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Effect of *Glomus intraradiaces and Glomus macrocarpium* on Growth and Copper Uptake by Maize Grown in Soil Experimentally Contaminated with Copper

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Abstract: Pot experiment was conducted to study the influence of various arbuscular mycorrhizal(AM) fungi species as a bioremediation agent for soil contaminated with Copper (Cu). Maize plant (*Zea mays* L.) - was grown in a calcareous soil and supplemented with five Cu addition levels of 0, 2, 4, 6 and 8 mM kg^{-1} soil in the form of $CuSO_4.5HO_2$. Two AM fungal inocula namely), *Glomus intraradiaces* and *Glomus macrocarpium* were used in this study under unsterilized soil conditions. The plants were harvested after 60 days of growth. Mycorrhizal colonization rate, plant dry weight (DW) and Cu concentration were determined. The Cu uptake and uptake efficiency, translocation efficiency and phytoextraction efficiency were calculated. The plants treated with *Glomus* spp had higher mycorrhizal colonization rates than *Glomus macrocarpium* -treated plants. Two mycorrhizal species increased shoot and root DW and *Glomus intraradiaces* was more ef fective. Mycorrhizal plants accumulated more copper in roots but large reductions in shoots. The uses of AM fungal for bioremediation of the contaminated soil lead to more absorption of Cu in plant. The comparisons of the Two AM fungal species indicate that the AM fungal represented by *Glomus intraradiaces* can benefit against potentially toxic Cu and therefore play a vital role in bioremediation of Cu-contaminated soils.

Key words: *Glomus intraradiaces* · *Glomus macrocarpium* · Copper · Bioremediation · *Zea mays* L. · Calcareous soil

INTRODUCTION

Among soil microorganisms, Arbuscular Mycorrhizal (AM) fungi (AMF) provide a direct link between soil and roots and are renowned for their ability to improve plant mineral nutrients, including trace metals. The roles of AMF in HM bioremediation have attracted much attention in recent years [1, 2]. It has been demonstrated that plants colonized by the Arbuscular Mycorrhizal (AM) fungi are usually more tolerant to certain metals than plants without these symbionts [1], but mechanisms underlying this protection are largely unknown. It has been suggested that the AM effects on host plant's tolerance toheavy metals may depend on reduced metal uptake because of retention and immobilization in chitin or glomalin in the fungal wall [3,4] and reduced metal transfer from roots to shoots [5,6]. Other protective effects may include metal dilution in plant tissue as a result of increased root or shoot growth, uptake exclusion by precipitation or chelation in the rhizosphere [7] and phosphorus (P)- mediated effects on the host plant [8]. AM symbioses are well known for their enhancement of phosphorus uptake and this nutritional benefit has been speculated to account for plant's tolerance to heavy metals [9]. This possible mechanism, however, has not been experimentally demonstrated.

The aims of this work were (1) to test the ability of two AM fungi species to colonize maize grown in contaminated soil, (2) to evaluate the influence of mycorrhizae species on plant growth and uptake of Cu by maize plants grown in soils across a gradient of Cu concentrations from uncontaminated to potentially toxic levels and (3) to confirm whether AM fungi can be applied as an aid in amelioration toxicity produced by Cu contamination under calcareous soil conditions.

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MATERIALS AND METHODS

Soil: Surface calcareous soil sample (0-15 cm) was collected from El- Nubaria at km 59 Alexandria - Cairo desert road (Egypt). The sample was air- dried, ground to pass 2 mm sieve, thoroughly mixed and stored in airtight polyethylene containers. The soil (Typic Calciorthids) has the following general properties: pH, 8.4; organic matter, 2.94 g kg^{-1}; total $CaCO_3$, 23%; EC, 1.9 dSm^{-1}; clay content, 25.36%; silt content, 23.52%; sand content, 51.12 % ; available P, 3.5 mg kg^{-1} soil; , available Cu, 0.12 mg kg^{-1}; total nitrogen, 0.09% and total Cu, 12 mg kg^{-1}. The procedures used for soil analysis were those described by Page *et al.* [10].

