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Influence of Arbuscular Mycorrhizal Fungus (*Glomus etunicatum*) with Lettuce Plants under Zinc Toxicity in Nutrient Solution

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Abstract: The effects of zinc toxicity on growth, chlorophyll, total sugar and protein content and mineral content of lettuce plants infected or not by Arbuscular Mycorrhizal (Am) fungus *Glomus etunicatum* and treated with nutrient solution containing 0, 1.5, 3.5, 5.5, 7.5 mM $ZnSO_4$ were studied. The introduction of Zn caused a decrease in the inhibiting effect of zinc on dry weight of roots and shoots of lettuce plant infected by Am in contrary with non-Am plants. This increase observed in dry weight may be due to improvement of Phosphorous uptake by mycorrhizal fungi. The decrease in dry weight of non-Am plants may be because of inhibitory effects of zinc on growth. Chlorophyll and total sugar content decreased in both Am and non-Am plants, which indicate the toxic effect of Zn on photosynthesis and carbohydrate metabolism. Mycorrhizal plants due to changing the translocation of Zn and sequestering in the hypha could elevate the effects of Zn to some extent. Total protein content increased in Am plants, probably due to induction of antioxidant enzymes and some stress proteins but reduced in non-Am plants which maybe caused by toxic effects of Zn on protein synthesis. Alleviating the severe effects of Am fungus observed in this study aroused an interest in considering the role of Am fungi in protection and elevation the sever effects of heavy metals in plants.

Key words: Arbuscular mycorhiza, *Glomus etunicatum*, lettuce, zinc, chlorophyll, protein, soluble sugar

INTRODUCTION

The occurrence of heavy metals in soil may be beneficial or toxic to environments. Excess of metals may produce some common effects of individual metals on different plants and thus cause toxicity. Zn is an essential element which acts as a plant nutrient and is a constituent of metalloenzyme or a cofactor for several enzymes such as anhydrases, dehydrogenase, oxidase and peroxidase (Hewitt, 1981) and plays an important role in regulating the nitrogen metabolism, cell multiplication and photosynthesis in plants (Shier, 1994). It also plays and important role in the synthesis of nucleic acid and proteins. But at higher concentration, it is toxic.

High concentrations of available zinc in soils usually arise from various sources of pollution, including: atmospheric deposition from nearby industrial source such as a smelting works, flooding of alluvial soils with zinc-polluted river water and sediments, excessive application of zinc-rich materials, including pig and poultry manure from animals fed additional zinc and high zinc sewage sludge or industrial waste waters.

The general toxicity symptoms of zinc are stunting of shoot, curling and rolling of young leaves, death of leaf tips and chlorosis (Rout and Das, 2003).

Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus tolerance to heavy metal stress. These all appear to be involved primarily in avoiding the build-up of toxic concentrations at sensitive sites within the cell and thus preventing the damaging effects, rather than developing proteins that can resist the heavy metal effects. The strategies for avoiding heavy metal build-up are diverse. Extracellularly they include roles for mycorrhizas and for cell wall and extracellular exudates (Hall, 2001).

Providing a direct physical link between soil and plant roots, the arbuscular mycorrhiza (Am) fungi are important rhizospheric microorganisms. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003) and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that Am fungi can colonize plant roots in metal contaminated soil (Vogel-Mikus *et al*., 2005), while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that Am fungi increased shoot uptake of metals (Weissenhorn *et al*., 1995), while in severely contaminated soil, Am fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Li and Christie, 2001; Malcova *et al*., 2003).

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A number of different mechanisms may be involved in the interactions between mycorrhizal colonization and accumulation of heavy metals including tissue dilution of the toxic element due to interactions with P nutrition, sequestration of toxic metal in the fungus and development of tolerance of the fungus (Bevege *et al.*, 1975; Smith and Read, 1997) and production of chelating agents.

Therefore one of the objectives of this work was to study the effects of Am fungi on some growth parameter of lettuce plant under zinc toxicity.

MATERIALS AND METHODS

Surface sterilized seeds of lettuce (*Lactuca sativa* L.) were sown in the sterilized soil/sand mixture. The mycorrhizal inoculum used was stock culture *Glomus etunicatum* which was bulked in an open pot culture of *Zea mays* L. Mycorrhizal treatments were carried out by adding 20 g per pot of mycorrhizal inoculum from our stock culture collection which was placed below lettuce seeds. Non-mycorrhizal treatments received the same quantity of autoclaved inoculum.

