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Inoculation of *Glomus* sp. Fungi and *Pseudomonas* sp. A Tool for Bioremediation of Cadmium Contaminated Soil

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Abstract: Objective: Microbes are ubiquitous in nature, exploring them for cleanup the environment shows significant importance in bioremediation of heavy metals. Effects of dual inoculation of AM fungi with bacteria *Pseudomonas* on accumulation of Cd (II) in maize plants were studied at different levels (0, 75 and 125 ppm) of Cd (II) and at different stages of plant growth (30 DAS, 60 DAS and at harvest). **Materials and Methods:** Cadmium tolerant Arbuscular Mycorrhizal (AM) fungi *Glomus* sp. (AM_1) and bacteria *Pseudomonas* sp. (PS_1) were used along with the standard cultures. Both fungal and bacterial species were isolated from the soil polluted with high concentration of cadmium in Coimbatore district of Tamil Nadu. *Glomus mosseae* (AMs) and *Pseudomonas putida* (PSs) were the standard cultures used in the study for comparative evaluation. **Results:** Dual inoculation gives the highest root colonization percentage, AM spore count, population of *Pseudomonas* in the rhizosphere. In addition, the plant biometric characteristics were also stimulated by dual inoculation. Inoculation of *Glomus* sp. (AM_1) with *Pseudomonas* sp. (PS_1) (T_6) showed highest percentage of root colonization (92 %) at 125 ppm of Cd on both 30 and 60 DAS over control (T_1). Same treatment had shown increased dry biomass of 23.75 (g pL^{-1}) and it is on par with T_8. The treatment T_8 (*G. mosseae* (AM_8) with *Pseudomonas* sp. (PS_1)) had accumulated high level of Cd in root (3.1856 mg g^{-1}) on 60 DAS at 125 ppm. The treatment T_6 had lower level of Cd in grain (0.0024 mg g^{-1}) at 125 ppm of Cd and on par with T_8. Dual inoculation has shown significant difference with the sole inoculation of both the organisms and can be used efficiently for bioremediation of cadmium. Inoculation of *Pseudomonas* and AM fungi has the ability to remove the cadmium contamination from soil and make the environment free from pollution.

Key words: Cadmium, AM fungi, glomus mosseae, pseudomonas heavy metals, germination

INTRODUCTION

Rapid industrialization, urbanization, intensive agriculture and increased contamination of heavy metals in soil is a major concern in India. "Bioremediation" is the process that uses microorganisms or their enzymes to return the natural environment altered by contaminants to its original condition. Cadmium affects root growth more severely than shoot growth (Vitoria *et al*., 2001). An increase in total plant or in root biomass increased the uptake of Cd when grown at constant Cd concentrations in the soil (Nylund, 2003). AM fungi *Glomus mosseae* and *Glomus intraradices* effective in accumulating heavy metals in the root system reducing metal transfer to shoots and its phytotoxic effects, contributing to the phytoremediation of contaminated soils (Whitfield *et al*., 2004). Hussien *et al*. (2001) found that various species of *Pseudomonas* is relatively efficient in the bioaccumulation of heavy metals than other soil rhizobacteria. The present study was undertaken with the objective to study the efficiency of AM fungi and *Pseudomonas* sp. on accumulation of Cd(II) in maize plants.

MATERIALS AND METHODS

A pot culture experiment was conducted for about one year in the green house of the Department of Agricultural Microbiology, Tamilnadu Agricultural University, Coimbatore with the standard isolates *G. mosseae* and *P. putida*. Pots (30×28 cm size) were filled with 5 kg of double sterilized soil which was the mixture of red soil, sand and farmyard manure in the ratio of 2:1:1. Soil was mixed with the recommended dose of fertilizers, 300:20:200 of N:P:K mg kg^{-1} of soil. Quarter of N, full P and K was applied as basal and remaining N was applied at 45th days after sowing.

