The Role of Arbuscular Mycorrhizae in the Growth and Zinc Uptake of Wheat Plant Grown on a Calcareous Soil Contaminated with Zinc

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ABSTRACT

Pot experiment was carried out at the green house of Faculty of Agriculture (Saba bacha), Alexandria University. The experiment was conducted to study the influence of various arbuscular mycorrhizal fungi(AMF) species as a bioremediation agent for soil contaminated with Zinc. Wheat plant(*Triticum aesitivum*) - Giza 168 was grown on a calcareous soil and supplemented with six Zn addition levels of 0, 2, 4,6,8 and 10 mM kg^{-1} soil in the form of $ZnSO_4.7HO_2$. Four AM fungal inocula namely *Glomus spp* (mixed), *Glomus intraradiaces*, *Glomus macrocarpium*, and *Glomus fasciculatum*, the first one was isolated from contaminated soil and were applied to the soil. The plants were collected after 60 days from sowing. Mycorrhizal colonization rate, plant dry weight (DW), Zn concentrations and Zn uptake were determined and uptake efficiency, translocation efficiency and phytoextraction efficiency were calculated. The *Glomus spp*-treated plants had higher mycorrhizal colonization rates than other inoculation-treated plants. All mycorrhizal species increased shoot and root DW, *Glomus spp* (mixed) was more effective than the others. Mycorrhizal plants accumulated more zinc in roots, however appeared a large reductions in shoots. The use of AM fungal for phytoremediation of the contaminated soil lead to more absorption of zinc in plant. The comparisons of the four AM fungal species indicate that the AM fungal represented by *Glomus spp* (mixed) showed a beneficial effect in phytoremediation of Zn-contaminated soils.

Key words: arbuscular Mycorrhizal; zinc contamination; phyoremediation; calcareous soil

INTRODUCTION

Ecosystems have been contaminated with heavy metals due to various human and natural activities. The sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, sewage sludge amendments, and the use of pigments and batteries. All these sources cause accumulation of metals in our agricultural soils and pose threat to food safety issues and potential health risks due to soil-to- plant transfer of metals (Khan, 2005). One of these metals is zinc.

Zinc is involved in plant enzyme function, carbohydrate metabolism, protein synthesis, and tryptophan and indole acetic acid synthesis (Marschner, 1995).When soil Zn concentrations become elevated, foliage becomes chlorotic, plants exhibit altered photosynthetic physiology, and growth is reduced Borkert *et al.*, (1998)

The remediation of contamineted soils is important because these usually cover large areas that are rendered unsuitable for agricultural and other human use. Conventional soil remediation practices in the past have relied mainly on the excavation of the contaminated soil. However, physical displacement, transport and storage or alternatively soil washing are expensive procedures and leave a site behind devoid of any soil microflora. In contrast plants offer an inexpensive and sustainable on-site approach (Kramer, 2005), which relates to heavy metal detoxification mechanisms (Hall, 2002). Arbuscular mycorrhizal (AM) fungi occur in the soil of most ecosystems, including polluted soils. In some cases mycorrhizal plants can show enhanced uptake and root-to-shoot transport (phytoextraction) while in other cases AM fungi contribute to heavy metal immobilization within the soil (phytostabilization). The significance of AM fungi in soil remediation has lately been recognized (Gaur and Adholeya, 2004). Galli et al. (1994) suggested that AM fungi can play a crucial role in protecting roots from heavy metals, the efficiency of protection, however, differs between distinct isolates of AM and different heavy metals.

The aims of this work were 1) to assess the ability of four AM species to colonize wheat plant grown on Zn contaminated calcareous soil; 2) to evaluate the influence of the tested mycorrhizal species on plant growth and uptake of Zn by wheat plants grown on soils across a gradient of Zn 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 Zn contamination under calcareous soil conditions.

MATERIALS AND METHODS

1. Soil

Surface calcareous soil sample (0-15 cm) was collected from El- Nubaria region at km 59 Alexandria –Cairo desert road. This sample was air-dried, ground to pass to 2mm sieve and thoroughly

mixed. The soil was analyzed for the determination of the main properties as outlined by(Page et al., 1982). It has the following general properties: pH of 8.2; organic matter of 3.24 g kg^{-1}; total $CaCO_3$ of 24.6%; EC of 1.7 dSm^{-1}; clay content of 25.36%; silt content of 23.52%; sand content of 53.77 % ; available P of 3.5mgkg^{-1} soil; , available Zinc of 0.36 mg/kg^{-1}; total nitrogen of 0.09% and total Zn of 75mgkg^{-1}. The soil was enriched with Zn at the rates of 0, 2,4,6,8 and 10 mMkg^{-1} soil (corresponding to 0,130.76, 261.52, 392.28, 523.04 and 653.80 mgkg^{-1} soil) in the form of Zn $SO_4.7HO_2$. The Zn-treated soils were well mixed, and exposed to repeated drying and rewetting cycles for two weeks with distilled water dried, ground and stored in plastic pots for analysis .

