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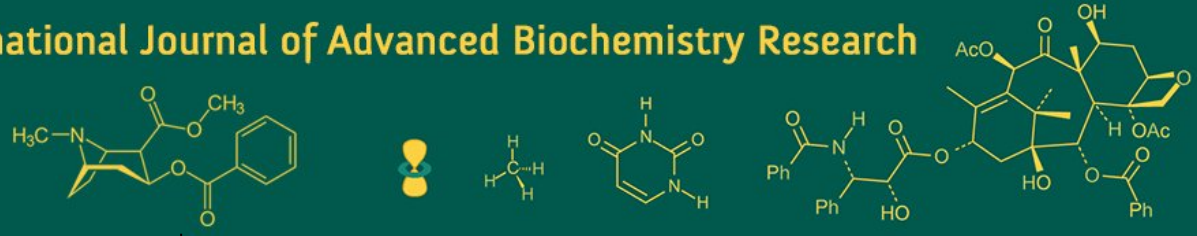

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Influence of Arbuscular mycorrhizal fungi and *Pseudomonas* in the activity of Phosphatase enzyme in maize (*Zea mays*) in response to cadmium contamination

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Abstract
Heavy metal cadmium (Cd^{2+}) tolerant Arbuscular Mycorrhizal fungi *Glomus* sp. (AM_1) and a bacteria *Pseudomonas* sp. (PS_1) both were isolated from the cadmium polluted soil of Coimbatore, Tamil Nadu. These isolates along with two standard cultures *G. mosseae* (AM s) and *P. putida* (PS s) in sole and combinations were used for green house experiment in Cd spiked soil at varying levels of Cd (0, 75 and 125 ppm). All the growth characters and phytochemical changes in the maize plant were studied on 30 and 60 DAS. *Glomus* sp. (AM_1) and *Pseudomonas* sp. (PS_1) inoculated plants had recorded the highest 21.6 per cent increased alkaline phosphatase activity over shoot at 125 ppm on 60 DAS. But acid phosphatase activity was increased in *G. mosseae* (AMs) and *Pseudomonas* sp. (PS_1) (7.12 µg of PNPP/g of fresh root tissue).

Keywords: Cadmium, enzyme activity, glomus, *Pseudomonas*, phosphatase

Introduction
Heavy metals are becoming a most significant pollutants in the environment and their toxicity creates a serious threat to the ecology. They are derived from various anthropogenic sources *viz*., wastes from industries, sewage, urban runoff, domestic and mining etc., and recreating activities like., boating and also from use of chemicals in agricultural production which has increasingly affected many different ecosystems (Macfarlane and Burchett, 2001) [4]. Cadmium is considered as a non-essential element which affects the plant growth and its development. It is also considered as a most significant soil pollutant because of its large solubility in water leads to be highly toxic (Pinto *et al*., 2004) [7]. Cadmium can also alter the uptake of minerals by plants and also reduce the population of soil microbes (Moreno *et al*., 1999) [5].

Cadmium Cd^{2+} can be very easily removed from the cell wall and subsequently immobilized in the cytoplasm is a reason for higher Cd-tolerance of roots (Inouhe *et al*., 1991) [2]. Numerous hydrolytic enzymes like acid phosphatase and alkaline phosphatase were involved in plant morphogenesis, as well as in cell wall metabolism (Sano *et al*., 2003) [9]. Landi *et al.* (2000) [3] reported that the available cadmium in the soil solution leads to decrease the activities of microbial enzymes. Both phosphatase were sensitive to heavy metals, however, alkaline phosphatase was more sensitive (Renella *et al*., 2003) [8]. *Pseudomonas putida* accumulate Cd in the medium as poly β hydroxylbutyrate granules and reduce the production of ethylene by ACC deaminase activity. *Glomus mosseae* and *Glomus intraradices* were enhanced the photosynthetic activity and plant growth in comparison to the non-mycorrhizal plants in metal contaminated area. AM fungi have been found colonizing plant growing on heavy metal contaminated habitats and can uptake the heavy metals and metals which were immobilized in mycelium. Therefore, AM fungi may be efficient in accumulating heavy metals in plant root system, thus reduce the metal translocation to shoots and contributing to the phytoremediation tool of heavy metal contaminated soils (Whitfield *et al*., 2004) [11].