Plants (three per pots) were grown for 60 days in a growth chamber in Urmia University with temperatures ranging from 19 to 25°C, a 16/8 h light/dark period and a relative humidity of 70-80%. One week after emergence of seed lings, plants received (30 mL per pot 3 times a week) modified Hoagland's nutrients solution (mg L^{-1}) with half P concentration, by adding 0 (control), 1.5, 3.5, 5.5, 7.5 mM $ZnSO_4$ to the nutrient solution. The plants were planted in February and harvested in March.

At the end of the experiment, plants were harvested and separated into roots and shoots to obtain the fresh and dry weights (dried at 70°C for 48 h). After acid digestion treatments, P plant tissue was determined calorimetric ally using the vanado-molybdate method, Total Zn in both roots and shoots of (Am and non-Am plants) was quantified by atomic absorption spectroscopy (Lott, 1956). The content of total chlorophyll in leaves was determined by the spectrophotometer according to smith and Benitez (Smith and Benitez, 1955). Total sugar content in shoots and roots were determined by anthrone method described by Fales (1951) and total protein was determined by Lowry *et al.* (1951). The percentage of mycorrhizal root length was calculated by a Gridline intersect technique (McGonigle *et al.*, 1990). The roots were washed under the cold water stream. Samples of washed roots were cut into approximately 1cm-segments, cleared with 10% KOH in boiling water bath for 15 min and stained with trypan blue for mycorrhizal colonization estimation.

STATISTICAL ANALYSIS

A statistical comparison of means examined with ANOVA and pearson correlation coefficient available in the SPSS statistical package.

RESULTS

The results presented in Fig. 1.A and B reveal the growth responses of lettuce plants grown under different Zn concentrations in the presence and the absence of Am fungi. However, Am fungi inoculations had a significant influence on root and shoot dry weight of lettuce plants, where the presence of Am fungi caused a decrease in the inhibiting effects of Zn on dry weight of roots and shoots in the studied plants. According to Fig. 1B roots and shoots dry weight of non-Am plants reduced in the presence of high Zn concentration in nutrient solution.

The data given in Fig. 2 show that chlorophyll (chl) content of Am and non-Am plants were generally reduced by increasing Zn concentration in nutrient solution. Am plants, had greater amount of chlorophyll than non-Am lettuce plants.

As shown in Fig. 3 different Zn concentration had significant effect on total protein content in lettuce plants. The protein content significantly increased in the

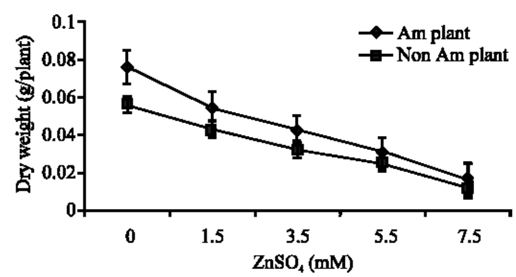

Fig. 1A: Effect of $ZnSO_4$ on root dry weight of mycorrhizal and non-mycorrhizal lettuce plants

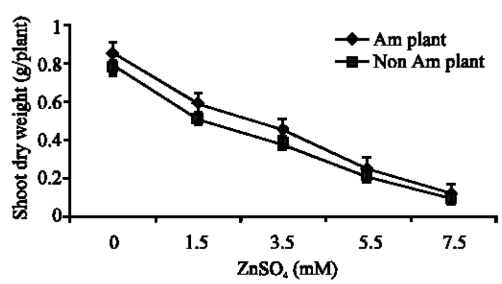

Fig. 1B: Effect of $ZnSO_4$ on shoot dry weight of mycorrhizal and non-mycorrhizal lettuce plants