2. *Mycorrhizal* inocula

Four arbuscular *mycorrhizal* fungi (AMF) species belonging to the genus *Glomus* were used in this study. The used species were *Glomus macrocarpium, Glomus fasciculatum, Glomus intraradiaces* and, *Glomus spp* (mixed).The first and second species were obtained from Department of plant nutrition at Göttingen-University, The third species was supplied from Department of plant pathology at Hanover University, Germany and the fourth species was obtained from Faculty of Agriculture., Ain Shams University of Cairo .

3. Pot experiment

Pot experiment was carried out at the green house of faculty of Agriculture (Saba Bacha), Alexandria University. Plastic pots of 30 cm depth and 13cm inside diameter with holes in their bottom, were filled with 1.2 kg of the Zn-enriched soil, leaving the upper 5cm without soil. Seeds of Wheat (*Triticum aesitivum* L.) - Giza 168 were surface-sterilized with 0.05% NaOCl solution and subsequently washed several times with distilled water and planted in each pot. About fifty grams of inoculum *Glomus intraradiaces* and 100 ml of an inoculum suspension (involving spores and colonized root segments) for the other species *Glomus macrocarpium, Glomus fasciculatum* and *Glomus spp* (mixed) were placed 2 cm below the seeds. Two weeks after planting, plants were thinned to 2 plants per pot. The soil of each pot was fertilized with 120 mg N kg^{-1} soil in the form of NH_4NO_3 , 150 mgK 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 Zn and AMF treatments were distributed in completely randomized design with three replicates. All pots were irrigated with tap water every three days to keep the soil at 70% of its field capacity by regular weighing of pots.

After 60 days from sowing, the plants were collected, after planting, samples of plants were oven dried at 70 C° for 48 hours, ground and dry weights of shoots and roots were recorded. Half g of the oven dried plant material was digested with H_2SO_4-H_2O_2 mixture according to (Lowther 1980). In the digested solution Zn was measured by atomic absorption spectrophotometer (A Analyst 400). In addition, samples of the soils were collected, at harvest from each pot and analyzed to estimate DTPA extractable Zn as described by (Lindsay and Norvell 1978).The staining method of (Phillips and Hayman 1970) was used for preparing root samples for microscopic observations. The gridlines intersect method of (Giovannetti and Mosse 1980) was used to estimate the mycorrhizae infection percentage using the following equation:

$$\text{AMF infection \%} = \frac{\text{Number of segments containing AMF}}{\text{Total number of examined segments}} \times 100$$

Also, the mycorrhizal dependency (MD) of plant growth was calculated according to the following formula (Plenchette *et al.*, (1983) :

$$\text{MD} = \frac{\text{(Dry mass mycorrhizal plant) - (Dry mass non-mycorrhizal plant)}}{\text{Dry mass mycorrhizal plant}} \times 100$$

The relative increase or decrease of zinc uptake of mycorrhizal plants relative to the non-mycorrhizal plants was calculated based on the following formula Wang *et al.* (2005):

$$\text{The relative Increase/ or decrease of Zn uptake} = \frac{\text{Zn uptake of mycorrhizal plants} - \text{Zn uptake of non-mycorrhizal plants}}{\text{Zn uptake of non-mycorrhizal plants}}$$

Also ,three aspects of plant Zn efficiency were assessed. according to Harper *et al.* (1997) were Zn uptake efficiency was calculated based on the ability of the root to take up Zn from the soil and the Zn translocation efficiency was computed as the ability of the plant to transport Zn to the shoot

$$\text{Uptake efficiency } (\mu g\ g^{-1}) = \text{Zn uptake of the plants/ root dry weight}$$

$$\text{Tanslocation efficiency} = \text{shoot Zn uptake/ root Zn uptake}$$

Another aspect was zinc phytoextraction efficiency which is calculated based on the ability of the root to transport Zn to shoot according to the following equation:

$$\text{Phytoextraction efficiency } \mu g\ g^{-1} = \text{shoot uptake/ root dry weight}$$

The obtained Data were subjected to statistical analysis of variance or regression (Snedecor and Cochran, 1972).

RESULTS AND DISCUSSION

Soil extractable Zinc

The effect of Zn application to the soil on the amounts of DTPA-extractable Zn before planting is shown in Figure.(1). Also, DTPA-extractable Zn after cropping without AMF inoculation and after cropping with the inoculation with the tested AMF

species are shown in Table (1). The amounts of the DTPA-Zn steadily and linearly increased with increasing the level of added Zn to the soil before planting. The values ranged from 0.20 $mgkg^{-1}$ soil without Zn application to 7.16 mg/kg soil at the higher rate of Zn application (10 mM/kg soil). (Sims and Johnson 1991) reported that the levels of DTPA-Zn in most soils that required by most crop plants were between 0.3 to1.4 mg/kg soil. Thus, the higher rates of Zn application may be toxic to the wheat plants.