The soil sample was enriched with Cu at the rate of 0, 2, 4, 6 and 8 mM kg^{-1} soil (corresponding to 0, 127.08, 254.16, 381.24 and 508.32 mg Cu kg^{-1} soil) in the form of $CuSO_4.5HO_2$. The different soil treatments were well mixed and exposed to repeated drying rewetting cycles for two weeks, then stored to measure DTPA-extractable Cu (available Cu) and for carrying on pot experiment [11].

Mycorrhizal Inocula: Two arbuscular mycorrhizal (AM) fungi species belonging to the genus *Glomus* were used in this study. These species were *Glomus macrocarpium* and *G. intraradiaces* two AM species were obtained from Hanover University (Germany) and were propagated several times on maize plants grown in a sandy soil for 10 weeks.

Pot Experiment: Pot experiment was carried out at sunlight green house with natural light and day/night temperature of 17/22°C and relative humidity of 40-65 %. Plastic pots, 60 cm deep and 40 cm in diameter with holes in their bottom, were filled with 5 kg of the enriched soil with Cu leaving the upper 5 cm without soil. Seeds of maize (*Zea mays* L.) supplied from Maize Department Filed Crop Res Inst.ARC, were surface-sterilized by 0.05% NaOCl solution and subsequently washed with distilled water and planted in each pot. About fifty grams of inoculums for two arbuscular mycorrhizal (involving spores and colonized root segments) were placed 2 cm below the seeds. After two weeks, the plants were thinned to one plant per pot. The soil of each pot was fertilized with 120 mg N kg^{-1} soil in the form of NH_4NO_3, 150 mg K kg^{-1} soil in the form of K_2SO_4 and 30 mg P kg^{-1} soil in the form of Ca $(H_2PO_4)_2$. The Cu levels and AM fungi treatments were distributed in completely randomized design with three replicates. All pots were irrigated with tap water every three days to keep the soil moisture at 70% of its water holding capacity during the experimental period [11].

After 60 days of growth, shoots and roots of maize were harvested separately. Sub samples of fresh roots were taken to assess mycorrhizal colonization. Roots and shoots were rinsed with tap water and then rinsed with deionized water, oven drying at 70°C for 48 h, weighed and then ground in a stainless mill. A 0.5 g of dried plant materials was digested in H_2SO_4-H_2O_2 mixture according to Lowther [12] and Cu concentration was determined in the digested solution by atomic absorption spectrophotometer. Also, samples of the soil were collected at harvest from each pot and analyzed to estimate available Cu as described by Lindsay and Norvell [13]. The mycorrhizal infection percentages was estimated after staining [14] using the gridlines intersect method of Giovannetti and Mosse [15].

The mycorrhizal dependency (MD) of plant growth was calculated according to the following formula [16]:

$$MD = \frac{\text{Dry mass of AM plants - Dry mass of non-AM plants}}{\text{Dry mass of AM plants}} \times 100$$

Three aspects of plant Cu efficiency were assesd. According to Harper *et al.* [17], Cu uptake efficiency was calculated based on the ability of the root to uptake up Cu from the soil and the Cu translocation efficiency was computed as the ability of the plant to transport the Cu to the shoot.

Uptake efficiency (μg g^{-1}) = Cu uptake of the plants/ root dry weight (1)

Tanslocation efficiency = shootCu uptake/ root Cu uptake (2)

The third aspect of Cu phytoextraction efficiency was calculated based on the ability of the root to transport Cu to shoot according to the following equation:

Phytoextraction efficiency (μg g^{-1}) = shoot uptake/ root dry weight (3)

The obtained Data were statistically analyed according to [18].

