

Original Research Article

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Effect of Zinc and Microbial Inoculation on Soil Enzyme Activities for Maize (*Zea mays* L.) in Black Soil

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ABSTRACT

Keywords

Maize, *Glomus intraradices* (AM), Zinc solubilizing, *Bacillus* Sp., ZnSO₄, Soil enzymes, Dehydrogenase, Urease and Phosphatases

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Field experiment was conducted in black 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 ZnSO₄. Treatment consisted of two factors viz., Microbial inoculation (M₁: control, M₂: AM fungi, M₃: ZSB and M₄: M₂ + M₃) and graded levels of ZnSO₄ (S₁: 0, S₂: 12.5, S₃: 25, S₄: 37.5, S₅: 50 kg ha⁻¹ and S₆: 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 ZnSO₄ 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 ZnSO₄ 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 ZnSO₄ 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.

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). 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.

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). Soil biological activities have been suggested as one of the important indicator of soil quality (Dick, 1994). It has been proposed that the microbiological and biochemical status of a soil can be used as an early and sensitive indicator of soil ecological stress or restoration processes in both natural and agro-ecosystem (Ruf *et al.*, 2003). Among these microbiological and biochemical factors, soil enzymes have been suggested as potential indicator of soil quality due to their biological nature, simple measurement and rapid response to changes in soil management when compared to other biological properties (Ling *et al.*, 2010). 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).

Soil enzyme activities can be used as potential indicators of nutrient cycling processes and fertility management, particularly in long – term organic and conventional farming systems (Fliebach *et al.*, 2007). 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). Len hard (1956) introduced the concept of determining the metabolic activity of microorganisms in soil and other habitats by measuring DHA. The activity of the DHA is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Garcia *et al.*, 1997) because it is linked to viable cells. Soil DHA reflects the total range of oxidative activity of soil microflora and, consequently it may be a good indicator of microbiological activity in the soil (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). Phosphatases in P mineralization in soil and the response of these enzyme activities to change in environmental factors and agricultural management.

Materials and Methods

Experimental soil

The field experiment was conducted at Cotton Research Station (TNAU), Perambalur. The soil was clay, deep black in colour belonging to *Typic Chromustert* (Peelamedu series). The initial soil was moderately alkaline, non saline, medium in organic status and low, low and medium in available N, P and K, respectively. With regards to the available micronutrient status of the soil, DTPA-Fe, DTPA-Cu and DTPA-Mn were found to be in sufficient level but the availability of DTPA-Zn was found to be deficient.

Field experiments

Treatment consisted of two factors *viz.*, microbial inoculation (M₁: control, M₂:

Arbuscular Mycorrhiza fungi, M₃: Zinc Solubilizing Bacteria and M₄: M₂+M₃) and graded levels of ZnSO₄ (S₁: 0, S₂: 12.5, S₃: 25, S₄: 37.5, S₅: 50 Kg ha⁻¹ and S₆: 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 for maize viz., 250:75:75 kg N, P₂O₅ and K₂O 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 quantities of ZnSO₄ were applied basally as per the treatment schedule.

Data collection and analysis

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 (Lindsay and Norwell, 1978).

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.

Dehydrogenase

Soil dehydrogenase activity was determined by the method outlined by Casida *et al.*, (1964). Ten g of soil sample was taken in 50 mL tubes and it was moistened to 50 per cent of water holding capacity by adding distilled water. Then to each tube, 1 mL of 1 per cent 2,3,5– triphenyl tetrazolium chloride was added. The contents of each tube were then mixed thoroughly at 37± 1°C for 48 hours. After incubation period, 20 mL of methanol was added and stirred for 5 min. The resultant slurry was filtered through Whatman No 42 filter paper. Filtration was continued till disappearance of red colour in the soil. Then the volume of methanol filtrate was made upto 25 mL and the intensity of red colour was determined in a spectrophotometer at a wavelength of 485 nm with methanol as a blank. The dehydrogenase enzyme activity was expressed as (µg of TPF g soil⁻¹ day⁻¹).

Urease

Ten gram of dry and sieved soil was taken in a 100 ml volumetric flask. To this 1.5 ml of toluene was added, mixed well and incubated for 15 minutes. Then 10 ml of 10 per cent urea solution and 20 ml of citrate buffer were

added, mixed thoroughly, stoppered and incubated for 3 h at 37^o C. Then the volume was made up to 100 ml with distilled water, mixed by shaking immediately. The contents were filtered through Whatman No.1 filter paper and 1ml of filtrate was pipetted out into 50 ml volumetric flask. To this 9 ml of distilled water, 4 ml of phenate and 3 ml of NaOCl were added, mixed well and allowed to stand for 20 minutes. The volume was made up to 50 ml and mixed well. The bluish green colour developed was read at 630 nm. Simultaneously a blank was also run. The concentration of urease in the sample was obtained from the standard graph using diammonium sulphate and this enzyme activity was expressed as ($\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) (Bremner and Mulvaney, 1978).

