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RESEARCH PAPER

Effect of zinc and microbial inoculation on soil enzyme activities for maize (*Zea mays* L.) in red soil

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Abstract: A Field experiment was conducted in red soils in order to study the soil enzyme activities viz., Dehydrogenase, Urease and Phosphatases for maize using Arbuscular mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) in combination with graded levels of $ZnSO_4$. Treatment consisted of two factors viz., Microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: $M_2 + M_3$) and graded levels of $ZnSO_4$ (S_1: 0, S_2: 12.5, S_3: 25, S_4: 37.5, S_5: 50 kg ha^{-1} and S_6: 0.5 % foliar spray @ 45 and 65 DAS) replicated three times in FRBD. The combination of AM and ZSB had enhanced the soil enzyme activities viz., Dehydrogenase, Urease and Phosphatases at vegetative, tasselling and harvest stage of crop growth. However, the Dehydrogenase, Urease and Phosphatases activity was quantitatively increased during tasselling stage due to microbial population, root activities and root exudates higher during tasselling stage. Increased doses of $ZnSO_4$ application had positive effect on the soil enzyme activities viz., Dehydrogenase, Urease and Phosphatases. Combined application of AM and ZSB along with 50 kg of $ZnSO_4$ ha^{-1} had recorded the highest soil enzyme activities at vegetative, tasselling and harvest stages. However, the dehydrogenase, urease and harvest stage was slightly increased at tasselling stage than vegetative and harvest stage. The application of graded doses of $ZnSO_4$ in combination with AM and ZSB had also increased the soil enzyme activities viz., Dehydrogenase, Urease and Phosphatases, which in turn increased the fertility status of soil.

Keywords: Maize • Glomus intraradices (AM) • Zinc solubilizing *Bacillus* Sp. • $ZnSO_4$ • Soil enzymes • Dehydrogenase • Urease • Phosphatases

Introduction

Maize (*Zea mays* L.) is one of the world's most widely grown cereals, having great significance as human food, animal feed and raw material source for large number of industrial products (Gajendra *et al.,* 2015; Ayyar and Appavoo, 2016). In India, maize is grown in a wide range of environment, extending from extreme semiarid to sub humid and humid regions. It is grown in about 9.09 M ha and utilized for versatile purposes. It is a main source of calories and minerals for most rural populations (Sugany, 2015).

Soil enzymes are the mediators and catalysts of biochemical processes important in soil functioning such as nutrient mineralization and cycling, decomposition and formation of soil organic matter and decomposition of xenobiotics (i.e., pesticides). Specifically, oxidoreductores can provide information on the status of key reactions that participate in rate limiting steps of oxidation – reduction process of organic and inorganic materials in soils (Trasar–cepeeda *et al.,* 2000). Sustainability of agricultural systems has become an important issue all over the world. Many issues of sustainability are related to soil quality and its change with time (Karlen *et al.,* 1997; Ayyar and Appavoo, 2017). Soil biological activities have been suggested as one of the important indicator of

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soil quality (Dick, 1994; Ruf *et al.*, 2003; Ling *et al.*, 2010; Fliebach *et al.*, 2007). Soil enzyme activities are 'sensors' of soil degradation. Since they combine information about microbial status, and also from soil physio–chemical conditions (Aon and Colaneri, 2001). Numerous studies have been conducted to evaluate the potential use of enzymatic activity as an index of soil productivity or soil fertility (Alef and Nannipieri, 1995).

Dehydrogenase activity (DHA) has been recognized as important biochemical indicators in soil (Ryoichi and Senaratne, 2009). The activity of the DHA is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity because it is linked to viable cells (Garcia *et al.*, 1997; Skujin, 1976). Urea cannot be used in plant metabolism directly. The primary physiological role of urease is to allow the organism to use externally supplied and internally generated urea as a nitrogen source. Urease converts urea to products at a rate of at least 10-14 times faster than the urea spontaneous decomposition rate. Urease catalyses urea hydrolysis, thus making urea – N available to be assimilated into organic compounds (Hogan *et al.*, 1983). Phosphatases catalyse the hydrolysis of ester–phosphate bonds, leading to the release of phosphate (P), which can be taken up by plants or microorganisms (Moscatelli, 2005). It has been shown that the activities of phosphatases (like those of many hydrolyses) depend on several factors such as soil properties, soil organism interactions, plant cover, leachate inputs, and the presence of inhibitors and activators (Speir and Ross, 1978; Suganya *et al.*, 2019).