After planting with AMF inoculation, the DTPA-Zn values at all Zn application rates were higher than those of without AMF inoculation. It is clear also, that the AMF species varied in their effects on the DTPA-Zn. The species *G.interaradices* was more effective mostly than the other species in increasing the DTPA-Zn. In the same time, no significant difference between the mean values of DTPA-Zn over all Zn application rates for the soils after planting with *G.intraradiaces, G. fasciculatum* and *G. macrocarpium* species.

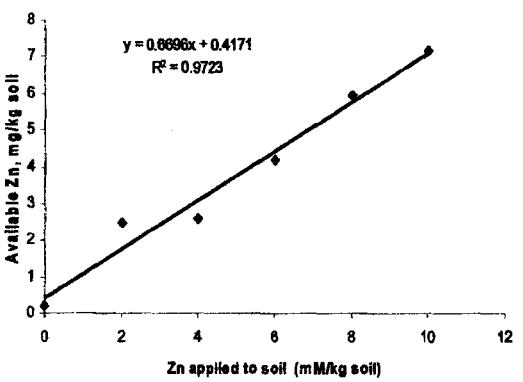

Fig. 1: Available Zn in soil before planting of wheat

Increasing the available amounts of Zn, at each level of added Zn to the soil after planting with the different AMF species inoculation, can be explained on the basis that hyphae mycelium increases the total absorption surface of infected plants and this improves its access of immobile elements such as P, Cu, and Zn Ortas *et al.*, (1996) and Giri *et al.*, (2005) in areas beyond the root's depletion zone. These results agree with those reported by Giri *et al.* (2005) who found that in alkaline soils, mycorrhizal hyphae lead to decrease alkalinity of the rhizosphere soil from 8.5 to 7.4 by organic acids exudation , which caused solubilizing the immobile elements such as P, Cu, and Zn. The obtained data indicate to the higher buffer capacity of the calcareous soil to be contaminated with heavy metals, wherever it received more than 10mM Zn/kg soil and the determined available Zn was 7.21 mg/kg soil. So the Egyptian calcareous soil have not any problems with heavy metal contamination.

Root colonization rate

Table (2) showed that mycorrhizae infection percentages increased with the mycorrhizae inoculation, and the highest colonization rates (51-53% of root length) occurred in the absence of Zn addition by both inocula *G. spp (mixed)* and *G. intraradices*.

The highest rate of Zn Table (2) decreased colonization to only 9-13%, but in the case of *G. macrocarpum* and *G. fasciculatum* the appearance and degree of infection was sharply decreased by Zn additions and colonizations, were closely similar. Also, the highest colonization percentages were observed for *G. spp(mixed)* at DTPA extractable Zn of 0.20 and 2.47 mg/kg soil before cropping or inoculation respectively Table (1). Although all roots of wheat plants were spontaneously colonized by AM fungi, there were significant differences between the control and AM treatments with an order of *G. spp* > *G. intraradices* > *G. macrocarpum* > *G. fasciculatum*. Plants in the control treatment (non AM) were not colonized at 4, 6, 8, and 10 mM/kg applied Zn to the soil. Also the result showed that at addition of 10 mM/kg of Zinc in soil, no colonization was observed with *G.macrocarpum* species. These findings are consistent with Marques et al. (2006) who used four different species of AM fungi *(G. spp.* BEG140, *G. claroideum,G. mosseae* and *G. intraradices*) and found that high levels of Zn concentration in soil decrease the AM fungi colonization. The data obtained in this study showed that the extent of infection was reduced progressively and regularly by additions of zinc into soil.

Plant growth

Table (2) revealed that shoot and root dry weights of wheat significantly decreased with increasing Zn application rate. These findings are consistent with the results of Diaz *et al.* (1996) who found that increasing doses of Zn or Pb reduced plant biomass. The addition of increasing doses of Zn reduced logarithmically plant biomass in the nonmycorrhizal plants up to 6 mM/kg Soil. With increasing the added Zn the growth of plants inhibited. This inhibition may be attributed to the effect of zinc on the photosynthesis, which is the key process in plant development Jamal *et al.* (2002) .

Taking into accounts that plants were growing in a soil with low P content (3.5 mg/kg soil) and with different doses of Zn applied, the dry weight of inoculated plants were significantly larger than those of the non-inoculated ones Table (2).