Phosphatase

Five g of soil sample was taken in boiling tube. To this 10 mL of distilled water, 0.25 mL of toluene and 1 mL of 10 mM para nitro phenyl phosphate (PNP) were added and incubated at room temperature for 1 hour. Then 5 mL of 0.5 M CaCl_2 and 20 mL of 0.5 M NaOH were added. The content was filtered using Whatman No 42 and volume was made up to 50 mL with distilled water. The intensity of yellow colour developed was read at 420 nm in spectrophotometer (Varian Cary 50 UV-visible spectrophotometer). The activity of phosphatase was calculated using standard graph and this enzyme activity was expressed as ($\mu\text{g PNP g}^{-1}$ of soil h^{-1}) (Bremner and Tabatabai, 1969).

Results and Discussion

Dehydrogenase activity

Microbial inoculation had marked influence on dehydrogenase activity over control in black soil (Figure 1). The highest dehydrogenase activity was recorded for M₄

(39.5 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}) followed by M₃ (36.4 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}) and M₂ (33.8 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}) over control during vegetative stage (Table 1). Graded doses of Zn application increased the dehydrogenase activity. The highest dehydrogenase activity was noticed for S₅ (50.3 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}) followed by S₄ (44.1 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}), S₃ (37.3 $\mu\text{g TPF g}^{-1}$ of soil day^{-1}) and S₂ (31.7 $\mu\text{g TPF g}^{-1}$ 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, 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. Subramanian *et al.*, (2009) found that Zn nutrition appears to increase dehydrogenases activity of inoculated and uninoculated soils which suggest that Zn is essential for microbial population.

Urease activity

Microbial inoculation, M₄ had recorded highest urease activity (5.5 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) followed by M₃ (4.5 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) and M₂ (4.2 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) over control (Figure 1). Irrespective of microbial inoculations, S₅ had highest urease activity (6.0 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) followed by S₄ (5.5 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}), S₃ (4.7 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) and S₂ (4.2 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil h^{-1}) over control (Table 2).

Initial soil characters

Taxonomy	Typic Chromustert
Textural class	Clay
pH (1:2.5 soil : water)	8.34
EC (dS m⁻¹)	0.30
Free CaCO₃ (g kg⁻¹)	90.4
Organic Carbon (g kg⁻¹)	5.10
CEC (c mol (p⁺) kg⁻¹)	36.2
Alkaline KMnO₄- N (kg ha⁻¹)	221
Olsen- P (kg ha⁻¹)	20.0
Neutral N NH₄Oac- K (kg ha⁻¹)	245
DTPA Zn (mg kg⁻¹)	0.80
DTPA Cu (mg kg⁻¹)	2.52
DTPA Fe (mg kg⁻¹)	10.2
DTPA Mn (mg kg⁻¹)	3.45

