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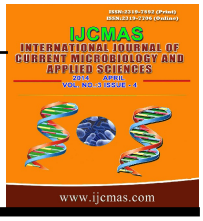

Original Research Article

In vitro Efficacy of Mycorrhizae on Heavy Metal Toxicity in Zea Mays

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A B S T R A C T

This study was conducted to know the efficacy of mycorrihizae on heavy metal toxicity in *Zea Mays*. Heavy metal toxicity is an increasing problem in agriculture due to industrialization and urbanization. The application of mycorrhizal for crops was found to be promising and useful in reducing heavy metal toxicity and enhancing nutrient uptake. The efficacy of VAM in reducing the heavy metal toxicity is scantly studied and seldom exploited in heavy metal polluted soil. The present study aims at understanding the role of VAM in the reduction of heavy metal toxicity using nitrate reductase system and the role of VAM in the alleviation, absorption and translocation of heavy metal.

K e y w o r d s

Mycorrhizae, Heavy Metal, Zea Mays; nitrate reductase

Introduction

Heavy metals such as zinc, cadmium, nickel, manganese are introduced into the ecosystem mainly as a result of pollution by industries. Soil at a 0-20 cm depth are found to be rich in heavy metals especially copper (Leep, and Dickinson, 1987) uptake and accumulation of heavy metal at toxic level often cause phytotoxicity in many plants which limit plant growth cause physiological disorder (Clement, et al., 1974) especially bio-memmbranes and metabolism. Besides there are many reports which indicates that heavy metal inhibit many important enzymes like nitrate reductase (Lee, et al., 1963). As the heavy metals interfere with plant growth and yield of crops, several studies have been conducted to alleviate the inhibitory effect of heavy metal by exogenous supply of several compounds as it may indirectly help to overcome the inhibitory effect. Application of mycorrhizal for crops was found to be promising and useful in reducing heavy metal toxicity and enhancing nutrient uptake. The VAM help their hosts its growth by absorption of phosphorus and other immobile elements from soil, protection against disease, drought, salinity and heavy metal toxicity (Harley, and Smith, 1983).Efficiency of VAM in heavy metal toxicity is scantly studied and seldom exploited in heavy metal polluted soil (Arines, et al., 1989). More extensive studies should be carried out to establish the quality of VAM and to understand the mode of action of VAM essential.

Materials and Methods

Seed Treatment

Seeds of uniform size were surface sterilized with 0.1% mercuric chloride (Austin, et al., 1969) for 2-3 minutes. After that the seeds were rinsed with distilled water and air dried for further uses.

Preparation of Pots for culture:

The pots used for the experiment were washed with tap water and dried in the sunlight. The pots were filled with garden soil and sand at the ratio 1:1. The pots were autoclaved for 2-3 hours at 20 Psi pressure (Raju, et al., 1990)

Culture and Assay Method

In each pot 7-8 seeds were sown about 2 cm below the surface of the soil. VAM inoculum course (2g) were placed 2 cm below the seeds. The inoculum course consists of spores of *Glomus aggregatum, Gigaspora margarita.* Different concentrations of Zinc without VAM represented the control. Nutrient solution (Cakmak, and Merschner, 1986) was added in the pot at the rate of 300 ml/pot of alternative days. Experiments were carried out following (2 level x 2 factor) factorial experimental design to establish the effect of zinc and VAM and the seedlings physiology of *Zea mays,* for each treatment 3 replicates were allotted randomly.

In addition to this, experiments were also carried out to establish inhibit the effect of zinc to *Zea mays*. Three treatments (T_1 = control, T_2 = 75 mM, T_3 = 100mM, T_4=125mM) were given to three experiment units. The plants were grown for 40 days, by which time the inoculated plants established typical mycorrhizal infection (Leake and Read, 1989.). Fresh root samples were stained with cotton blue to ensure the establishment of VAM in the test plants before starting the zinc treatment (Philips and Hagemann ,1970).The assay technique was done by nitrate reductase assay in the leaves and roots (Srivastava, and Mathur, 1981) and soluble protein estimation was done using Lowry et al (1951) . The zinc estimation was done using Peach and Tracey et al. (1956).