The inhibition of plant growth of mycorrhizal plants with Zn addition was more pronounced with *G.macrocarpum* than with the other species as can be noted from the higher slope of the regression equation Table (3). The effectiveness of the fungi to improve plant growth varied according to the levels of Zn in soil. At high doses of Zn addition *G.*

Table 1: The amounts of DTPA extractable Zn in soil after planting of wheat as affected by AMF species inoculation

Applied Zn(mM/kg)	DTPA-extractable Zn , mg/kg soil					Mean of Zn rate
	without inoculation	*Glomus spp(mixed)*	*G.intraradices*	*G.fasciculatum*	*G.macrocarpum*	
0	0.24	0.40	0.30	0.29	0.26	**0.30**
2	2.53	2.92	3.05	2.76	3.27	**2.91**
4	3.88	4.41	4.31	4.80	5.50	**4.58**
6	5.03	6.76	6.80	7.35	6.61	**6.51**
8	6.56	7.49	8.26	7.54	7.68	**7.51**
10	7.21	7.56	8.82	8.33	7.93	**7.97**
Mean of AMF	**4.24**	**4.92**	**5.26**	**5.18**	**5.21**	
AM inoculation L.S.D$_{0.05}$				0.26		
Zn rates L.S.D$_{0.05}$				0.28		
InteractionAM×Zn L.S.D$_{0.05}$				* 0.64		

macrocarpum was less efficient in stimulating the growth of wheat plants than the other species as can be deduced from the slopes of the regression equations showed in Table (4) This fungi showed lower percentages of colonization in the presence of increasing doses of added Zn than the other species Table (2) which suggests a certain degree of tolerance for *G.spp(mixed)* to this metal. On other words, the results presented here indicated that the fungus *G.spp (mixed)* were more effective than the other species in protecting the wheat plants against Zn toxicity. Thus, the AM fungus seems to protect the host plant from moderate Zn pollution by immobilizing Zn in the mycelium.

It is clear also that, the four tested species had significant effects in enhancing plant growth (Table 2). Shoot and root dry weights increased by 77% and 125% in *G.spp(mixed)* treatment, 67% and 104 % in *G.intradices* , 71% and 79% in *G.fasciculatum* and 30% and 66 % in *G.macrocarpum* treatments relative to the control plants, respectively (average values over the different Zn application rates)

The wheat plants showed high mycorrhizal dependency (MD) with AM fungi, and *G. ssp* (mixed) increased not only the shoot and root biomass but also the colonization of wheat plants; thus it could be a promising AM inoculation for phytoremediation of Zn-contaminated soils.

The MD of wheat inoculated with *G. spp (mixed)* , *G. intraradices* ,*G. fasciculatum* and *G.macrocarpum* were 47 % , 44 %, 42 % and 28 %, respectively (average values over the different Zn application rates).

Zinc concentration

Table (4) showed that zinc concentrations in wheat shoots and roots significantly increased with mycorrhizae inoculation species. However Zn concentration was higher in the roots than in the shoots. This strategy may be very important for mycorrhizal plants surviving on metal contaminated soils. Mycorrizal plants have various detoxification mechanisms including the retention of toxic metals in roots and the subsequent reduction of translocation to shoots Tullio *et al.*, (2003); Christie *et al.*, (2004); Janouskova *et al.*(,2005). As well as various detoxification pathways, such as chelation/immobilization of metals by extraradical mycelium, or compounds secreted by the fungus (glomalin) in the soil, precipitation in polyphosphate granules in the soil, adsorption to fungal cell walls, and chelation of metals inside the fungus (Gaur and Adholeya, 2004).

Table (4) also showed that Zn concentration in shoots and roots of wheat significantly increased with increasing Zn concentration in soil except at the high levels of added zinc. The high concentrations of zinc in soil affected mycorrhizae

Table 2: The effect of Zn rates and inoculation with different AMF species on mycorrhizal infection and wheat growth

AMF. type	Zn rate mM/kg soil	AMF infection%	Wheat plant growth (g/plant).		
			Roots Dry weigth	Shoots Dry weigth	Whole plant
Non-AMF	0	2.20	2.17	5.11	7.28
	2	1.10	1.80	4.88	6.68
	4	0	0.90	3.16	4.06
	6	0	0.62	2.38	3.00
	8	0	0	0	0
	10	0	0	0	0
Mean		**0.55**	**0.92**	**2.59**	**3.50**
G.spp (mixed)	0	51.06	3.01	5.86	8.87
	2	52.20	3.10	5.33	8.43
	4	35.50	2.30	5.60	7.90
	6	36.60	1.77	4.58	6.35
	8	26.63	1.21	3.20	4.41
	10	13.30	0.98	2.88	3.86
Mean		**35.88**	**2.06**	**4.58**	**6.64**
G.intraradices	0	53.30	2.29	5.96	8.25
	2	42.80	2.91	5.10	8.01
	4	27.73	1.89	5.08	6.97
	6	19.96	1.91	3.80	5.71
	8	24.40	1.27	3.50	4.77
	10	8.86	0.97	2.50	3.47
Mean		**29.51**	**1.87**	**4.32**	**6.20**
G.fasciculatum	0	22.16	2.64	5.87	8.51
	2	14.43	2.60	5.10	7.70
	4	16.63	2.11	5.30	7.41
	6	9.96	1.25	4.85	6.10
	8	11.10	0.78	3.54	4.32
	10	3.30	0.48	1.95	2.43
Mean		**12.93**	**1.64**	**4.44**	**6.08**
G.macrocarpum	0	18.86	2.90	5.22	8.12
	2	22.06	2.50	5.29	7.79
	4	17.73	2.60	5.05	7.65
	6	18.86	0.82	2.68	3.50
	8	6.63	0.30	1.78	2.08
	10	0	0	0	0
Mean		**14.02**	**1.52**	**3.37**	**4.86**
L.S. D $_{0.05}$		0.90	0.18	0.27	0.29
Mean effect of Zn rate	0	**29.51**	**2.60**	**5.60**	8.21
	2	**26.51**	**2.58**	**5.14**	7.72
	4	**19.52**	**1.96**	**4.84**	6.80
	6	**17.08**	**1.27**	**3.66**	4.93
	8	**13.75**	**0.71**	**2.40**	3.12
	10	**5.10**	**0.49**	**1.47**	1.95
L.S. D $_{0.05}$		0.99	0.19	0.29	0.31
Interaction (AMxZn rate)		*	*	*	*
L.S. D $_{0.05}$		2.21	0.43	0.65	0.70