RESULTS AND DISCUSSION

Before cropping, The amount of the DTPA-Cu steadily and linearly increased with increasing the level of added Cu to the soil is shown in Fig. 1. The low availability of Cu in soil solution might be explained by the soil properties (height PH and $CaCO_3$ content). Also, DTPA-extractable Cu after cropping without and with two AM fungi inoculation are found in Table 1. After cropping with AM fungi inoculation, the DTPA-Cu values at all Cu application rates were higher than those of cropping without AM fungi inoculation. In the same time, no significant difference between the mean values of DTPA-Cu over all Cu application rates for the soils after cropping with *G. intraradiaces* and *G. macrocarpium* species. Table 2 showed that Under different copper concentrations the roots of non-inoculated plants were not infected with mycorrhizal fungi. The infection rates of *G. intraradiaces* were the highest under different concentrations of Cu. The mycorrhizal infection rates of the two fungi greatly changed when Cu concentrations in soil were less than 4 mM Cu kg^{-1} soil, but the high levels of Cu in soil inhibit mycorrhizal colonization particularly *G. macrocarpum* which completely eliminate AM colonization at 6 and 8 mM Cu kg^{-1}soil. these results were in agreement with those obtained by Lius *et al.* [19] who reported that high levels of Cu inhibit spore germination and mycorrhizal colonization.. AM fungal ecotypes from different habitats may exhibit different degrees of metal tolerance [8]. Our results showed that *G. intraradices* was more tolerant relatively to Cu and slightly colonized maize roots even when 8 mM Cu kg^{-1} soil was added 14 %. Also the results in Table 2 revealed that shoot and root dry weights of maize significantly decreased with increasing Cu application rate. Compared to the non-mycorrhizal treatment, two AM fungi species increased significantly the shoot and root dry weights (average values over Cu rates. (With high Cu doses (6 and 8 mM Cu kg^{-1} soil mycorrhizal colonization was so severely depressed and both shoot and root dry weights were decreased with two AM fungi species. The inhibition of plant growth of mycorrhizal plants with 8 mM Cu kg^{-1} soil addition level was more pronounced with *G.macrocarpum* which showed lower colonization than that *G. intraradiaces* (Table 2).

According to Smith and Read [20], a number of different mechanisms may be involved in the interactions between mycorrhizal colonisation and accumulation of heavy metals, including tissue dilution of the toxic element due to interactions with P nutrition (and increased yield), sequestration of the toxic metal in the fungus and development of tolerance by the fungus. In our experiment, tissue dilution is likely to have been important because mycorrhizal infection had a relatively big effect on plant yield, taking in consideration that the plants were growing in a soil with low P content (3.5 mg kg^{-1}) and different doses of applied Cu, the dry weight of the inoculated plants were significantly higher than those of the non-inoculated ones (Table 2). Li *et al.* [21] stated that AM fungi often improved plant Cu nutrition.

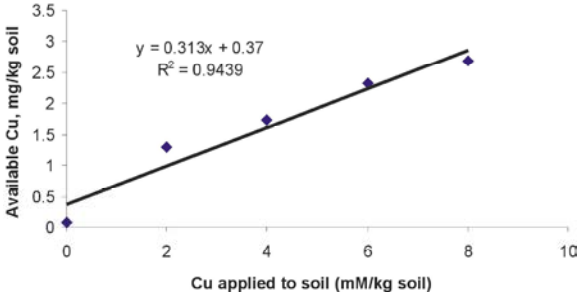

Fig. 1: Available Cu in soil before cropping of maize.

This inhibition may be attributed to the effect of Cu on photosynthetic and respiratory electron transfer and the activity of various oxidative enzymes [22]. Also, this inhibition may be due to reduced carbon fixation, altered nutrient acquisition and reduced growth [23].

The results indicated also that, the fungus *G.intraradices* was more effective than *G.macrocarpum* in protecting the maize plants against Cu toxicity. Thus, the AM fungus seems to have various heavy metal detoxification mechanisms including the retention of toxic metals in roots and the subsequent reduction of translocation to shoots [24].

Average shoot and root dry weights of AM-treated plants increased by 92% and 74% in *G.intradices* and 51 % and 24% in *G.macrocarpum* treatments compared to the non-inoculated plants, respectively. The mycorrhizal dependency (MD) of maize plants inoculated with *G. intraradices* and *G.macrocarpum* were 42%, 19 %, for roots and 48%, 34% for shoots respectively. These results showed high MD on AM fungi and could be a promising AM inocula for successful bioremediation. MD was calculated according to the data in Table 2.

Compared with non-mycorrhizal treatment, data showed that AM fungi species increased significantly Cu concentration in shoots and roots of maize plants.

Table 1: Available Cu in soil after cropping of maize as affected *Glomus intraradiaces and Glomus macrocarpium* inoculation owed by the same letter are not significantly different

	Available Cu, mg kg^{-1} soil			
Cu rate mM kg^{-1} soil	Without inoculation	*G.intraradices*	*G.macrocarpum*	Mean of Cu rate
0	0.85	0.98	1.03	0.95 e
2	2.23	2.59	2.37	2.40 d
4	2.59	3.12	2.82	2.84c
6	3.47	3.71	3.76	3.65b
8	3.68	4.00	3.91	3. 86 a
Mean of AM fungi inoculation	2.56 b	2.88 a	2.78 a	
AM × Cu				
L.S.D$_{0.05}$	0.232			

Means followed by the same letter are not significantly different at LSD$_{0.05}$ level of probability.