Arbuscular mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) enhance the Organic carbon, Biomass carbon and nutrient availability in soil and it is source of food for the microorganism present in the soil. Microbial population increase the enzyme activity in soil in turn improves the soil fertility. Keeping these facts in mind, the present investigation was attempted to study the effect of graded levels of Zn and microbial inoculation on soil enzyme activities for maize (*Zea mays* L.) in red soil.

Materials and methods

Experimental site and soil characteristics

A field experiment was conducted in red soil during 2014 at farmer's field, Pullanviduthi, near National Pulses Research Centre (TNAU), Vamban, Pudukkottai district. It lies between 8° 30′ to 10° 40′ North latitude and 78° 24′ to 79° 40′ East longitude and is at a height of 600 MSL. The soil was Sandy loam, red in colour belonging to *Udic Rhodustalf* (Mudukulam series). The details of soil characteristics are given in Table 1. The initial soil was neutral in reaction, non saline, low status of OC and CEC. The available N was low, P and K were found to be medium. The DTPA-Fe, DTPA-Cu and DTPA-Mn were found to be in sufficiency level while the availability of DTPA-Zn was found to be deficient.

Treatment design and crop management

Treatment consisted two factors *viz.,* microbial inoculation (M_1: control, M_2: AM fungi, M_3: ZSB and M_4: M_2+M_3) and graded levels of $ZnSO_4$ (S_1: 0, S_2: 12.5, S_3: 25, S_4: 37.5, S_5: 50 kg ha $^{-1}$ and S_6: 0.5% foliar spray @ 45 and 65 DAS) replicated three times in FRBD. Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges by adopting a spacing of 60 x 25 cm along with vermiculite based mycorrhizal inoculum (*Glomus intraradices* TNAU-03-08) 2 g per hill at a depth of 5 cm. Zinc solubilizing bacteria was applied at 2 kg ha^{-1} after mixing it with 25 kg each of sand and farm yard manure. The recommended fertilizer prescription for maize *viz.,* 250:75:75 kg N, P_2O_5 and K_2O kg ha^{-1} was followed. The full dose of P and K were applied basally and N was applied at three splits *viz.,* basal (25%), vegetative (50%) and tasselling (25%) stages. Calculated

Table 1. Initial soil characters

Taxonomy	Udic Rhodustalf
Textural class	Sandy loam
pH (1:2.5 soil : water)	6.69
EC (dS m^{-1})	0.07
Free $CaCO_3$ (g kg^{-1})	20.1
Organic carbon (g kg^{-1})	3.53
CEC (c mol (p$^+$) kg^{-1})	12.6
Alkaline $KMnO_4$- N (kg ha^{-1})	210
Olsen- P (kg ha^{-1})	15.4
Neutral N NH_4Oac- K (kg ha^{-1})	208
DTPA Zn (mg kg^{-1})	0.82
DTPA Cu (mg kg^{-1})	2.57
DTPA Fe (mg kg^{-1})	14.2
DTPA Mn (mg kg^{-1})	8.30

quantities of $ZnSO_4$ were applied basally as per the treatment schedule.

Sampling and analytical methods

Composite surface (0-15 cm) soil samples were collected from the site before the experiment and analysed for their physical, physio–chemical and chemical properties. Soil samples were also collected from each of the plots at vegetative (30th DAS), tasselling (60th DAS) and harvesting stage of crop for analyse. The samples were air dried and passed through 2 mm sieve prior to analysis. The pH and EC were determined in 1:2.5 (soil:water) suspension using digital pH meter. Organic carbon (OC) content of soil was determined by chromic acid wet oxidation method (Walkley and Black, 1934); available nitrogen content in soil was determined by alkaline permanganate method (Subbiah and Asija, 1956); available Phosphorus content in soil was estimated using olsen's extraction method (Olsen *et al.*, 1945); available potassium in soil was analysed neutral normal ammonium acetate method as outlined by Jackson (1973); exchangeable calcium and magnesium in soil was determined by versenate titration–neutral normal ammonium acetate extract (Jackson, 1973) and available micronutrient (Zn, Cu, Fe and Mn) in soil was estimated by Atomic Absorption Spectrophotometer (AAS) after extracting soil samples with DTPA (Diethelene Triamine Penta Acetic Acid) extractant (Linday and Norwell, 1978).

Soil dehydrogenase activity was determined by the method outlined by Casida *et al.* (1964). Soil Urease enzyme was determined by the method outlined by Bremner and Mulvaney, 1978. Soil Phosphatase enzyme was estimated by Bremner and Tabatabai, 1969. Data were statistically analysed using ANOVA package method of Gomez and Gomez (1984) and least significant difference (LSD) at 5% probability level was computed to compare the treatments.