* Significant at 0.05 probability level.

Table 3: Regression analysis of whole plant growth of wheat inoculated with the tested *Glomus* species, versus Zn addition to soil

Factor	Regression equation Y =Whole Plant X = Zn rate levels	Correlation coefficient (r)*
Non-AM	$\hat{Y}$= - 0.8214x+7.6105	r = - 0.977
G.spp (mixed)	$\hat{Y}$= - 0.5523x+9.3981	r = - 0.973
G.intraradices	$\hat{Y}$= - 0.4983x+8.6881	r = - 0.988
G.fasciculatum	$\hat{Y}$= - 0.5979x+9.0676	r = - 0.968
G.macrocarpum	$\hat{Y}$= - 0.884x+9.2767	r = - 0.953

* All correlations are significant at P <0.05 level

development and decreased degree of infection Table (2) with wheat roots . These result are agree with those reported by (Gildon and Tinker 1983) who found that the degree of infection of onions with the vesicular-arbuscular mycorrhizal fungus *G. mosseae* was strongly reduced by additions of zinc to the soil and could be completely eliminated by heavy rates of application.

Also, Marques *et al.* (2006) found that high levels of Zn decreased the AM colonization. The results in Tables (1 and 2) showed that *G. fasciculatum* and *G.macrocarpum* are very sensitive and reduced AM colonization by high Zn levels and decreased Zn concentrations in the roots as compared with the other species at the higher levels of Zn. The correlation coefficients for each AM inoculation for each treatment and Zn concentrations of shoots and roots are shown in Table (5). Galli *et al.* (1994) suggested that mycorrhizal fungi could play a crucial role in protecting plant root from heavy metals. The efficiency of protection, however, differed between fungi species, plant species, and contamination levels.

The higher R/S Zn- ratio Table (4) meaning the ability of root tissue to accumulate Zn and consequently low Zn translocation from root to shoot. The results showed that Zn R/S ratio was varied in the inoculated and in the non-inoculated plants at low rates of Zinc but R/S take opposite trend and become zero in non-inoculated plants at higher rate of 8 and 10 mM/kg soil of Zn . Root to shoot ratio of Zn concentration Table (4) indicating that concentration of root-Zn for *G. spp* (mixed) were between 2-9 folds higher than those in shoots at all Zn application rates to soil. Again *G.intraradices* reached concentration of Zn in roots approximately between 1-7 folds higher than those in shoots, while reached between 1-12 folds when wheat plant inoculated with *G. fasciculatum* and *G.macrocarpum* reached between 2-11 folds higher than in shoots. These data elucidate the ability of root tissues to accumulate heavy metals at higher application of zinc. These results agree with those of Khan *et al.* (2000) who found that little translocation of heavy metals absorbed by mycorrhizal maize seedling grown in contaminated soil, to the shoot.

Zinc uptake

The Zn uptake Table (6) reflects the effects of AMF fungal inocula on both plant biomass and Zn concentrations in soil and calculated according to the data in Tables (2 and 4) The data in Table (6) also revealed that Zn uptake in wheat shoots and roots significantly increased with more Zn added for both non-mycorrhizal and mycorrhizal treatments. Shoot and root uptake in mycorrhizal plants were significantly higher compared to non-mycorrhizal plants when Zn added with different levels. With non-mycorrhizal plants Zn uptake increased with the application of 2, 4 and 6 mM/kg soil only and became zero at 8 and 10 mM/kg as aresult of Zn toxicity, Consequently plant growth inhibition (dying the plants). The inoculation with the AMF species increased generally the uptake with increasing Zn application up to 6 mM/kg soil for roots and 4 mM/kg soil for shoots and decreased at the other Zn levels.

The increase / or decrease of Zn uptake into shoots and roots of mycorrhizal plants relative to the uninoculated plants were calculated according to the data in Table (6). Shoot Zn uptake by *G.spp (mixed), G.intraradices , G.fasciculatum* and *G.macrocarpum*- treated plants was increased by 387%, 355%, 133%, and 67%, respectively. Similarly, In comparison with the control plants, root Zn uptake increased by 566%,478%,277% and 256% respectively (average values over the different Zn application rates).