Table 2: The effect of Cu rates on *Glomus intraradiaces* and *Glomus macrocarpium* infection and maize plant growth.

			Plant (g plant^{-1})	
AM fungi type	Cu rate mM kg^{-1} soil	AM fungi infection, %	Roots DW	Shoots DW
Non-inoculation	0	10.81	3.44	6.20
	2	0.00	1.87	3.55
	4	0.00	0.95	2.03
	6	0.00	0.00	0.00
	8	0.00	0.00	0.00
Mean		2.16 c	1.25c	2.36 c
G.intraradices	0	56.20	3.41	6.33
	2	35.53	2.64	5.76
	4	24.13	2.35	4.89
	6	17.20	1.57	3.78
	8	14.30	0.87	1.94
Mean		29.47 a	2.17 a	4.54 a
G.macrocarpum	0	54.00	2.71	5.43
	2	22.60	2.20	4.92
	4	18.58	1.92	4.21
	6	0.00	0.93	3.23
	8	0.00	0.00	0.00
Mean		19.04 b	1.55 b	3.56 b
Mean effect of Cu rate	0	40.34 a	3.19a	6.00 a
	2	19.38 b	2.24 b	4.74 b
	4	14.24 c	1.74 c	3.71 c
	6	5.73 d	0.83 d	2.34 d
	8	4.77 e	0.29e	0.65 e
AM x Cu rate L.S. D $_{0.05}$		3.15	0.66	0.98

Means followed by the same letter are not significantly different at LSD$_{0.05}$ level of probability

Significant differences were observed in shoot Cu concentration between two inoculation treatments , but Cu concentration in roots were higher in two inoculation treatments than in shoots and there was a significant difference in root Cu concentrations between two AM fungi species. The results showed that *G. intraradices* increased the Cu concentration in roots and shoots than *G.macrocarpum*. This indicated that the accumulation were closely related not only to AM fungal species, but also to the availability of Cu in soil (Table 1).

The concentrations of Cu was much higher in the roots than in the shoots, thereby that extra-radical hyphae of AM fungus can transport Cu from soil to plant, but transfer from fungus to plant is restricted due to fungal

Table 3: Means of Cu content as affected by *G. intraradiaces* and *G. macrocarpium* fungi species and Cu application rate.

AM fungi type	Cu rate (mM kg^{-1} soil)	Cu content µg $plant^{-1}$ Roots	Shoots
Non-inoculation	0	1.17	0.81
	2	67.71	29.22
	4	36.80	21.56
	6	0	0
	8	0	0
Mean		21.14c	10.32c
G.intraradices	0	1.77	1.79
	2	152.12	56.56
	4	176.98	90.91
	6	157.66	82.10
	8	104.71	60.45
Mean		118.65a	58.40a
G.macrocarpum	0	0.84	1.36
	2	65.91	37.05
	4	115.78	62.98
	6	79.98	64.08
	8	0	0
Mean		52.5b	33.10b
Mean effect of Cu rate	0	1.26e	1.32d
	2	95.25b	40.94b
	4	109.85a	58.48a
	6	79.21c	48.73b
	8	34.90d	20.15 c
AM x Cu rate L.S.D 0.05		13.06	6.29

Means followed by the same letter are not significantly different at $LSD_{0.05}$ level of probability

Table 4: Copper Uptake efficiency, Phytoextraction efficiency and Translocation efficiency of maize plants as affected by Cu rate and two AM fungi species.

