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# The Role of Arbuscular Mycorrhizae *Glomus* Spp (mixed) and *Glomus fasciculatum* in Growth and Copper Uptake of Maize Grown in Soil 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 spp*(mixed) and *Glomus fasciculatum* 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 content were determined. The 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 and *Glomus spp* (mixed) was more effective than the other. Mycorrhizal plants accumulated more copper in roots but large reductions in shoots. The uses of AM fungal for phytoremediation of the contaminated soil lead to more absorption of Cu in plant. The comparisons of the twoAM fungal species indicate that the AM fungal represented by *Glomus spp* (mixed) can benefit against potentially toxic Cu and therefore play a role in bioremediation of Cu-contaminated soils. Such results underline the importance of indigenous AM fungi, which are presumably more adapted to heavy metals.

**Key word:** *Glomus spp* · Bioremediation · Calcareous soil · Copper · *Zea mays* L · Mycorrhizal dependency

## INTRODUCTION

The use of rhizosphere microbes such as arbuscular mycorrhizal (AM) fungi in bioremediation of heavy metal contaminated soils has attracted more attention recently [1]. AM fungi provide direct links between soil and roots and consequently may have an essential contribution to plant growth by improving mineral nutrition and enhancing plant tolerance to stress [1,2]. Furthermore, AM fungi also affect metal uptake by plants from soil and translocation from root to shoot, however, mycorrhizal effects may depend on elements, plant and fungal species/ecotypes [3].

Copper (Cu) plays an important role in photosynthetic and respiratory electron transfer, the activity of various oxidative enzymes and pollen formation [4]. When Cu levels are elevated in soils, however, plants become chlorotic and exhibit reduced carbon fixation, altered nutrient acquisition and reduced growth [5]. Although Cu is toxic to plant and fungal symbionts at high concentrations [6], AM fungi have been reported to occur widely in Cu mine spoils and Cu contaminated soils [7]. This indicates that there may be some AM fungal species tolerant to Cu and suggests their potential use in bioremediation. The remediation of such soils is important because these usually cover large areas that are rendered unsuitable for agricultural and other human use.

The aims of this work were (1) to test the ability of four 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*. [8].

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.

**Mycorrhizal Inocula:** Two arbuscular mycorrhizal (AM) fungi species belonging to the genus *Glomus* were used in this study. These species were *Glomus fasciculatum* and *G. spp* (mixed). The first one was obtained from Hanover University (Germany) and the last one consisted of a mixture several AM fungal species and isolated from soil contaminated with sewage water (supplied from Ain Shams University Egypt),

**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.*) - 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 *Glomus fasciculatum* and *G. spp* (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 [9].

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 [10] 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 [11]. The mycorrhizal infection percentages was estimated after staining [12] using the gridlines intersect method of Giovannetti and Mosse [13].

The mycorrhizal dependency (MD) of plant growth was calculated according to the following formula [14].

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

Three aspects of plant Cu efficiency were assesd. According to Harper *et al*. [15], 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.

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

$$\text{Tanslocation efficiency= shootCu uptake/ root Cu uptake} \quad (2)$$

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:

$$\text{Phytoextraction efficiency } (\mu g\ g^{-1}) = \text{shoot uptake/ root dry weight} \quad (3)$$

The obtained Data were statistically analyed according to [16].

## RESULTS AND DISCUSSION

The amount of the DTPA-Cu steadily and linearly increased with increasing the level of added Cu to the soil before cropping is shown in Fig. 1. The values ranged from 0.12 mg Cu $kg^{-1}$ soil without Cu application to 2.81 mg Cu $kg^{-1}$ soil with the application rate of

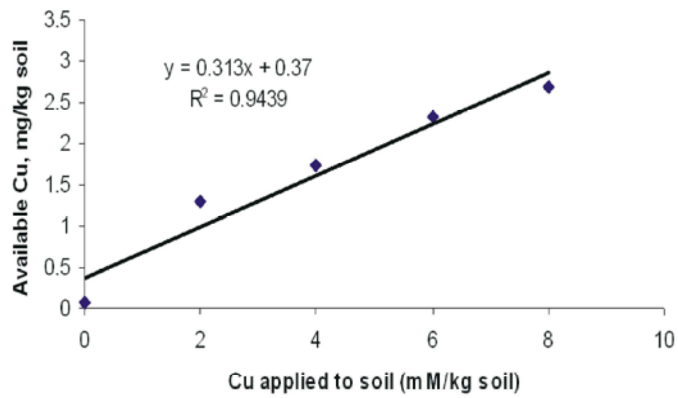


Fig. 1: Available Cu in soil before cropping of maize.