Zinc uptake efficiency and phytoextraction efficiency were increased with increasing the mounts of added Zn to the soils but showed the opposite trend at the higher level of Zn in soil Figure (2)

Compared with the non-mycorrhizal plants Zn uptake efficiency and phytoextraction efficiency of mycorrizal plants were higher with all Zn addition levels Figure (2). Also zinc translocation efficiency was lower in mycorrhizal plants than in non-mycorrhizal ones at all Zn addition levels Figure (2)

Table 4: Zinc content of wheat plants as affected by arbuscular mycorrhizal fungus species and Zn application rate

AMF type	Zn rate mM/kg soil	Zinc content mg/kg		Roots/Shoots (R/S)
		Roots	Shoots	
Non-AMF	0	0.25	0.19	1.31
	2	50.60	8.94	5.66
	4	83.80	10.08	8.31
	6	91.80	14.46	6.34
	8	0	0	0
	10	0	0	0
Mean		**37.74**	**5.61**	
G.spp (mixed)	0	1.55	0.72	2.15
	2	118.20	16.41	7.20
	4	157.00	18.20	8.62
	6	205.70	37.21	5.52
	8	220.40	31.06	7.10
	10	130.80	26.42	4.95
Mean		**138.94**	**21.67**	
G.intraradices	0	0.27	0.23	1.17
	2	84.00	19.00	4.42
	4	134.30	21.40	6.28
	6	206.60	30.91	6.68
	8	202.00	30.30	6.66
	10	148.00	20.82	7.10
Mean		**129.20**	**20.44**	
G.fasciculatum	0	0.52	0.46	1.13
	2	95.46	10.71	8.91
	4	117.40	12.31	9.54
	6	184.25	15.00	12.28
	8	94.15	12.52	7.52
	10	89.50	9.66	9.27
Mean		**96.88**	**10.11**	
G.macrocarpum	0	0.65	0.32	2.03
	2	110.30	11.17	9.87
	4	146.40	13.11	11.17
	6	134.70	15.50	8.70
	8	93.80	9.20	10.20
	10	0	0	0
Mean		**80.98**	**8.22**	
L.S. D $_{0.05}$		4.35	1.54	
Mean effect of Zn rate	0	**0.65**	**0.38**	1.71
	2	**91.71**	**13.25**	6.92
	4	**127.78**	**15.02**	8.51
	6	**164.61**	**22.62**	7.28
	8	**122.07**	**16.62**	7.34
	10	**73.66**	**11.38**	6.47
L.S. D $_{0.05}$		4.76 *	1.68 *	
Interaction (AM x Zn rate)L.S. D $_{0.05}$		10.65	3.76	

* Significant at 0.05 probability level.

Table 5: Regression analysis of Zn shoots and roots concentrations of wheat plants inoculated with the tested arbuscular mycorrhizal fungus species, versus Zn addition to soi

Factor	Regression equation Y=Plant Zn concentrations X= Zn rate levels	Correlation coefficient (r)*
Zn Shoot vs. .Zn addition		
Non-AMF	$\hat{Y}$= -0.3341x+7.2824	r= - 0.197
G.spp (mixed)	$\hat{Y}$=2.7351x+7.9943	r= 0.794
G.intraradices	$\hat{Y}$=2.0909x+9.989	r= 0.703
G.fasciculatum	$\hat{Y}$=0.7731x+6.2443	r= 0.571
G.macrocarpum	$\hat{Y}$=-0.0731x+8.5824	r= 0.041
Zn Root vs. .Zn addition		
Non-AM	$\hat{Y}$= -2.0721x+48.102	r= - 0.178
G.spp (mixed)	$\hat{Y}$= 14.308x+67.402	r= 0.682
G.intraradices	$\hat{Y}$= 16.642x+45.984	r= 0.799
G.fasciculatum	$\hat{Y}$= 7.2546x+60.607	r= 0.460
G.macrocarpum	$\hat{Y}$= -0.9207x+85.579	r= - 0.053

* All correlations are significant at $P < 0.05$ level.