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Cd application: The experimental soil was spiked with (Cd) at the rate of 75 and 125 mg kg^{-1} of soil in the form of cadmium chloride ($CdCl_2$). Pot mixture was incubated with desired concentrations of Cd for about 12 h before sowing.

Inoculation method:

- Pure inoculum of *G. mosseae* (AMs) and *Glomus* sp. (AM_1) were applied @ 50 g/pot (containing 8-10 spores/g of inoculum) as a thin layer, 5 cm below the seeds prior to sowing
- Two isolates of *Pseudomonas* viz., PS_1 and *Pseudomonas putida* (PSs) were used for the study. Fourty eight hours old broth cultures (10^9 cells mL^{-1}) of the selected isolates were inoculated at the rate 50 mL broth/pot prior to sowing

Seeds and sowing: Maize seeds (Var. CO 1) obtained from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore and used for this study. The seeds were surface sterilized with 0.1% mercuric chloride for 3 minutes before sowing. The seeds were sown 3 cm below the surface and three plants were maintained in each pot.

Treatment details: Two factors were taken for pot culture experiment. Main factor was Cd concentrations and it was denoted as C_1;0 ppm (control), C_2;75 ppm and C_3;125 ppm, and the second factor was the single and combinations of microorganisms. It was denoted as T_1 to T_9. Three replications were maintained for each treatment.

Sampling: Plant samples were collected randomly at 30th and 60th day after sowing and used for analysis. In the last sample Cd content in cob was also analysed.

Analytical methods

Estimation of Cd content in root and grain samples (USEPA, 1979): Three hundred mg of plant samples viz., shoot and root samples were taken in a conical flask. 10 mL of acid mixture of HNO_3 and H_2O_2 (1:1) was added to each sample. Then the whole content was transferred to microwave digester for digestion. The temperature was maintained at 200°C for 15 min followed by 110°C for 15 min and then cooled for another 15 min. A clear solution was obtained; it was diluted with 2 mL of distilled water. The sample was filtered through What man No. 1 filter paper and volume was made up to 25 mL. Readings were taken in Atomic Absorption Spectrometer (AAS).

The AM root colonization percentage and total spore count was estimated at 30 and 60 DAS in all the treatments. The AM colonization was estimated by adopting the procedure described by Phillips and Hayman (1970). AM fungal spores were collected from the rhizosphere soil samples by wet sieving and decantation technique (Gerdemann and Nicolson, 1963). *Pseudomonas sp.* population was estimated by serial dilution and plating. Plant samples were allowed for sun drying one day and then dried at 60 to 70°C and the dry biomass was obtained. The data were subjected to statistical analysis by variance ($p = 0.05$) with mean separation by Least Significant Difference (LSD) as per the methods detailed by Panse and Sukhatme (1978). The analysis for microbial population count was based on the log and transformed values.

RESULTS AND DISCUSSION

Population of *Pseudomonas* sp.: *Pseudomonas* sp. population was estimated on 30 and 60 DAS of maize rhizosphere soil at different Cd levels. With the increased concentration of Cd the population was significantly reduced in all the treatments. The combined inoculation reduced the population reduction at high Cd level. The highest population was recorded in T_7 (*Glomus* sp. (AM_1) with *P. putida* (PS_S)) on both 30 and 60 DAS (Table 1). *Pseudomonas* has the high efficiency in tolerating Cd contamination when inoculated with AM fungi (Katarina *et al*., 2004).

Estimation of AM colonization percentage and total spore count: When the maize roots were observed for AM colonization incremental levels of Cd reduced root colonization percentage irrespective of the treatments. Inoculation of *Glomus* sp. (AM_1) with *Pseudomonas* sp. (PS_1) (T_6) showed highest percentage of root colonization (92 %) at 125 ppm of Cd on both 30 and 60 DAS over control (T_1). The combined inoculation of AM with *Pseudomonas* sp. enhanced the percentage at high level of Cd than sole treatment (Fig. 1). The same treatment had shown the highest number of spores (109 and 139 spores 50 g^{-1} of soil) at 125 ppm of Cd on respective 30 and 60 DAS over control T_1 (10 and 15 spores 50 g^{-1} of soil) (Table 2). Mycorrhizal root colonization varies with heavy metals, concentrations and period of exposure. It had been shown to be delayed, reduced, and even eliminated (Liao *et al*., 2003). In our experiment AM colonization percentage was decreased with increasing Cd concentrations but had been shown