Cu rate mM Cu Kg^{-1} Soil	AM fungi species Non- AM	*G.intraradices*	*G.macrocarpum*
Cu rate efficiency µgg^{-1} DW			
0	0.58	1.04	0.81
2	51.83	79.05	46.80
4	61.43	114	93.10
6	0	152.71	155.00
8	0	189.84	0.00
Phytoextraction efficiency µgg^{-1} DW			
0	0.24	0.52	0.50
2	15.63	21.42	16.84
4	22.70	60.00	32.80
6	0.00	73.34	69.00
8	0.00	0.00	0.00
Translocation efficiency			
0	0.69	1.01	1.62
2	0.43	0.37	0.56
4	0.59	0.51	0.54
6	0.00	0.52	0.80
8	0.00	0.58	0.00

immobilization in special parts of the roots, therefore enhances plant growth as well as increased tolerance heavy metals by reduced metal translocation to the above-ground organs of the plant [25,26]. In general, AM fungi inoculation can improve plant Cu nutrition [21, 27].

The root to shoot ratio of Cu concentration (R/S) which calculated from Table 3 were varied in the inoculated and in the non-inoculated plants.. The R/S ratio of Cu concentration indicated that concentration of root Cu for *G.intraradices* was between 2-6 fold higher than those in shoots at all Cu application rates to soil, while reached between 1-4fold higher than those in shoots when maize plant inoculated with *G.macrocarpum*. These data elucidate the ability of root tissues to accumulate Cu in line with Gonzalez-Chavezet *et al.* [28] who found that AM fungal inoculation decreases Cu concentrations in plant shoots, thus exerting a protective effect on the host plants against heavy metal toxicity and leading to higher plant yields.

The data in Table 3 revealed that Cu content in maize shoots and roots significantly increased with increasing Cu levels added to soil for both non-mycorrhizal and mycorrhizal treatments and the interactions between them were also significant for shoot and root Cu content. Statistically, shoot and root content in mycorrhizal plants were significantly higher compared to non-mycorrhizal plants when Cu added with different levels. With non-mycorrhizal plants Cu content of shoots and roots increased with the application of 2 and 4 mM Cu kg^{-1} soil only and became zero at 6 and 8 mM Cu kg^{-1} soil because of the plant growth inhibition (dying the plants).

The inoculation with two AM fungi species increased generally the content with increasing Cu application up to 4 mM kg^{-1} soil for roots and shoots and decreased at the other Cu levels. The addition of 6 and 8 mM Cu kg^{-1} soil depressed severely the mycorrhizal colonization (Table 2). The obtained results indicate that all AM fungi inoculations may be not suitable for Cu phytoextraction by maize, but show a potential role in phytostabilization of soil moderately polluted by Cu because of higher root Cu content by mycorrhizal plants (Table 3). Furthermore, AM fungi can protect host plants from Cu toxicity and improve P nutrition. Also, the interaction effect between Cu addition levels to soil and the inoculation with AM fungi species showed significant trend on Cu content of shoots and roots.

Copper uptake efficiency and phytoextraction efficiency were increased with increasing the amounts of added Cu to the soils but showed the opposite trend at 8 mM Cu kg^{-1} soil level particularly in *G.macrocarpum* treatment (Table 4). Compared with the non- mycorrhizal

plants Cu uptake efficiency and phytoextraction efficiency of mycorrizal plants were higher with all Cu addition levels except in *G.macrocarpum* treatment where their values became zero at 8 mM Cu kg^{-1} soil level. Also Cu translocation efficiency in mycorrhizal or non-mycorrhizal plants was lower at all Cu addition levels in soil but higher with zero Cu level (Table 4). The AM fungi colonized roots have a reduced ability to take up Cu from soil and to translocate it to shoots under conditions of Cu contamination. It has been pointed out that in soil with different Cu contamination levels there may be a critical Cu concentration below which Cu uptake is enhanced by AM fungi and above which there is inhibition of Cu translocation to shoots [11]. Similarly, our results showed a critical Cu contamination level below which AM fungi improve plant Cu nutrition and above which AM fungi depress plant Cu uptake from soil or Cu translocation to the aerial parts of host plants. Obviously, this critical value may vary with a variety of factors and needs further study.

In summary, we investigated the protective effect of AM against growth and uptake of potentially toxic Cu by growing maize in experimentally contaminated soil. *G.intraradices* showed the highest tolerance to this contamination. A field experiment to confirm its application effects is also needed in naturally contaminated soil.