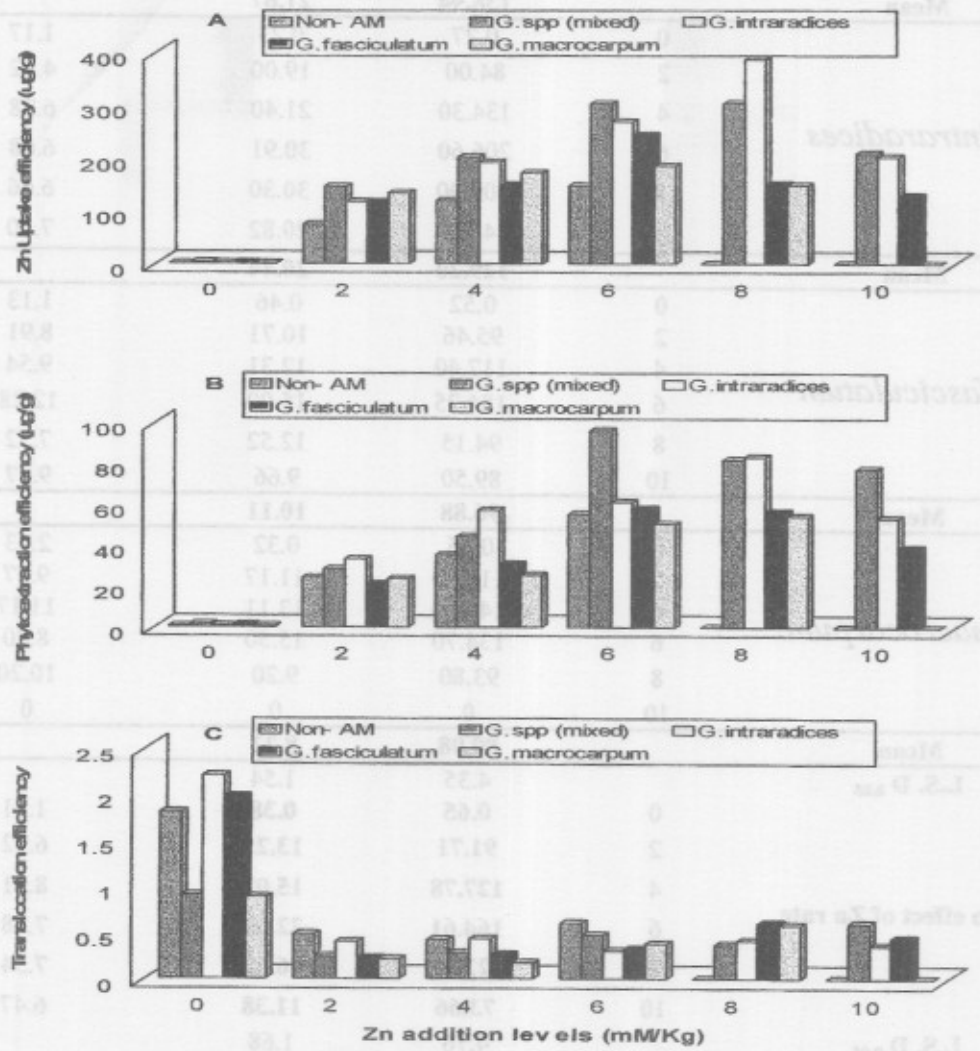

Fig. 2: Zn uptake efficiency (A), phytoextraction efficiency (B) and translocation efficiency (C) of wheat plants under inoculation with four mycorrhizal fungus

Table 6: Means of zinc uptake as affected by arbuscular mycorrhizal fungus species and Zn application rate

AMF. type	Zn rate mM/kg soil	Zinc uptake µg /plant		
		Roots	Shoots	Total Zn uptake (µg /plant)
Non-AMF	0	0.54	0.97	1.51
	2	91.08	43.63	134.71
	4	75.42	.31.85	107.27
	6	56.92	34.41	91.33
	8	0	0	0
	10	0	0	0
Mean		**37.33**	**18.48**	**55.80**
G.spp (mixed)	0	4.67	4.22	8.89
	2	366.42	87.46	453.88
	4	361.10	102.00	463.10
	6	364.00	170.42	534.42
	8	266.68	99.39	366.07
	10	128.18	76.1	204.28
Mean		**248.51**	**89.93**	**338.44**
G.intraradices	0	0.62	1.37	1.99
	2	244.44	96.90	341.34
	4	253.82	108.71	362.53
	6	394.61	117.46	512.07
	8	256.54	106.05	362.59
	10	143.56	52.05	195.61
Mean		**215.60**	**80.42**	**296.02**
G.fasciculatum	0	1.37	2.70	4.07
	2	248.2	54.62	302.82
	4	247.7	65.24	312.94
	6	230.31	72.75	303.06
	8	73.44	44.3	117.74
	10	42.96	18.84	61.80
Mean		**140.66**	**43.07**	**183.74**
G.macrocarpum	0	1.88	1.67	3.55
	2	275.70	59.00	334.70
	4	380.64	66.20	446.84
	6	110.45	41.54	151.99
	8	28.14	16.37	44.51
	10	0	0	0
Mean		**132.80**	**30.80**	**163.6**
L.S. D $_{0.05}$		15.47	4.36	3.24
Mean effect of Zn rate	0	**1.82**	**2.19**	**4.00**
	2	**245.16**	**68.32**	**313.49**
	4	**263.74**	**74.8**	**338.54**
	6	**231.26**	**87.32**	**318.57**
	8	**125.00**	**53.22**	**178.18**
	10	**63.00**	**29.40**	**92.34**
L.S. D $_{0.05}$		16.95	4.78	3.55
Interaction (AM x Zn rate)		*	*	*
L.S. D $_{0.05}$		37.91	10.69	7.95

* Significant at 0.05 probability level

The results showed that AMF colonized roots have a reduced ability to take up Zn from soil and to translocate it to shoots compared to the uncolonized roots exhibited Zn contamination.