Table 1: Available Cu in soil after cropping of maize as affected by *Glomus spp*(mixed) and *Glomus fasciculatum* inoculation

| Cu rate mM $kg^{-1}$ soil | without inoculation | *Glomus spp* | *G.fasciculatum* | Mean of Cu rate |
|---|---|---|---|---|
| 0 | 0.85 | 1.21 | 1.06 | 0.69 e |
| 2 | 2.23 | 2.79 | 2.50 | 2.51 d |
| 4 | 2.59 | 3.22 | 2.91 | 2.91 c |
| 6 | 3.47 | 3.91 | 3.56 | 3.65b |
| 8 | 3.68 | 4.49 | 4.07 | 4.08a |
| Mean of AM fungi inoculation | 2.56 c | 3.12 a | 2.82 b | |
| AM × Cu | | | | |
| L.S.$D_{0.05}$ | | 0.265 | | |

8 mM Cu $kg^{-1}$ soil. 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 AM fungi inoculation and after cropping with the inoculation with the tested AM fungi species 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. It is clear also, that the AM fungi species varied in their effects on the DTPA-Cu and the species *G. spp* (mixed) was more effective mostly than *G. fasciculatum* in increasing the DTPA-Cu. In the same time, there are significant difference between the mean values of DTPA-Cu over all Cu application rates for the soils after cropping with *G. fasciculatum* and *G. spp* (mixed).

Our result in Table 2, showed that mycorrhizae infection percentages increased with the mycorrhizae inoculation with an order of *Glomus spp(mixed)* > *G. fasciculatum* > non AM fungi. The highest colonization rate (60% of root length) occurred in the absence of Cu addition by G. spp (mixed). Root colonization rates significantly differ between three treatments that received highest rates of Cu.The high levels of Cu in soil inhibit mycorrhizal colonization particularly *G. fasciculatum* which completely eliminate AM colonization. These results were in agreement with those obtained by Lius *et al*. [6] who found that high levels of Cu inhibit spore germination and development and mycorrhizal colonization. However, both pot experiments and field investigations have found that AM fungi colonized plant roots extensively even in Cu-contaminated soils or soils contaminated with Cu and other metals [7,17]. AM fungal ecotypes from different habitats may exhibit different degrees of metal tolerance and those from heavy metal contaminated soils are usually more tolerant. Several AM fungi tolerant to Cu have been reported [17,18]. Our results showed that *G. spp* (mixed) was tolerant to Cu and easily colonized maize roots even when 8Mm $kg^{-1}$ Cu was added. This AM fungal *G. spp* (mixed) was isolated from a 50-year-old soil irrigated with sewage water, where it may have evolved a strong tolerance to heavy metals.

Also 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). 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).

Table 2: The effect of Cu rates on *Glomus spp*(mixed) and *Glomus fasciculatum* infection and maize plant growth.

| AM fungi type | Cu rate mM $kg^{-1}$ soil | AM fungi infection, % | Plant ( g $plant^{-1}$) 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.spp* (mixed) | 0 | 60.10 | 3.65 | 7.03 |
| | 2 | 43.40 | 3.73 | 6.42 |
| | 4 | 33.57 | 2.32 | 5.41 |
| | 6 | 21.55 | 1.82 | 4.62 |
| | 8 | 19.00 | 1.16 | 2.63 |
| Mean | | 35.52 a | 2.54 a | 5.22a |
| *G.fasciculatum* | 0 | 55.40 | 2.51 | 5.78 |
| | 2 | 34.37 | 2.06 | 5.01 |
| | 4 | 23.20 | 1.43 | 4.63 |
| | 6 | 4.47 | 1.03 | 3.72 |
| | 8 | 0.00 | 0.00 | 0.00 |
| Mean | | 23.49 b | 1.41 b | 3.66 b |
| Mean effect of Cu rate | 0 | 42.10 a | 3.20 a | 6.34 a |
| | 2 | 25.92 b | 2.55b | 5.00 b |
| | 4 | 18.92 c | 1.57c | 4.02c |
| | 6 | 8.67 d | 0.95 d | 2.78 d |
| | 8 | 6.33 e | 0. 39 e | 0.88 e |
| AM x Cu rate | | | | |
| L.S. $D_{0.05}$ | | 3.21 | 0.52 | 0.93 |

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

The results indicated also that, the fungus *G.spp* (mixed) and *G.fasciculatum* were more effective than non-mycorrhizal treatment 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 [19].