Result and discussion

Dehydrogenase activity

Microbial inoculation had marked influence on dehydrogenase activity over control (Figure 1). The highest dehydrogenase activity was recorded for M_4 (37.1 µg TPF g^{-1} of soil day^{-1}) followed by M_3 (32.3 µg TPF g^{-1} of soil day^{-1}) and M_2 (30.9 µg TPF g^{-1} of soil day^{-1}) over control during vegetative stage (Table 2). Graded doses of Zn application increased the dehydrogenase activity. The highest dehydrogense activity was noticed for S_5 (47.0 µg TPF g^{-1} of soil day^{-1}) followed by S_4 (40.4 µg TPF g^{-1} of soil day^{-1}), S_3 (35.6 µg TPF g^{-1} of soil day^{-1}), S_2 (27.7 µg TPF g^{-1} of soil day^{-1}) and S_6 (20.3 µg TPF g^{-1} of soil day^{-1}) over control. Similar trend was noticed at tasselling and harvest stage. The dehydrogenase activity showed slight increase at tasselling stage than vegetative and harvest stages due to microbial population,

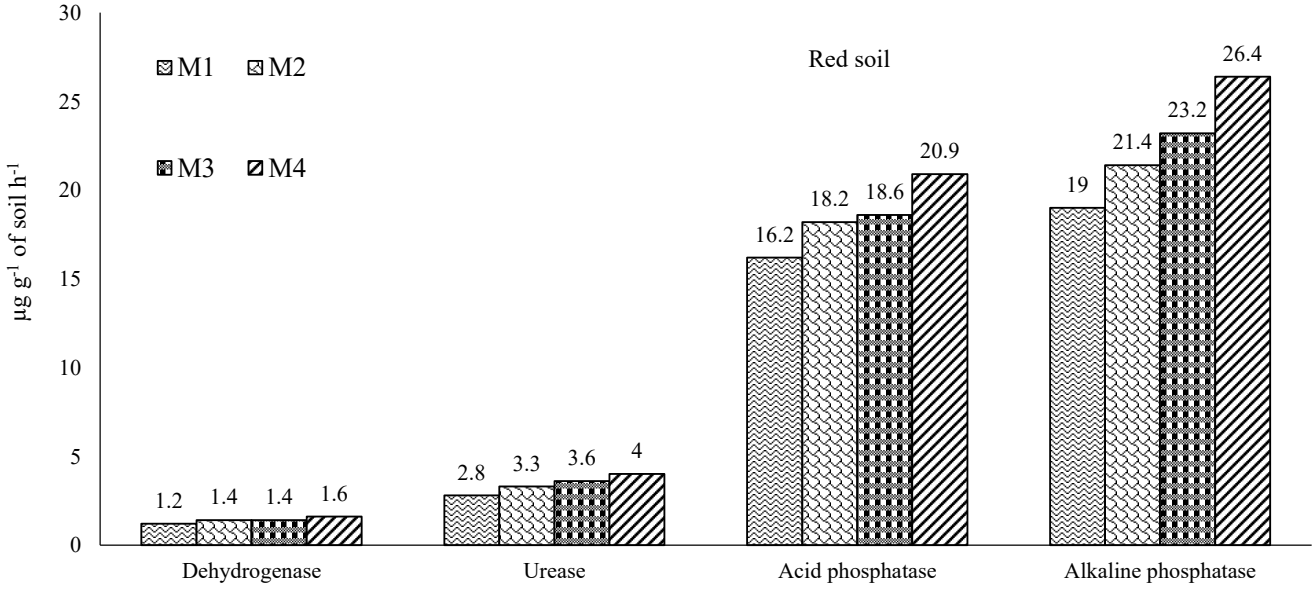

Figure 1. AMF and ZSB on soil enzyme activities during tasselling stage
M_1 – Control; M_2 – Arbuscular Mycorrhizal Fungi; M_3 – Zinc Solubilizing Bacteria; M_4 – M_2 + M_3