Materials and Methods

An experiment was conducted in the green house of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore using the standard cultures *Glomus mosseae* and *Pseudomonas putida*. 5 kg of sterilized soil (double) was filled in the pots (30x28 cm size) and mixed with 300:20:200 of N: P: K mg/kg of soil (recommended dose). ¼th N and 100 % P and K was mixed in the soil (basal dose). ¾th of N was applied at 45th DAS. Cadmium (Cd^{2+}) was applied @ 75 and 125 mg/kg of soil as $CdCl_2$ before 12 h of sowing. All the microorganisms *G. mosseae* (AM s) and *Glomus* sp. (AM $_1$) were applied as a thin layer below 5 cm to the seeds @ 50g per pot having 8-10 spores /g and 48 h old liquid cultures of bacteria *viz*., *Pseudomonas* sp. (PS $_1$) and *Pseudomonas putida* (PS s) were applied @ 50 ml per pot with a cell load of 10^9 cells/ml. Surface sterilized (0.1 % $HgCl_2$ for 3 minutes before sowing) maize seeds - var. CO 1 were sown and three plants per pot was maintained. Factorial experiment with 3 replications was used. Cd $^{2+}$ was supplied as $CdCl^2$ with three concentrations (Cd0 ppm, Cd75 ppm and Cd125 ppm) as factor 1 and microorganisms as single and combinations were factor 2. (T_1 to T_9). Randomly collected plant samples (30 and 60 DAS) were used for estimating phosphatase activity.

Treatment details

T_1 (UI) – Un-inoculated control
T_2 - PS $_1$ – *Pseudomonas* sp.,
T_3 - PS $_S$ – *Pseudomonas putida*
T_4 - AM $_1$ – *Glomus* sp.,
T_5 - AM $_S$ – *Glomus mosseae*
T_6 – T_2+T_4 (PS $_1$ – *Pseudomonas* sp., + AM $_1$ – *Glomus* sp.)
T_7 – T_3 + T_4 (PS $_S$ – *Pseudomonas putida* + AM $_1$ – *Glomus* sp.,)
T_8 – T_2+T_5 (PS $_1$ – *Pseudomonas* sp., + AM $_S$ – *Glomus mosseae*)
T_9 – T_3+T_5 (PS $_S$ – *Pseudomonas putida* + AM $_S$ – *Glomus mosseae*)

Enzyme activity

Both acid and alkaline phosphatase activities were measured in root and shoot as per method of Morton (1952) [6]. Ten g of maize shoot and root were ground with acid washed sand using a pre-chilled pestle and mortar using 20 ml grinding medium (0.2 M acetate buffer for acid phosphatase or 0.2 M borate buffer for alkaline phosphatase). The homogenate was passed through four layers of cheese cloth and filtrate was centrifuged at 3000 rpm for 5 min. the supernatant was used for estimating phosphatase activity. The enzyme phosphatase hydrolyzed paranitro phenol phosphate. The yellow coloured (in alkaline medium 8.5 pH) released para nitrophenol was estimated at 725 nm. (pH 4.5 for acid phosphatase).

Results and Discussion

Phosphatase activity

Activity of acid phosphatase was measured in both shoot and root of maize plants on 30 and 60 DAS. It was observed that the acid phosphatase activity was high with increasing levels of Cd on both 30 and 60 DAS. The treatment T_8 (*G. mosseae* (AM $_S$) and *Pseudomonas* sp. (PS $_1$)) had recorded the highest acid phosphatase activity in root (7.12 µg of PNPP/g) Table. 1, it was slightly reduced (6.86 µg of PNPP/g) Table. 2 in shoot at 125 ppm on 30 DAS. The combined inoculation of *G. mosse*ae (AM s) and the isolate (PS $_1$) had shown 248.2 and 131 % more in enzyme activity over its sole application of *G. mosse*ae in shoot and root on 30 DAS respectively.

Table 1: Effect of microbial consortium of AMF and *Pseudomonas* sp. on acid phosphatase activity in root of maize crop on 30 and 60 DAS at different Cd levels Cd0 ppm, Cd75 ppm and Cd125 ppm

