 2002; Malcova et al., 2003; Andrade et al., 2009; Punamiya et al., 2010; Zhang et al. 2010 and Achakzai et al., 2012.**

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