Table 2. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on soil dehydrogenase activity (µg TPF g^{-1} of soil day^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	16.8	23.7	30.5	34.1	41.8	16.8	27.3	17.2	25.9	31.5	35.6	42.6	18.0	28.5	15.1	24.3	29.2	33.3	40.4	15.3	26.3
M_2	19.5	28.4	34.1	38.3	45.2	19.6	30.9	20.9	29.5	36.8	40.1	47.6	21.4	32.7	17.5	27.2	32.8	39.9	42.6	18.7	29.8
M_3	20.3	26.2	37.6	42.0	47.4	20.1	32.3	22.3	30.2	38.2	42.9	50.3	22.5	32.7	19.0	25.5	36.2	41.8	46.7	19.5	31.5
M_4	24.1	32.5	40.2	47.3	53.6	24.7	37.1	25.7	33.6	41.0	48.6	56.5	25.8	38.5	23.8	31.4	39.7	45.8	51.4	23.9	36.0
Mean	20.2	27.7	35.6	40.4	47.0	20.3	31.9	21.5	29.8	34.4	41.8	49.3	21.9	33.1	18.9	27.1	34.5	40.2	45.3	19.4	30.9
			SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)		
M			0.62		1.3					0.61		1.3					0.59		1.2		
S			0.71		1.5					0.74		1.5					0.71		1.5		
M X S			1.47		3.1					1.47		3.0					1.42		3.1		

Table 3. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on soil Urease activity (µg NH_4 – N g^{-1} of soil h^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	1.6	2.1	2.5	3.2	3.9	1.6	2.5	1.9	2.4	2.9	3.6	4.1	1.9	2.8	1.4	1.9	2.2	3.0	3.5	1.4	2.2
M_2	1.9	2.5	3.0	3.7	5.0	1.9	3.0	2.1	2.7	3.3	4.2	5.4	2.1	3.3	1.6	2.2	2.9	3.5	4.8	1.6	2.8
M_3	2.0	2.4	3.5	4.2	5.3	2.0	3.2	2.3	2.9	3.8	4.6	5.7	2.3	3.6	1.8	2.1	3.2	4.0	4.9	1.8	3.0
M_4	2.3	3.0	3.8	4.8	6.0	2.3	3.7	2.6	3.2	4.1	5.3	6.4	2.6	4.0	2.0	2.8	3.5	4.6	5.5	2.0	3.4
Mean	2.0	2.5	3.2	4.0	5.1	2.0	3.1	2.2	2.8	3.5	4.4	5.4	2.2	3.4	1.7	2.3	3.0	3.8	4.7	1.7	2.8
			SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)		
M			0.04		0.1					0.14		0.3					0.04		0.1		
S			0.04		0.1					0.17		0.4					0.03		0.1		
M X S			0.09		0.2					0.35		0.7					0.05		0.1		

Table 4. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on soil acid phosphatase activity (µg p-nitrophenol g^{-1} of soil h^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	12.3	13.4	15.1	16.5	17.0	12.3	14.4	14.1	15.8	16.6	17.3	18.9	14.2	16.2	10.4	13.0	13.9	15.1	16.5	10.9	13.3
M_2	14.4	16.3	17.8	19.1	20.2	14.4	17.0	15.2	17.9	18.8	20.3	21.4	15.3	18.2	13.3	15.2	17.4	18.0	19.5	13.7	16.2
M_3	14.8	16.6	18.1	19.3	20.2	14.8	17.3	16.0	18.3	19.1	20.4	21.9	16.0	18.6	13.8	15.2	17.8	18.1	19.8	13.8	16.3
M_4	15.5	19.3	21.6	22.4	23.9	15.6	19.7	17.2	20.2	22.7	23.5	24.8	17.0	20.9	14.3	18.8	20.3	21.9	23.7	14.3	18.9
Mean	14.3	16.4	18.2	19.3	20.3	14.3	17.1	15.6	18.1	19.3	20.4	21.8	15.6	18.5	13.0	15.6	17.4	18.3	19.9	13.2	16.2
			SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)		
M			0.33		0.7					0.35		0.7					0.31		0.6		
S			0.33		0.7					0.40		0.8					0.37		0.8		
M X S			0.73		1.5					0.86		1.8					0.74		1.6		

Table 5. Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on soil alkaline phosphatase activity (µg p-nitrophenol g^{-1} of soil h^{-1})