Treatments/ Concentration of Cd (ppm)	Acid phosphatase activity in root (µg of PNPP/g)					
	30 DAS			60 DAS		
	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm
T_1(UI)	0.19	1.43	2.01	0.52	1.35	2.82
T_2 (PS $_1$)	0.83	2.65	3.12	1.32	2.62	3.95
T_3 (PS $_S$)	0.54	1.52	2.91	0.91	2.10	3.32
T_4 (AM $_1$)	1.24	2.13	3.87	2.39	3.50	4.30
T_5 (AM $_S$)	0.98	1.47	3.08	2.95	4.15	5.12
T_6 (AM $_1$ x PS $_1$)	2.13	4.79	6.98	3.20	4.52	5.36
T_7 (AM $_1$ x PS $_S$)	1.95	3.90	5.09	2.61	3.91	4.62
T_8 (AM $_S$ x PS $_1$)	2.91	5.87	7.12	3.52	5.37	6.58
T_9 (AM $_S$ x PS $_S$)	1.76	3.61	5.41	2.96	3.92	4.12
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.082	0.165		0.087	0.176	0.082
C	0.047	0.095		0.050	0.101	0.047
T x C	0.142	0.285		0.152	0.304	0.142

The overall activity was significantly increased on 60 DAS at 0 ppm and 75 ppm. There was some reduction of acid phosphatase activity at 125 ppm on 60 DAS than 30 DAS in both shoot and root. The same treatment has shown the highest enzyme activity in root (6.58 µg of PNPP/g) on 60 DAS also. The percentage increase over its sole inoculation of *G. mosseae* was 40 and 28.5 in shoot and root at 125 ppm on 60 DAS respectively. The combined inoculation was significantly increased the acid phosphatase activity than the sole treatments.

Table 2: Effect of microbial consortium of AMF and *Pseudomonas* sp. on acid phosphatase activity in shoot of maize crop on 30 and 60 DAS at different Cd levels

Treatments/ Concentration of Cd (ppm)	Acid phosphatase activity in shoot (µg of PNPP/g)					
	30 DAS			60 DAS		
	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm
T_1(UI)	0.04	1.08	1.65	0.18	0.92	2.09
T_2 (PS $_1$)	0.37	1.32	2.17	0.64	1.81	1.36
T_3 (PS $_S$)	0.15	0.94	2.18	0.36	1.45	2.71
T_4 (AM $_1$)	0.91	1.82	2.63	1.84	2.69	3.60
T_5 (AM $_S$)	0.57	1.19	1.97	2.07	3.61	4.42
T_6 (AM $_1$ x PS $_1$)	1.28	3.16	6.12	2.65	3.84	4.79
T_7 (AM $_1$ x PS $_S$)	1.13	2.65	4.10	2.10	2.32	4.08
T_8 (AM $_S$ x PS $_1$)	1.84	4.91	6.86	2.94	4.18	6.19
T_9 (AM $_S$ x PS $_S$)	1.04	3.10	5.08	2.41	2.80	3.91
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.067	0.134		0.070	0.142	0.067
C	0.038	0.077		0.040	0.082	0.038
T x C	0.116	0.232		0.122	0.246	0.116

Alkaline phosphatase activity was measured in both shoot and root of maize plants on 30 and 60 DAS. It was observed that the alkaline phosphatase activity was increased with increasing levels of Cd on both 30 and 60 DAS. The treatment T_8 (*G. mosseae* (AM $_S$) and *Pseudomonas* sp. (PS $_1$)) had recorded the highest alkaline phosphatase activity in

root (6.03 µg of PNPP/g) Table. 3, it was highly reduced (4.72µg of PNPP/g) in shoot Table. 4 at 125 ppm on 30 DAS. The percentage increase in root over shoot was 27.7. But the sole inoculation of *G. mosseae* had shown 293.3 and 181.7 per cent of reduced enzyme activity over its combined inoculation with *Pseudomonas* sp. (PS $_1$) in shoot and root on 30 DAS at 125 ppm.

Table 3: Effect of microbial consortium of AMF and *Pseudomonas* sp. on alkaline phosphatase activity in root of maize crop on 30 and 60 DAS at different Cd levels

Treatments/ Concentration of Cd (ppm)	Alkaline phosphatase activity in root (µg of PNPP/g)					
	30 DAS			60 DAS		
	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm
T_1(UI)	0.43	1.02	1.75	0.45	1.21	1.84
T_2 (PS $_1$)	0.74	1.92	3.98	1.62	2.35	2.91
T_3 (PS $_S$)	1.23	1.97	2.56	0.91	1.32	1.75
T_4 (AM $_1$)	1.56	2.46	4.21	1.55	2.61	3.13
T_5 (AM $_S$)	0.98	1.62	2.14	2.15	2.83	3.92
T_6 (AM $_1$ x PS $_1$)	2.54	3.41	5.84	3.65	5.91	7.20
T_7 (AM $_1$ x PS $_S$)	2.12	3.76	5.06	2.92	3.41	4.52
T_8 (AM $_S$ x PS $_1$)	2.87	4.93	6.03	3.12	5.42	6.35
T_9 (AM $_S$ x PS $_S$)	2.34	4.12	5.31	3.51	4.61	5.92
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.077	0.154		0.087	0.174	0.077
C	0.044	0.089		0.050	0.100	0.044
T x C	0.133	0.267		0.151	0.302	0.133