Average shoot and root dry weights of AM-treated plants increased by 121% and 103 % in *G.spp* (mixed), 55% and 13 % in *G.fasciculatum* treatments compared to the non-inoculated plants. The mycorrhizal dependency (MD) of maize plants inoculated with *G. spp* (mixed) and *G. fasciculatum* were 51% and 11%, for roots and 55% and 36% for shoots respectively. These results showed high MD on AM fungi and *G.spp* could be a promising AM inocula for successful bioremediation. MD was calculated according to the data in Table 2. In general, on soils without background contamination, AM inoculation can improve plant Cu nutrition [20, 21]. Under heavy metal contamination, AM fungal inoculation decreases heavy metal concentrations in plants especially in shoots, thus exerting a protective effect on the host plants against heavy metal toxicity and leading to higher plant yields [22, 23]. On the other hand, it has also been observed that AM inoculation either increased heavy metal content in plants, leading to an inhibitory effect on plant biomass [24, 25], or sometimes, enhanced both metal concentrations and plant biomass [26, 27]. Our results showed that AM inoculation with *G. fasciculatum* significantly improved plant Cu nutrition with no Cu added, while inoculation decreased plant Cu concentrations when Cu was added particularly at higher Cu addition levels.

The results showed that, *G.spp* (mixed) increased the Cu concentration in roots and shoots than *G. fasciculatum*. 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 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 [28, 29].

Table 3: Means of Cu content as affected by *Glomus spp*(mixed),*Glomus fasciculatum* 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 | 10.32 c |
| *G.spp* (mixed) | 0 | 2.45 | 2.60 |
| | 2 | 224.96 | 75.11 |
| | 4 | 209.08 | 95.32 |
| | 6 | 200.95 | 107.14 |
| | 8 | 163.37 | 78.66 |
| Mean | | 160.20a | 71.80a |
| *G.fasciculatum* | 0 | 0.73 | 1.79 |
| | 2 | 79.33 | 44.04 |
| | 4 | 85.69 | 81.49 |
| | 6 | 91.38 | 75.55 |
| | 8 | 0 | 0 |
| Mean | | 51.43b | 40.60b |
| Mean effect of Cu rate | 0 | 1.45e | 1.73e |
| | 2 | 124.00b | 49.46c |
| | 4 | 110.05a | 66.12a |
| | 6 | 97.44c | 60.80b |
| | 8 | 54.46d | 26.22 d |
| AM x Cu rate | | | |
| L.S.D 0.05 | | 19.17 | 4.78 |

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.spp* (mixed) | *G.fasciculatum* |
|---|---|---|---|
| Cu rate efficiency $\mu g g^{-1}$ DW | | | |
| 0 | 0.58 | 1.39 | 1.00 |
| 2 | 51.83 | 80.45 | 60.00 |
| 4 | 61.43 | 131.21 | 117.00 |
| 6 | 0 | 169.28 | 162.00 |
| 8 | 0 | 208.65 | 0.00 |
| Phytoextraction efficiency $\mu g g^{-1}$ DW | | | |
| 0 | 0.24 | 0.71 | 0.71 |
| 2 | 15.63 | 20.14 | 21.40 |
| 4 | 22.70 | 41.09 | 60.00 |
| 6 | 0.00 | 58.90 | 73.34 |
| 8 | 0.00 | 67.81 | 0.00 |
| Translocation efficiency | | | |
| 0 | 0.69 | 1.06 | 2.45 |
| 2 | 0.43 | 0.33 | 0.56 |
| 4 | 0.59 | 0.46 | 0.95 |
| 6 | 0.00 | 0.53 | 0.83 |
| 8 | 0.00 | 0.48 | 0.00 |

The Cu content reflecting the effects of AMF fungal inocula on both plant biomass and Cu concentrations in soil, 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 and there are significant difference between the values of *G.spp* (mixed) and *G.fasciculatum*. 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 8 mM Cu $kg^{-1}$ soil particularly With *G.fasciculatum* because of the plant growth inhibition (dying the plants).

The inoculation with the 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 8mM Cu $kg^{-1}$ soil depressed severely the mycorrhizal colonization except for *G. spp*. (Table 2). The obtained results indicate that two 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, Many studies have confirmed that improved mineral nutrition, especially P, is another way in which AM fungi can increase plant growth and metal tolerance [17,30]. Our results showed that AM inoculation significantly improved shoot and root P nutrition at all five Cu addition levels but the effect was small at 6 and 8 mM Cu $kg^{-1}$ soil added (unpublished data), in accordance with the results observed in plant biomass. With 6 and 8 mM Cu $kg^{-1}$ soil added, mycorrhizal colonization was so severely depressed (Table 2),

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.fasciculatum* 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.fasciculatum* 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 [9]. 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. spp*(mixed) showed the highest tolerance to this contamination compare with *G.fasciculatum* treatment and has an applicative prospect in contaminated soil with Cu. A field experiment to confirm its application effects is also needed in naturally contaminated soil.

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