Treatments	Vegetative stage							Tasselling stage							Harvest stage						
	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean	S_1	S_2	S_3	S_4	S_5	S_6	Mean
M_1	14.3	15.6	18.2	20.1	21.8	14.3	17.4	16.0	18.3	19.5	21.7	22.6	16.1	19.0	13.8	16.6	17.0	19.3	21.1	14.0	17.0
M_2	17.8	19.0	20.5	24.1	25.2	17.9	20.8	18.2	19.3	21.0	24.7	26.8	18.3	21.4	17.4	18.5	20.4	24.0	24.6	17.5	20.4
M_3	18.5	20.2	22.3	25.2	26.9	18.5	21.9	19.8	21.5	24.3	26.6	27.1	19.8	23.2	18.0	19.2	21.5	25.2	26.3	18.0	21.4
M_4	20.0	21.3	23.2	27.7	30.8	20.0	23.8	20.8	25.6	27.8	30.4	33.0	20.8	26.4	19.7	20.8	22.9	26.0	29.8	19.7	23.2
Mean	17.7	19.0	21.1	24.3	26.2	17.7	21.0	18.7	21.2	23.2	25.9	27.4	18.8	22.5	17.2	18.8	20.5	23.6	25.5	17.3	20.5
			SEd		CD (0.05)					SEd		CD (0.05)					SEd		CD (0.05)		
M			0.40		0.8					0.43		0.9					0.39		0.8		
S			0.48		1.0					0.53		1.1					0.45		0.9		
M X S			0.96		2.0					1.06		2.2					0.95		2.0		

root activities and root exudates higher during tasselling stage. Dehydrogenase activity is only present in viable cells; it is thought to reflect the total range of oxidative activity of soil microflora and consequently may be considered to be a good indication of microbial activity. It is understandable that the mycorrhizal fungus inoculated soils had higher organic carbon status and biomass carbon that may have provided adequate C source for the proliferation of heterotrophic microorganism which eventually increased dehydrogenase activity. Zn nutrition appears to increase dehydrogenases activity of inoculated and uninoculated soils which suggest that Zn is essential for microbial population (Subramanian *et al.*, 2009).

Urease activity

Microbial inoculation, M_4 had recorded highest urease activity (4.0 µg NH_4 – N g^{-1} of soil h^{-1}) followed by M_3 (3.6 µg NH_4 – N g^{-1} of soil h^{-1}) and M_2 (3.3 µg NH_4 – N g^{-1} of soil h^{-1}) over control during tasselling stage (Figure 1). Irrespective of microbial inoculations, S_5 had highest urease activity (5.1 µg NH_4 – N g^{-1} of soil h^{-1}) followed by S_4 (4.0 µg NH_4 – N g^{-1} of soil h^{-1}), S_3 (3.2 µg NH_4 – N g^{-1} of soil h^{-1}), S_2 (2.5 µg NH_4 – N g^{-1} of soil h^{-1}) and S_6 (2.0 µg NH_4 – N g^{-1} of soil h^{-1}) over control during vegetative stage (Table 3). The trend of results obtained during tasselling and harvest stages of crop growth on urease activity was similar to that of vegetative stage. However, the urease activity was quantitatively increased during tasselling stage probably due to higher amount of root exudates which lead to higher microbial population resulting in increased urease activity. The change in microbial community and release of root exudates as a result of mycorrhizal colonization modified the soil enzymes activities (Ayyar *et al.*, 2019).

Phosphatase activity

During vegetative stage, among the microbial inoculations, M_4 had registered highest acid and alkaline phosphatase were 19.7 and 23.8 µg p-nitrophenol g^{-1} of soil h^{-1} followed by M_3 (17.3 and 21.9 µg p-nitrophenol g^{-1} of soil h^{-1}) and M_2 (17.0 and 20.8 µg p-nitrophenol g^{-1} of soil h^{-1}) over control (Figure 1). Among the graded doses of $ZnSO_4$ application S_5 had highest acid and alkaline phosphatase (20.3 and 26.2 µg p-nitrophenol g^{-1} of soil h^{-1}, respectively) followed by S_4, S_3, S_2 and S_6 over control. Similar trend was noticed at tasselling and harvest stage (Table 4 & 5). However, the tasselling stage, acid and alkaline phosphatase was slightly higher which may probably due to plant metabolic and root activities. Soil phosphatase plays on important role in the P nutrition of plants because it mediates the release of inorganic phosphorus from organically bound phosphorus. Phosphatase enzymes are also directly involved in the acquisition of phosphorus by plants. Mycorrhizal colonization and ZSB *viz., Bacillus* sp. has been shown to influence phosphatase activity (Subramanian *et al.*, 2008).

Conclusion

The soil enzyme activities *viz*., dehydrogenase, urease, acid and alkaline phosphatase were high with the application of M_4 treatment followed by M_3. Irrespective of microbial inoculations, graded doses of $ZnSO_4$ application increased the soil enzyme activities. Fungal root colonization was found to be more in soil. M_4 (AM fungi + ZSB) had highest root colonization. Irrespective of the microbial inoculations, increasing doses of $ZnSO_4$ application had increased the root colonization over lower doses of $ZnSO_4$.

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