Results and Discussion

Effect of different concentration of zinc on nitrate reductase of *Zea mays*

The inhibitory role of zinc and the concentration effect of roots and leaves of *Zea mays*were expressed in Table 1 and figure 1. The results obtained clearly shows that the increasing concentration of zinc decreases the nitrate reductase activity in *Zea mays.* Zinc interferes with protein synthesis by altering the RNA polymerase and ribosomes (Falchuck, et al., 1977) have been proposed. These Studies indicate that the zinc is capable of altering nitrate reductase activity both through the protein and carbohydrate metabolism. This experiment thus concludes that zinc adversely affect the nitrate reductase in linear fashion, lower the concentration of zinc promotes the nitrate reductase activity and its efficiency is more in leaves than roots.

Nitrate reductase activity in the presence and absence of Glomus aggregatum

The comparative results on nitrate reductase activities as affected by different

concentrations of zinc in the presence and absence of VAM were shown in Table no 2 and Figure 3.

Results of this experiment and subsequent statistical analysis point out that VAM is able to recover the toxic effect of zinc significantly, only upto 75 mM. At higher concentration of 125 mM VAM has no efficacy to reduce the toxic effect of zinc. The results suggest that VAM can be used only in the soils which are moderately polluted with zinc. However the nitrate reductase activity of inoculant plants distinctly higher than stressed plants. Thus we find that the enhanced nitrate reductase activity in inoculated plants was only due to higher availability of nitrogen at the enzyme site and this supports the hypothesis of Shivashankar and Rohini Iyer (1988)

Nitrate reductase activity in the presence and absence of *Gigaspora margarita*

The comparative results on nitrate reductase activities as affected by different concentrations of zinc in the presence and absence of VAM were shown in Table no 2 and Figure 2

The results indicated that the unstressed condition of *Gigaspora margarita* significantly enhanced the nitrate reductase activity than the*Glomus aggregatum*. 100 mM and above levels VAM fails to improve the zinc effect significantly, thus *Gigaspora margarita* has the capacity to recover the zinc effect upto certain limit may be 75 mM level. Thus presence study indicates that the heavy metal affects the nitrate reductase activity by two ways are by inhibiting the nitrate reductase synthesizing mechanism and by reducing the available nitrate at the site of nitrate reductase (Senthil kumar and Arockiasamy, 1994).

Accumulation of Zinc in the presence of *Glomus aggregatum*

The results of this study were expressed in table no 4. And shows the accumulation of zinc in the seedlings increased with the increase in the concentration of the medium. At 75 mM 13% increase of zinc content was observed in leaves and at 100 mM about 18% zinc was accumulated in the leaves and only 35 % in the roots.When the concentration was increased the zinc retained in the root system was more (18 to 35%).

This indicates that whether the plant has some mechanism or endogenous adaptation to check the flow of zinc to the leaves and at 125 mM concentration zinc was accumulated more in the roots than the leaves.

Accumulation of Zinc in the presence of *Gigospora margarita*

The result of this study was shown in table no 5. The results indicate the accumulation of zinc in the seedlings increased with the increase in the concentration of the medium. At 100 mM concentration about 8 % of zinc was accumulated in the leaves and 30 % in roots. When the concentration was increased, the zinc retained in the root system was more (18 to 35 %).

At 125 mM concentration zinc was accumulated more in the roots than the leaves. The zinc content in the leaves represented that transported zinc and the zinc content in the roots represented the retained zinc. At higher concentration zinc level retained in the root, thus this

Table.1 Effect of different concentration of zinc on nitrate reductase of *Zea mays*

S.No	Concentration	Nitrate reductase (mM)		% of Control		Significance	
		L	R	L	R	L	R
1	0						
2	75 mM	0.051±0.008	0.012±0.003	-	-	-	-
3	100 mM	0.067±0.0016	0.011 ±0.0024	-	75	S	S
4	125 mM	0.046±0.008	0.008±0.0024	90.1	75	S	S

Significance @ 5% level L = Leaves R = Roots

Table.2 Effect of *Glomus aggregatum* on nitrate reductase in the presence and absence of different concentrations of zinc