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REFERENCES

1. Gaur, A. and A. Adholeya, 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current. Sci. India. 86: 528-534.
2. Khan, A.G., 2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. Journal of Trace Elements in Medicine and Biology 18. 355-364.
3. Khan, A.G., C. Kuek, T.M. Chaudhry and Khoo, C.S. Hayes, 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere, 41: 197-207.
4. Gonzalez-Chavez, M.C., R. Carrillo-Gonzalez, S.F. Wright and K.A. Nichols, 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ. Pollut. 130: 317-323.
5. Joner, E.J., R. Briones and C. Leyval, 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil, 226 227-234.
6. Christie, P., X.L. Li and B.D. Chen, 2004. Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. Plant Soil, 261: 209-217.
7. Kaldorf, M., A.J. Kuhn, W.H. Schröder, U. Hildebrand and H. Bothe, 1999. Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J. Plant Physiol. 154: 718-728.
8. Wang, F.Y., X.G. Lin and R. Yin, 2005a. Heavy metal uptake by arbuscular mycorrhizas of *Elsholtzia splendens* and the potential for phytoremediation of contaminated soil. Plant and Soil. 269: 225-232.
9. Diaz, G. C. Azcón-Aguilar and M. Honrubia, 1996. Influence of arbuscular mycorrhiza on heavy metal (Zn and Pb) uptake and growth of *Lygaeum spartum* and *Anthyllis cytisoides*. Plant Soil 180, 241-249.
10. Page A.L., R.H. Miller and D.R. Keeny, 1982. Methods of Soil Analysis (Part 2). American Society of Agronomy, In. Madison, Wisconsin, USA.
11. Wang, F.Y., 2007. Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. Pedobiologia, 51: 99-109.
12. Lowther, J.R., 1980. Use of a single sulfuric acid-hydrogen peroxide digest for the analysis of Pinus radiata needles. Commun. Soil Sci. Plant Anal., 11: 175-188.
13. Lindsay, W.L. and W.A. Norvell, 1978. Development of DTPA soil test for Zn, iron,manganese and Copper. Soil. Sci. Soc. Am. J., 42: 421-428.
14. Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc., 55: 158-160.

15. Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist. 84: 489-500.
16. Plenchette, C.J. A. Fortin and V. Furlan, 1983. Growth responses of phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. Applied and Environmental Microbiology. 66: 2526-2530.
17. Harper, F.A., S. Smith and M. Macnair, 1997. Can an increased copper requirement in copper-tolerant *Mimulus guttatus* explain the cost of tolerance? I. Vegetative growth. New Phytologist. 136: 455-467
18. Snedecor, W. and W.G. Cochran, 1974. Statistical Method. 6th Edition. The Iowa State College Press. Iowa, U.S.A.
19. Luis, M., I. Carvalho, M. Cacador and Amelia Martins-Loucao, 2006. Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. Plant and Soil, 285: 161-169.
20. Smith, S.E. and D.J. Reed, 1997. In: Mycorrhizal Symbiosis, second ed. Academic Press, London, pp. 589.
21. Li, X.L., H. Marschner and E. George, 1991. Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. Plant and Soil, 136: 49-57.
22. Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edition, Academic Press, London, UK.
23. Borkert, C.M., F.R. Cox and M.R. Tucker, 1998. 'Zinc and copper toxicity in peanut, soybean, rice and corn in soil mixtures', Commun. Soil. Sic. Plant Anal., 29: 2991-3005.
24. Tullio, M., F. Pierandrei, A. Salerno and E. Rea, 2003. Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium-polluted and unpolluted soil. Biol. Fertil. Soil. 37: 211-214.
25. Schutzendubel, A. and A. Polle, 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany, 53: 1351-1365.
26. Zhang, X.H., Y.G. Zhu, B.D. Chen, A.J. Lin, S.E. Smith and A.E. Smith, 2005. Arbuscular mycorrhizal fungi contribute to the resistance of upland rice to combined metal contamination of soil. Journal of Plant Nutrition, 28: 2065-2077.
27. Liu, A., C. Hamel, R.I. Hamilton, B. Ma and D.L. Smith, 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhiza maize (*Zea mays* L.) grown in soil at different P and micronutrient. Mycorrhiza, 9: 331-336.
28. Gonzalez-Chavez, C., J. D'Haen, J. Vangronsveld and J. C. Dodd, 2002. Copper sorption and accumulation by the extraradical mycelium of different *Glomus spp*. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant and Soil. 240: 287-297.