It has been pointed out that in soil with different Zn contamination levels there may be a critical Zn concentration below which Zn uptake is enhanced by AM fungi and above which there is inhibition of Zn translocation to shoots Chen *et al.*, (2003). It is clear that, there may be a critical Zn contamination level which AM fungi improve plant Zn nutrition and above which AM fungi depress plant Zn uptake from soil. Obviously, this critical value may vary with a variety of factors such as the AM species and the contamination levels. In the present study, the four AM fungi species had different effects on Zn uptake by wheat plants. The Zn partitioning in the wheat cultivars may occur because more Zn was immobilized in the roots by all of mycorrhizal species, especially by *G. spp* (mixed) and *G.intraradices*, thus relatively less of the Zn could be translocated to the shoot.

In conclusion, the protective effect of AM against uptake of potentially high level of Zn by wheat grown in Zn contaminated soil showed that *Glomus spp* (mixed) represented the highest tolerance to this contamination among the tested four AM fungi isolates. This AM fungi has an applicative prospect in contaminated soil. However, soil is a complex substrate and there are many other factors influencing the growth of wheat, so this work cannot provide a counter measure to solve the problem of contaminated soil. However, evidence for the potential role of AM fungi in facilitating wheat growth in contaminated soil with Zn has been provided by the present work.

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الملخص العربى

دور فطر الميكورهيزا فى نمو وإمتصاص الزنك بنبات القمح النامي فى أرض جيرية ملوثة بالزنك

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يهدف هذا البحث تقييم ودراسة تاثير التلقيح بانواع مختلفة من فطريات الميكورهيزا على تقليل الاثر الضار لعنصر الزنك عند زيادة تركيزه فى التربة على نمو النبات ، وتراكم هذا العنصر فى انسجتها.

ولتحقيق ذلك أجريت تجربة اصص باستخدام ارض جيرية لوثت بـ ٦ مستويات من الزنك وهى صفر ، ٢،٤،٦،٨،١٠ ملليمول زنك /كجم تربة فى صورة كبريتات الزنك مع استخدام نبات القمح صنف (جيزة ١٦٨) واربعة لقاحات من فطر الميكورهيزا وهى *Glomus spp* (mixed) ، *Glomus intraradiaces* ، *Glomus macrocarpium* و *Glomus fasciculatum*

وقد تم توزيع المعاملات فى تصميم عشوائى كامل بثلاث مكررات.

وقد تم اخذ العينات النباتية بعد ٦٠ يوم من الزراعة وتحليلها لمعرفة محتواها من الزنك.

وتوضح نتائج الدراسة بان تقل معدلات الاصابة بفطريات الميكورهيزا فى جذورنبات القمح بزيادة معدلات اضافة الزنك، وقد وصلت الى اعلى قيمة لها ٥١-٥٣% لفطرى *Glomus spp* و *Glomus intraradiaces* فى حالة عدم اضافة الزنك كذلك ادى التلقيح بفطر الميكورهيزا الى زيادة معنوية فى كل من الوزن الجاف للمجموع الجذرى والخضرى لنبات القمح وبقيم اكبر مماحدث للنباتات غير الملقحة وفى نفس الوقت لوحظ ان الزيادة فى معدلات اضافة الزنك اعلى من ٦ مليمول/ كجم تربة ادت الى انخفاض دور الميكورهيزا كما زاد معدل امتصاص العنصر تحت الدراسة بواسطة النبات نتيجة المعاملة بالميكورهيزا وقد ادى التلقيح بهذه الفطريات الى زيادة محتوى النبات بل وحدث تراكم معنوى للزنك فى المجموع الجذرى والخضرى اذا ماقورنت بالنباتات الغير ملقحة بعد ٦٠ يوم من الزراعة.

كذلك توضح نتائج الدراسة ان تركيز العنصر فى المجموع الجذرى اكبر من المجموع الخضرى فى النبات تحت الدراسة ، وعموما فان اعلى تركيز للزنك فى المحصول لوحظ عند معدل الاضافة ٦ مليمول/كجم تربة فى النباتات الملقحة بخليط *Glomus spp* و*Glomus intraradiaces* وفى نفس الوقت ياخذ عكس الاتجاه السلبق مع معدلات الاضافة الاعلى من ٦ مليمول زنك/كجم.

مما سبق تشير النتائج المتحصل عليها الى اهمية استخدام فطر الميكورهيزا فى الاراضى الملوثة بالزنك ويمكن اعتبارها طريقة تطبيقية واعدة فى زراعة الاراضى الملوثة بالعناصر الثقيلة والحد من انتشار الامراض للانسان و الحيوان والمحافظة على نظافة البيئة.