S.No	Concentration	Nitrate reductase (mM)		% of Control		Significance	
		L	R	L	R	L	R
1	0	0.038±0.0016	0.005 ±0.0024	-	-	-	-
2	75 mM	0.054±0.008	0.07±0.0016	-	-	S	S
3	100 mM	0.052±0.008	0.004±0.008	-	80	N.S	S
4	125 mM	0.044±0.0024	0.003 ±0.008	-	60	N.S	S

Significance @ 5% level L = Leaves R = Roots

Table.3 Effect of *Gigaspora margarita* on nitrate reductase in the presence and absence of different concentrations of zinc

S.No	Concentration	Nitrate reductase (mM)		% of Control		Significance	
		L	R	L	R	L	R
1	0	0.060±0.0024	0.008 ±0.0016	-	-	-	-
2	75 mM	0.070±0.0033	0.015 ±0.0016	85.7	53.3	S	S
3	100 mM	0.052±0.0024	0.012 ±0.008	86.6	75	S	S
4	125 mM	0.045±0.0016	0.006 ±0.0024	99.2	-	S	S

Significance @ 5% level L = Leaves R = Roots

Table.4 Total zinc levels in the roots & leaves of *Zea mays* treated with different concentration of zinc in the presence and absence of *Glomus aggregatum*

S.No	Zinc Treatment	Zinc level mg/g dry wt.				Total		% of Total			
		L		R		L	R	V_0		V_1	
		V_0	V_1	V_0	V_1	V_0	V_1	L	R	L	R
1	75 mm	0.02	0.13	0.02	0.34	0.05	47	2	2	13	34
2	100 mM	0.08	0.18	0.30	0.35	0.38	43	8	30	18	35
3	125 mM	0.12	0.23	0.10	0.53	0.22	76	12	10	23	53

L= Leaves R= Roots

Table.5 Total zinc levels in the roots &leaves of *Zea mays* treated with different concentration of zinc in the presence of *Gigospora margaratia*

S.No	Zinc Treatment	Zinc content mg/g dry wt.			% of Total	
		L	R	Total	L	R
1	75 mM	0.20	0.35	0.55	20	55
2	100 mM	0.33	0.44	0.77	33	77
3	125 mM	0.35	0.46	0.81	35	81

L= Leaves R= Roots

Figure.1 Seedlings treated with 75mM, 100mM and 125mM zinc concentrations.

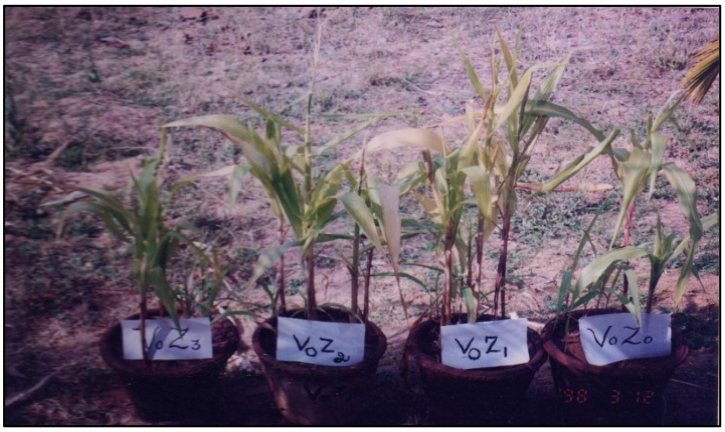


Figure.2 Seedlings inoculated with *Gigaspora margarita* treated with different concentrations of zinc

Figure.3 Seedlings inoculated with *Glomus aggregatum* treated with different concentrations of zinc

Figure.4 The cross section of the root showing the hyphal entry through epidermis X 150