Similar increasing trend was observed in all the treatments on 60 DAS also. But the treatment T_6 (*Glomus* sp. (AM $_1$) with *Pseudomonas* sp. (PS $_1$)) had recorded the highest enzyme activity in root (7.20 µg of PNPP/g) over control (1.84 µg of PNPP/g) at 125 ppm on 60 DAS. The same treatment showed 21.6 per cent increased enzyme activity over shoot at 125 ppm on 60 DAS. The combined inoculation significantly increased the alkaline phosphatase activity than the sole treatments.

Table 4: Effect of combined inoculation of AM fungi and *Pseudomonas* sp. on alkaline phosphatase activity in shoot of maize crop on 30 and 60 DAS at different Cd levels

Treatments/ Concentration of Cd (ppm)	Alkaline phosphatase activity in shoot (µg of PNPP/g)					
	30 DAS			60 DAS		
	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm	Cd_0 ppm	Cd_{75} ppm	Cd_{125} ppm
T_1(UI)	0.21	0.63	1.16	0.32	0.84	1.13
T_2 (PS $_1$)	0.56	0.82	1.82	0.67	1.72	2.25
T_3 (PS $_S$)	0.74	0.61	1.72	0.62	0.94	1.46
T_4 (AM $_1$)	0.93	1.28	2.93	1.09	1.86	2.61
T_5 (AM $_S$)	0.48	0.96	1.20	1.27	2.11	2.74
T_6 (AM $_1$ x PS $_1$)	1.86	2.17	3.49	2.38	4.27	5.92
T_7 (AM $_1$ x PS $_S$)	1.49	2.58	3.08	1.73	2.18	3.19
T_8 (AM $_S$ x PS $_1$)	2.04	3.19	4.72	2.18	4.38	5.14
T_9 (AM $_S$ x PS $_S$)	1.96	3.28	3.98	3.10	3.91	4.18
	SEd	CD (0.05)		SEd	CD (0.05)	
T	0.051	0.103		0.065	0.132	0.051
C	0.029	0.059		0.038	0.076	0.029
T x C	0.089	0.179		0.114	0.228	0.089

Both acid phosphatase and alkaline phosphatase are specifically induced in the presence of mycorrhizal when compared to uninoculated control. Under phosphorus starvation plant cells produced acid phosphatase with a pronounced preference for phosophoenol pyruvate (Duff *et al*., 1989) [1]. Apical part of the roots showed inhibition of acid and alkaline phosphatase at higher concentration (50, 250 and 1000 µg/ml) (Siroka *et al*., 2004) [10]. Our results showed that both the enzymes were increased with increasing Cd levels. The combinations of AM fungi with *Pseudomonas* sp. increased acid phosphatase to 7.12 µg of PNPP per gram of fresh root tissue over control 2.01 µg of PNPP per gram of fresh root tissue at 125 ppm of Cd on 30 DAS.

There was a slight increase in acid phosphatase activity at 1000 µg/ml of Cd in coleoptiles was detected by Siroka *et al*., (2004) [10]. In case of alkaline phosphatase it was 7.2 µg of PNPP per gram of fresh root tissue at T_6 over control 1.84 µg of PNPP per gram of fresh root tissue on 60 DAS.

Conclusion

As discussed in the results the cadmium toxicity in soil and also in plants grown in metal contaminated area was significantly lowered by the inoculation of AM fungi along with *Pseudomonas* sp. Enzyme activity was higher with higher levels of Cd^{2+} in all the treatments and at all the stages. The treatment T_6 (*Glomus* sp. (AM $_1$) and *Pseudomonas* sp. (PS $_1$)) had recorded the highest 21.6 per cent increased alkaline phosphatase activity over shoot at 125 ppm on 60 DAS. Acid phosphatase activity was increased in T_8 (*G. mosseae* (AM $_S$) and *Pseudomonas* sp. (PS $_1$)) (7.12 µg of PNPP/g of fresh root tissue). The phytochemical changes in the plant could have facilitated the accumulation of Cd^{2+} in the roots and also reduced its translocation to above ground parts. Hence, the microbial consortium can be exploited for bioremediation of heavy metal Cd^{2+} contaminated soil as a cost-effective tool.

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