Table 1: Effect of Cd treatment on Pseudomonas sp. population found in maize rhizosphere

	Pseudomonas sp. (×105 CFU/g ODS)					
	30 DAS			60 DAS		
Treatments	C1: 0 ppm	C2: 75 ppm	C3: 125 ppm	C1: 0 ppm	C2: 75 ppm	C3: 125 ppm
T1: UI	1.19	1.1	1.02	3.69	3.01	3.12
T2: PS_1	4.56	3.71	2.63	7.06	6.56	6.16
T3: PS_S	4.47	3.1	2.9	6.36	6.47	6.73
T4: AM_1	4.16	2.28	0.91	6.91	6.16	6.41
T5: AM_S	3.19	2.12	1.72	5.69	5.36	5.27
T6: AM_1×PS_1	6.18	4.72	3.94	9.42	8.18	8.43
T7: AM_1×PS_S	6.92	5.1	4.82	8.68	8.92	9.69
T8: AM_S×PS_1	4.6	2.8	3.64	7.1	6.59	6.9
T9: AM_S×PS_S	5.18	4.19	2.98	7.68	7.19	7.37
Mean	4.49	3.24	2.73	6.95	6.49	6.68
T	SED	CD (0.05)		SED	CD (0.05)	
C	0.01	0.02		0.01	0.02	
T×C	0.005	0.011		0.005	0.011	

Values rare mean of three replications, UI: Uninoculated control, ODS: Oven dried soil, DAS: Days after sowing, AM1: *Glomus* sp., AMS: *Glomus mosseae*, PS1: *Pseudomonas* sp., PSS: *Pseudomonas* putida

Table 2: Effect of Cd treatment on AM spore count in maize rhizosphere soil

	AM spore (No. /50 g soil)					
	30 DAS			60 DAS		
Treatments	C1: 0 ppm	C2: 75 ppm	C3: 125 ppm	C1: 0 ppm	C2: 75 ppm	C3: 125 ppm
T1: UI	20	13	10	26	22	15
T2: PS_1	23	18	15	35	22	15
T3: PS_S	25	21	18	40	33	28
T4: AM_1	95	56	49	110	86	79
T5: AM_S	80	80	67	125	110	97
T6: AM_1×PS_1	118	117	109	163	147	139
T7: AM_1×PS_S	114	115	90	159	145	120
T8: AM_S×PS_1	100	98	85	145	128	115
T9: AM_S×PS_S	104	105	92	149	135	122
Mean	75.44	69.22	59.44	105.78	92	81.11
T	SED	CD (0.05)		SED	CD (0.05)	
C	1.847	3.704		2.5	5.012	
T×C	1.066	2.138		1.443	2.894	

Values rare mean of three replications, UI: Uninoculated control, ODS: Oven dried soil, DAS: Days after sowing, AM1: *Glomus* sp., AMS: *Glomus mosseae*, PS1: *Pseudomonas* sp., PSS: *Pseudomonas* putida