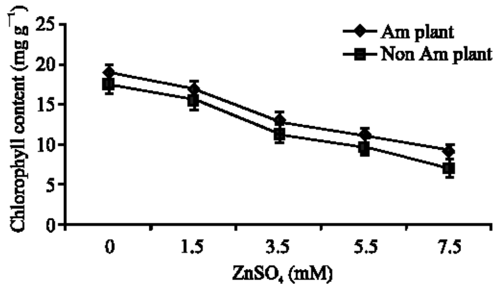

Fig. 2: Chlorophyll content of mycorrhizal and non-mycorrhizal lettuce plants

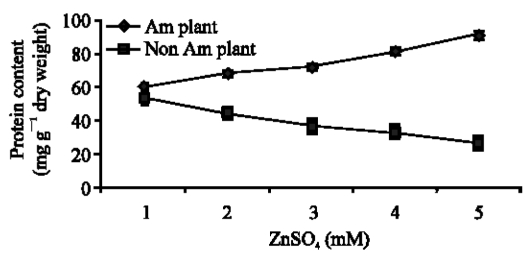

Fig. 3: Protein content of mycorrhizal and non-mycorrhizal lettuce plants

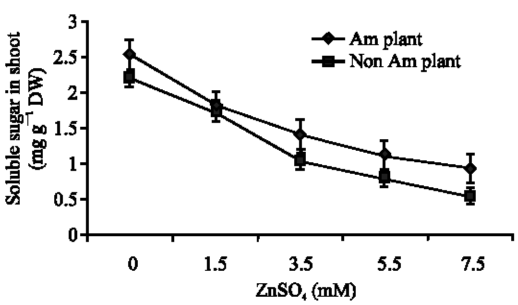

Fig. 4: Soluble sugar content in shoot of mycorrhizal mycorrhizal lettuce

presence of Am fungi and reduced in the absence of fungi in non-Am plants.

The results of Fig. 4 showed that soluble sugar content decreased by increasing Zn concentration in nutrient solution, However, Am plants in comparison with non-Am plants had greater amount of sugar.

The data in the present investigation show that the phosphorus content of non-Am plants was generally decreased by increasing in Zn concentration in nutrient solution. Am inoculation had a significant effect on phosphorus content where the value of this element obtained in the Am plants remained greater than those given by non-Am plants.

According to atomic absorption spectrophotometry roots of Am plants had greater amount of Zn than shoots.

The percentage of root length colonized by Am fungus with grid line intersect method showed a decreased in colonization by increasing in Zn concentration in nutrient solution.

DISCUSSION

The data of the present study showed that dry weight of roots and shoots of lettuce plants would increase in the presence of Am fungi. These results indicated that the known beneficial effects of plant mycorrhization appeared to be mainly due to improvement of P uptake by mycorrhizal fungus. Shetty *et al.* (1994) pointed out that growth inhibition in plants grown under high levels of zinc, was due to interference of zinc with phosphorous uptake by plants. They also reported that the application of Vesicular Abuscular Mycorrhiza (VAM) fungi at contaminated sites increased plants biomass even at elevated of zinc in the soil.

As shown in Fig. 2, chlorophyll content of Am and non-Am lettuce plants decreased in the presence of high Zn concentrations. Zinc induced inhibition of Fe translocation from root to tops which is needed for chlorophyll synthesis and therefore causes chlorosis in plant. Chavan and Banerjee (1980) reported that Zn toxicity appear to be due to Fe deficiency. White (1976) observed that increased levels of Zn in soil greatly increased translocation of Mn to tops which indicates the appearance of chlorosis.

They hypothesized that Zn and Mn interfere with Fe utilization in the leaves for chlorophyll synthesis. In the present study, Am plants possess greater amount of chlorophyll in comparison with non-Am plant. Mycorrhizae fungi, has been shown to alter the pattern of Zn translocation from root to shoot (Shetty *et al.*, 1994) An inhibition of Zn translocation to shoots was also reported in mycorrhizal maize seeding (Kahn *et al.*, 2000).

The data of the present study showed that total protein content increased in the presence of mycorrhizae fungi but it reduced in the absence of fungi. Based on these results, the mechanisms related to physiological interactions between Am fungus and letuce plants involve increased protein synthesis as well as induction of antioxidant enzymes to avoid heavy metal-mediated oxidative stress.

This finding supports results from some studies reporting the role of stress proteins such as phytochelatins and metallothioneines (Rauser, 1999; Clements, 2001) as possible mechanisms for protection against high toxic heavy metals. (Ott *et al.*, 2002;

Burleigh *et al*., 2003; Tong *et al*., 2004). In non-mycorrhizal lettuce plants, reduction in total protein content may be due to the toxic effects of zinc on cellular metabolism and protein synthesis.

According to Fig. 4, reduction in soluble sugar in both Am and non-Am plant may be due to a decrease in chlorophyll content and the abnormal structure of chloroplasts, which leads to low photosynthesis efficiency. Mycorhizal plants alleviate the sever effect of Zn by changing the translocation of zinc and sequestering it in their hypha, so the toxic effects of Zn on photosynthesis and carbohydrate metabolism might decrease.

But reduced amount of phosphorous which observed in non-Am plants may be due to interference of zinc with phosphorous uptake by lettuce plants.

The great amount P in mycorrhizal plants, emphasizes the enhancement of P uptake from the soil and its translocation to plants by the extrardial mycelium of Am fungi (Ryan and Angus, 2003; Rufkyikri *et al*., 2004; Vogel-Mikus *et al*., 2005).

These results clearly indicate the beneficial effect of Am fungi in the protection of plants and alleviation the toxic effect of heavy metals.

Therefore additional researches are needed to explore the behavior of Am fungi in various plant species and families for plant protection under heavy metal stress.

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