Fig.1 AMF and ZSB on soil enzyme activities

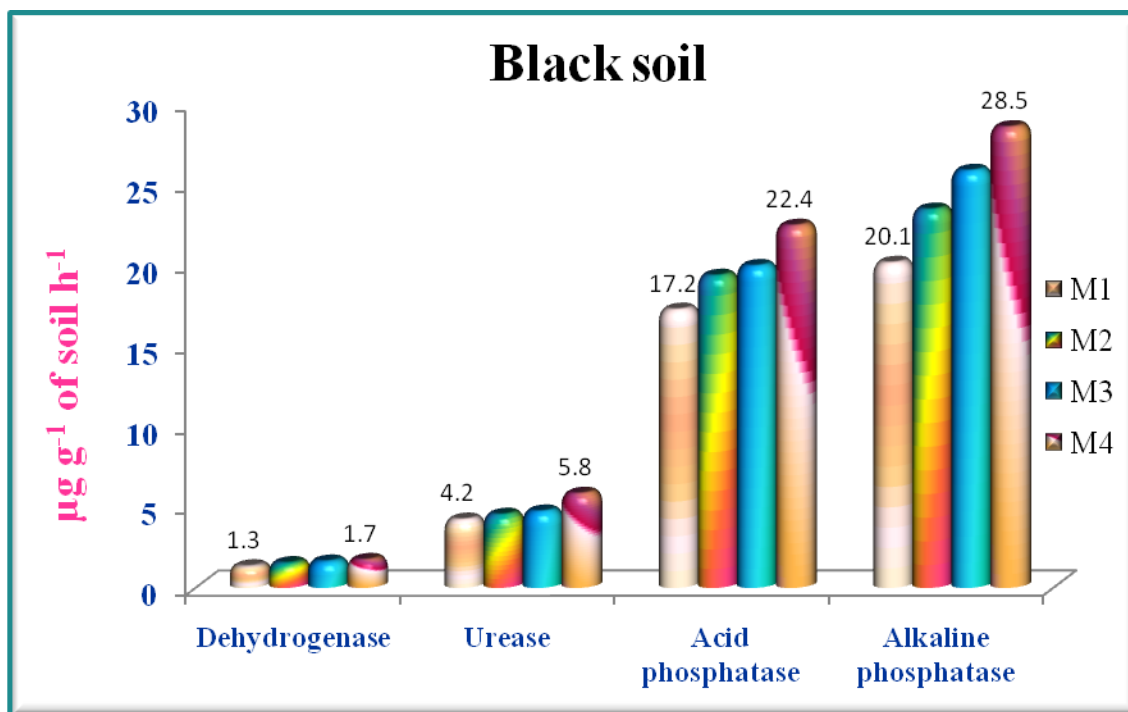


Table.1 Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on soil dehydrogenase activity ($\mu\text{g TPF g}^{-1}$ of soil day $^{-1}$)

Treatments	Vegetative stage							Tasselling 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
M ₁	17.3	26.4	32.6	35.2	42.8	17.5	28.6	18.7	27.4	34.7	37.4	44.8	18.8	30.3
M ₂	22.0	31.3	35.6	44.5	47.2	22.0	33.8	22.8	32.6	37.1	45.4	48.0	22.9	34.8
M ₃	23.0	34.2	39.6	46.1	52.3	23.1	36.4	23.1	35.0	41.5	46.9	53.2	23.2	37.2
M ₄	26.0	34.7	41.2	50.4	58.9	26.0	39.5	26.8	35.2	41.7	51.0	59.4	26.6	40.1
Mean	22.1	31.7	37.3	44.1	50.3	22.2	34.6	22.9	32.6	38.8	45.2	51.4	22.9	35.6
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.67		1.4					0.68		1.4			
S		0.82		1.7					0.82		1.7			
M X S		1.64		3.4					1.63		3.4			

Treatments	Harvest stage						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean
M ₁	16.4	25.4	31.9	34.7	41.6	16.5	27.8
M ₂	21.5	30.4	34.7	43.3	46.7	21.9	33.1
M ₃	23.1	33.6	38.2	46.0	51.8	23.1	36.0
M ₄	25.5	34.0	40.9	48.4	58.1	25.6	38.8
Mean	21.6	30.9	36.4	43.1	49.6	21.8	33.9
		SEd		CD (0.05)			
M		0.65		1.4			
S		0.79		1.7			
M X S		1.59		3.3			

Table.2 Graded levels of Zn with Arbuscular Mycorrhizal fungi and zinc solubilizing bacteria on soil urease activity ($\mu\text{g NH}_4 - \text{N g}^{-1}$ of soil h^{-1})

Treatments	Vegetative stage							Tasselling 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
M₁	3.1	3.6	4.1	4.7	5.1	3.0	3.9	3.3	3.8	4.4	4.9	5.3	3.2	4.2
M₂	3.3	3.8	4.3	5.0	5.6	3.3	4.2	3.5	4.0	4.6	5.3	5.8	3.5	4.5
M₃	3.5	4.1	4.6	5.4	5.9	3.5	4.5	3.7	4.3	4.9	5.6	6.1	3.7	4.7
M₄	4.0	5.1	5.7	6.8	7.3	4.0	5.5	4.2	5.3	6.1	7.2	7.8	4.2	5.8
Mean	3.5	4.2	4.7	5.5	6.0	3.5	4.5	3.7	4.4	5.0	5.8	6.3	3.7	4.8
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.03		0.1					0.07		0.1			
S		0.04		0.1					0.08		0.1			
M X S		0.08		0.2					0.05		0.1			

Treatments	Harvest stage							
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean	
M₁	2.9	3.5	3.8	4.4	4.9	2.8	3.7	
M₂	3.0	3.4	4.0	4.6	5.1	3.0	3.9	
M₃	3.1	3.7	4.2	5.0	5.4	3.1	4.1	
M₄	3.7	4.7	5.2	6.3	6.7	3.6	5.0	
Mean	3.2	3.8	4.3	5.1	5.5	3.1	4.2	
		SEd		CD (0.05)				
M		0.03		0.1				
S		0.03		0.1				
M X S		0.06		0.1				