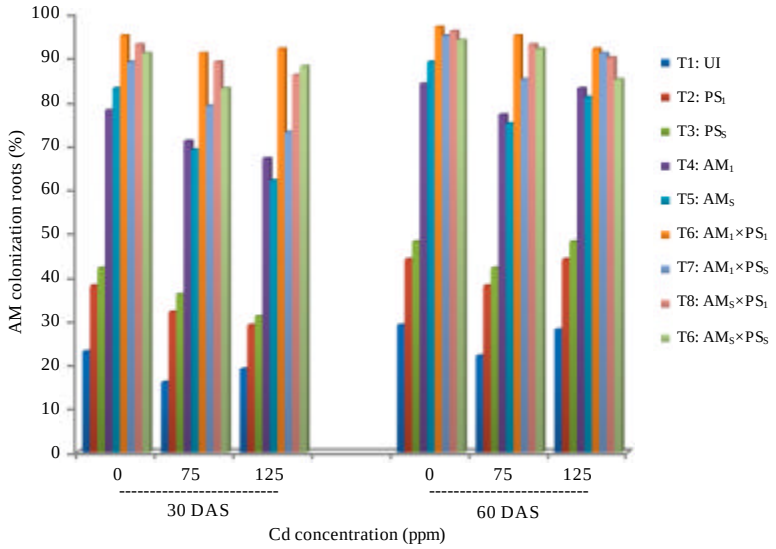

Fig. 1: Effect of Cd on AM colonization in maize plant

that this percentage was increased on 60 DAS when compared to 30 DAS. Our results are in agreement with those obtained by Tullio *et al.* (2003) who reported that the mycorrhizal infection percentage and the spore numbers are decreased by increasing the Cd content of soil. Spore density was also reduced with increasing Cd levels but it had been shown that the spore density was increased on 60DAS over 30 DAS. But Khan *et al.* (2000) found that there was no adverse effect of heavy metal on spore numbers and AM colonization of maize.

Dry biomass of maize plantsL: The oven dried plants were weighed on 30 and 60 DAS. It was observed that the dry weight of the plants was reduced with increased levels of Cd in all the treatments on 30 DAS. The treatment (T_6) had shown increased dry biomass of 23.75 (g pL^{-1}) and it is on par with T_8 (Fig. 2). Cd stress causes significant decrease in fresh weight, dry weight, root length and shoot length (Malekzadeh *et al.*, 2007). At higher concentration of Cd mycorhizal plants showed increased shoot and root weight than non-mycorhizal plants (Liao *et al.*, 2003). In contrast to this, in the present study dry weight of the plant was decreased with increased levels of Cd. This may be due to the inhibition of root cell division or root elongation due to Cd toxicity. Heavy metal stress surely affects the plant growth, but the inoculation of AM fungi with *Pseudomonas* sp. in Cd contaminated areas there was less reduction in the overall plant growth. It agreed with the results of Lin *et al.* (2007), who reported that increased levels of Cd increased fresh weight and dry weight of wheat plants.

Cd content in root and grain of maize plant: The amount of applied Cd translocated in to the maize plant system was analyzed separately for root and grain. The Cd content in roots had increased with increasing levels of Cd in all the treatments. There was increase in the accumulation of Cd in roots on 60 DAS compared to 30 DAS. The treatment T_8 (*G. mosseae* (AMs) with *Pseudomonas* sp. (PS_1)) had accumulated high level of Cd in roots (3.1856 mg g^{-1}) on 60 DAS at 125 ppm (Fig. 3). The treatment T_6 had lower level of Cd in grain (0.0024 mg g^{-1}) at 125 ppm of Cd and on par with T_8 (Fig. 4). The combined inoculation of AM fungi with *Pseudomonas* sp. increased the root Cd accumulation. Latif (2008) had reported increased accumulation of Cd in root than shoot and this accumulation has increased with long duration of exposure. In mycorrhizal plants the accumulation of metal was more in roots but the transfer of metal from root to shoot was restricted (Chen *et al.*, 2004). Mycorrhizal colonization may alter the morphological and physiological changes in the roots (Atkinson *et al.*, 1994), with subsequent modifications in the mycorrhizosphere and this influenced the metal mobility and uptake by the plants at elevated levels of soil pH. Metal concentration in maize grains was increased with increasing Cd levels, but in treatments with both AM and *Pseudomonas* the concentration of metal in grain was reduced. Reduced translocation of