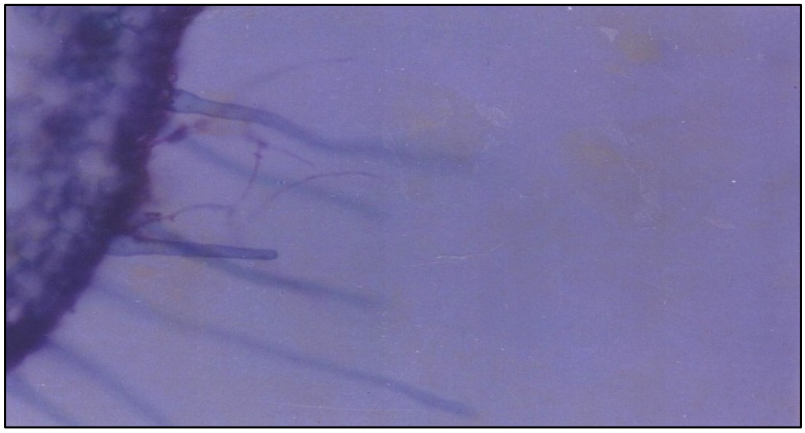


Figure.5 The cross section of the roots showing the *Gigaspora margarita* X 650

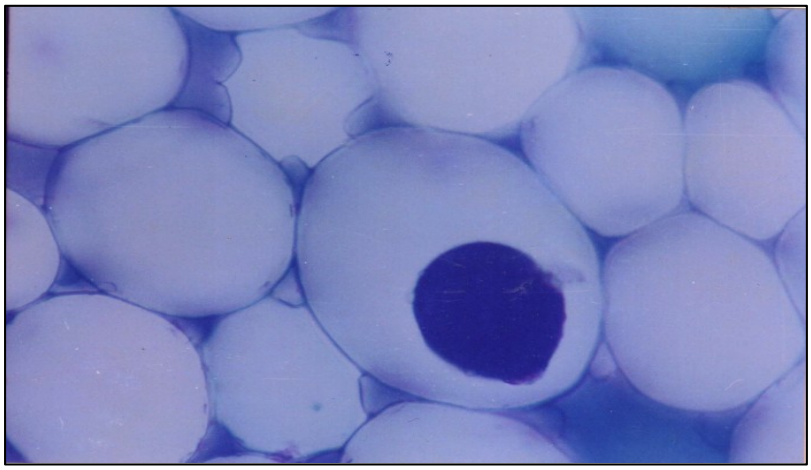
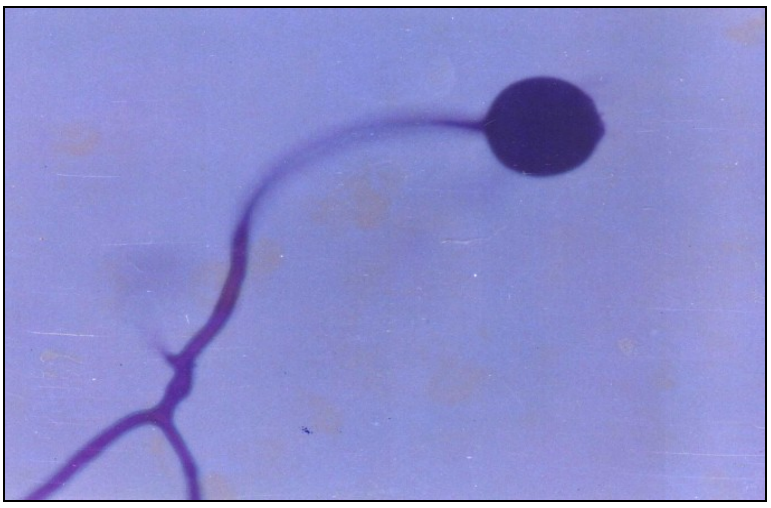


Figure.6 *Glomus aggregatum* spore with mycelium X 150



observation indicates that the plant itself has some mechanism to retain more zinc than the available zinc is more. In addition to the above effect presence of VAM also increases the zinc in the root system. The possibility of metal binding protein by VAM or by the host in response to VAM was thought to be the possible mechanism heavy metal alleviation by VAM. However some other mechanism have also been proposed to explain the alleviation of heavy metal toxicity by VAM, the cell wall of VAM could adsorb the heavy metal especially the ligand in the cell wall binds the heavy metal and reduce the amount of soluble, available, metabolically active zinc (Cumming, and Weinstein, 1990). It can adsorb some antagonizing elements or anions thus inactivate the zinc in the form of zinc phosphate (18).The fungal mycelium excretes some mucilaginous substance in response to heavy metal which binds and prevents being transported to shoot system (Robson, and Pitman, 1983). These studies can be revealed that the Mycorrhizae can be used as bio remediation agent for heavy metal polluted water and can be used for irrigation purposes to certain extent.

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