Table.3 Graded levels of Zn with Arbuscular Mycorrhizal fungi and zinc solubilizing bacteria on soil acid phosphatase activity ($\mu\text{g p-nitrophenol g}^{-1}$ of soil h^{-1})

Treatments	Vegetative stage							Tasselling 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
M ₁	15.0	16.2	16.5	17.9	19.0	15.0	16.6	15.3	16.4	17.6	18.9	19.4	15.3	17.2
M ₂	16.0	17.9	19.1	20.2	21.8	16.0	18.5	16.5	18.6	19.7	21.0	22.5	17.5	19.3
M ₃	17.3	18.2	19.0	20.8	21.7	17.3	19.1	18.1	19.0	19.9	21.4	22.7	18.0	19.9
M ₄	17.6	20.0	23.5	24.2	25.3	17.7	21.4	18.1	21.7	24.7	25.0	26.5	18.3	22.4
Mean	16.5	18.1	19.5	20.8	22.0	16.5	18.9	17.0	18.9	20.5	21.6	22.8	17.3	19.7
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.36		0.8					0.38		0.8			
S		0.43		0.9					0.46		1.0			
M X S		0.86		1.8					0.88		1.8			

Treatments	Harvest stage							
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean	
M ₁	14.8	16.0	16.2	17.3	18.9	14.8	16.3	
M ₂	15.2	17.5	18.3	19.9	21.2	15.3	17.9	
M ₃	16.8	17.7	18.5	20.6	21.1	16.9	18.6	
M ₄	17.4	19.9	22.8	23.6	24.9	17.4	21.0	
Mean	16.1	17.8	19.0	20.4	21.5	16.1	18.5	
		SEd		CD (0.05)				
M		0.35		0.7				
S		0.42		0.9				
M X S		0.85		1.8				

Table.4 Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc solubilizing bacteria on soil alkaline phosphatase activity ($\mu\text{g p-nitrophenol g}^{-1}$ of soil h^{-1})

Treatments	Vegetative stage							Tasselling 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
M₁	15.2	17.8	18.3	19.4	22.9	15.2	18.1	17.1	19.3	20.5	22.6	23.8	17.2	20.1
M₂	19.0	21.3	22.1	24.5	26.9	19.0	22.1	19.8	23.1	24.9	25.8	27.1	19.8	23.4
M₃	19.4	21.4	25.2	27.3	29.6	19.4	23.7	20.0	24.8	26.3	30.5	32.9	20.1	25.8
M₄	22.0	23.9	26.3	30.1	33.2	22.1	26.3	22.9	27.4	29.5	32.6	35.5	23.0	28.5
Mean	18.9	21.1	23.0	25.3	28.2	18.9	22.6	20.0	23.7	25.3	27.9	29.8	20.0	24.4
		SEd		CD (0.05)					SEd		CD (0.05)			
M		0.43		0.9					0.47		1.0			
S		0.51		1.1					0.56		1.2			
M X S		1.02		2.1					1.12		2.3			

Treatments	Harvest stage						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	Mean
M₁	14.5	17.0	17.4	20.4	22.9	14.6	17.8
M₂	18.7	20.4	21.9	24.0	26.8	18.7	21.8
M₃	19.0	21.1	24.3	26.2	28.5	19.0	23.0
M₄	21.8	23.2	25.9	29.2	32.5	21.8	25.7
Mean	18.5	20.4	22.4	25.0	27.7	18.5	22.1
		SEd		CD (0.05)			
M		0.42		0.9			
S		0.50		1.0			
M X S		1.03		2.2			

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.

Phosphatase activity

During vegetative stage, among the microbial inoculations, M₄ had registered highest acid and alkaline phosphatase were 21.4 and 26.3 µg p-nitrophenol g⁻¹ of soil h⁻¹ followed by M₃ (19.1 and 23.7 µg p-nitrophenol g⁻¹ of soil h⁻¹) and M₂ (18.5 and 22.1 µg p-nitrophenol g⁻¹ of soil h⁻¹) over control (Figure 1). Among the graded doses of ZnSO₄ application S₅ had highest acid and alkaline phosphatase (22.0 and 28.2 µg p-nitrophenol g⁻¹ of soil h⁻¹, respectively) followed by S₄, S₃ and S₂ over control. Similar trend was noticed at tasselling and harvest stage (Table 3 and 4). However, the tasselling stage, acid and alkaline phosphatase was slightly higher which may probably due to plant metabolic and root activities. Soil phosphatase plays an 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) have shown that the soil phosphatase activity in the maize rhizosphere increased by 2-3 times as that of uninoculated soil suggesting that this could reduce the pH and stimulate the availability of P.

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