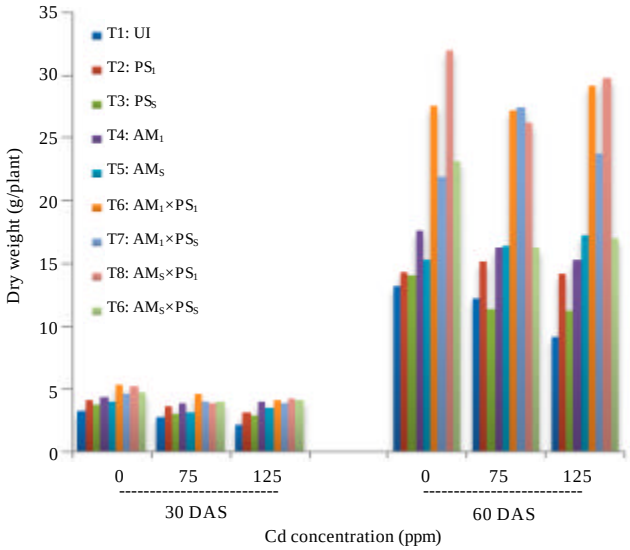

Fig. 2: Effect of Cd on dry biomass of maize plant

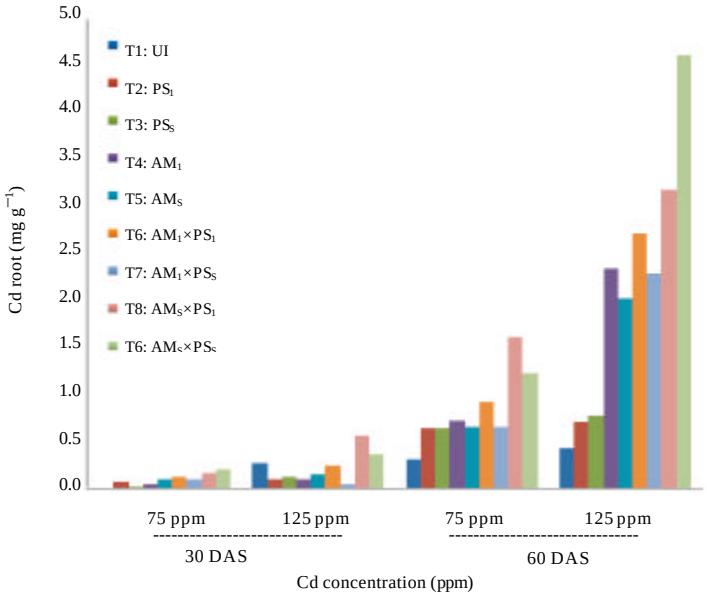

Fig. 3: Cd accumulation in maize root

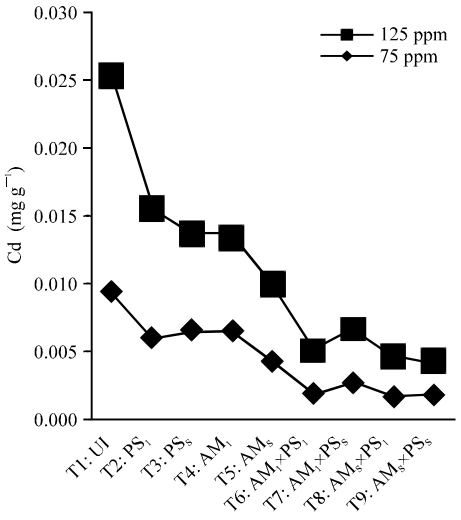

Fig. 4: Cd accumulation in maize grain

metal to the grains was observed in AM colonized plants as they restrict the transfer of metal from root to shoot and grain.

CONCLUSION

Based on the results discussed above cadmium toxicity in plants as well as in soil was reduced by the inoculation of AM fungi with *Pseudomonas* sp. The phytochemical changes in the plant facilitate the accumulation of Cd in the roots and reduced the translocation of Cd to shoots and economic parts. It can be concluded that consortium of microorganisms can be utilized for the bioremediation of Cd contaminated soil in a cost effective manner.

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