Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2073-2076
Received: 10-05-2019
Accepted: 12-06-2019

PV Geetha Sireesha
Dept. of Soil Science and Agricultural Chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

G Padmaja
Dept. of Soil Science and Agricultural Chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

CH Sreenivas
Dept. of Soil Science and Agricultural Chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

PC Rao
Dept. of Soil Science and Agricultural Chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Correspondence
PV Geetha Sireesha
Dept. of Soil Science and Agricultural Chemistry, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Fertilization regulates soil enzymatic activity under long term experiments of rice-rice cropping system

PV Geetha Sireesha, G Padmaja, CH Sreenivas and PC Rao

Abstract
Different fertilizers may affect soil enzymatic activity and soil fertility dynamics. These effects were investigated in a long term field experiment with rice-rice cropping system (*Oryza sativa* L.) at Godavari Alluvials (Vertic chromusters) in Andhra Pradesh. The enzyme activities *viz.*, urease (38.5, 46.2 µg of NH_4^+-N g^{-1} soil $2h^{-1}$), dehydrogenase (570.1, 596.3 µg of TPF g^{-1} soil day^{-1}) and acid (85.36, 87.96 µg p-nitrophenol g^{-1} soil h^{-1}) and alkaline phosphatase (138.7, 144.8 µg p-nitrophenol g^{-1} soil h^{-1}) were significantly higher in treatment T_4 (100 % RD of NPK + FYM @ 5 t ha^{-1}). The enzyme activity of soils, which is governed by microbial population is also significantly higher in INM treatments. Our results demonstrate that soil enzymatic activity acted as a useful indicator of soil fertility dynamics. Enzymatic activities were positively and significantly correlated with content of organic carbon.

Keywords: Dehydrogenase, INM, phosphatase and urease activity

Introduction
The role that microbial activity plays in ecosystem processes is significant because approximately 80% to 90% of soil processes are mediated by microorganisms (Nannipieri and Badalucco, 2003) [8]. Soil microbial population are the driving force behind regulating soil processes such as organic matter decomposition and nutrient cycling, it is imperative to have a better understanding of the factors that regulate its size, activity, and structure (Masto *et al.*, 2006) [7]. Soils containing a high microbial diversity are characteristic of a healthy soil-plant relationship, whereas those with low microbial diversity are characterized as an unhealthy soil that often hardly responds to environmental changes (Tejada *et al.*, 2011) [24].
Phosphatases find widely in bacteria to mammals, and indicate their importance in fundamental biochemical processes. The term phosphatase in soil is used to describe a group of enzymes that are responsible for the hydrolytic cleavage of a variety of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate. Acid and alkaline phosphatases particularly hydrolyse the ester bonds binding P to C (C-O-P ester bonds) in organic matter. During the process, inorganic P is released from organically bound P such as leaf litter, dead root systems, and other organic debris without concomitant release of C (Harrison, 1983) [3]. Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing (Tarafdar *et al.*, 2001) [23]. Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms, and thus in the cycling of it within the soil (Schneider *et al.*, 2001) [14].
Information on the nature of urease activity in soil was beneficial to develop and employ strategies for nitrogen management. Urease hydrolysis activity is elevated in aerobic condition, and its hydrolysis varied to the plant growth stage within green manure amendment to the crop (Pattnaik *et al.*, 1999) [12]. Urease activity is not well when the bioavailability in the soil is troubled Saliha *et al.* (2006) [13] confirm that urease activity increased along with microbial population in soil amended with liquid organic substrate.

Material and Methods
Site description: Field experiment was conducted in Godavari Alluvials (Vertic chromusters) at Andhra Pradesh Rice Research Institute, Maruteru in Andhra Pradesh. It was located at 81^0 44' E to 81^0 73'E Longitude, 16^0 37' N to 16^0 62' N Latitude and 5 m above mean sea level. The mean annual rainfall is 800-1100 mm falls under Godavari zone of Andhra Pradesh. The monthly mean maximum temperatures during the crop growth period ranged from 27.3 ^{0}C to 35.1 ^{0}C with an average of 30.5 ^{0}C, while the monthly mean minimum temperature ranged from 17.7 ^{0}C to 27.5 ^{0}C with an average of 23.1 ^{0}C. The total rainfall received during the crop growth period was 1319 mm distributed throughout the year and had a total of 62 rainy days.

Experimental design and treatments: This experiment was laid out in randomized block design with eight treatments and three replications. The treatment details during *kharif* and *rabi* were as follows:

T_1 – Control
T_2 – 100 % RD of NPK (120-60-40) + $ZnSO_4$ @ 40 kg ha^{-1}
T_3 – FYM @ 10 t ha^{-1}
T_4 – 100 % RD of NPK + FYM @ 5 t ha^{-1}
T_5 – 50% RD of NPK+ 50% N through Green leaf manure (*Calotropis* spp @ 2.8 t ha^{-1})
T_6 – 50 % RD of NPK + 50 % N through FYM (FYM @ 6.4 t ha^{-1})
T_7 – 50 % RD of NPK + 25 % N through Green leaf manure (*Calotropis* @ 1.9 t ha^{-1}) + 25% N as FYM (FYM @ 6.4 t ha^{-1})
T_8 – 50 % RD of NPK + *Azospirillum* @ 2.5 kg ha^{-1}

The organic sources and biofertilizers were applied at the time of field preparation. MTU 1061was selected and raised in the field with a spacing of 20 cm X 10 cm. The recommended fertilizer dose (120-60-40 kg N, P_2O_5 and K_2O ha^{-1}) was applied in the form of urea, diammonium phosphate and muriate of potash, respectively. Green leaf manure and farmyard manure were applied one week before sowing of crops as per the treatments. *Azospirillum* was applied long with FYM to the treatment plots.

Soil sample analysis: The initial soil was clay loam, neutral in reaction pH 7.0 (Jackson, 1973) [4], non saline in nature EC 1.09 dS m^{-1} (Jackson, 1973) [4], medium in organic carbon OC 0.55 % (Walkley and Black (1934) [25], high in available N 394 kg ha^{-1} (Subbiah and Asija (1956) [19], low in available P 17.0 kg ha^{-1} (Olsen *et al.*, 1954) and high in available K 384 kg ha^{-1} (Jackson, 1973) [4]. Urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai and Bremner (1972) [21]. Dehydrogenase activity in the soil was determined by the procedure given by Casida *et al.* (1964) [2]. The method involved spectrophotometric determination of the Tri Phenyl Formazon (TPF) produced when soil is treated with Triphenyl Tetrazolium Chloride (TTC). The acid and alkaline phosphatase activity was assayed by quantifying the amount of p-nitrophenol released and expressed as µg of p-nitrophenol released g^{-1} soil h^{-1} as described by Tabatabai and Bremner (1969) [20].

Statistical analysis: The data on the observations made were analyzed statistically by applying the technique of analysis of variance for randomized block design as suggested by Panse and Sukhatme (1978) [11].

Results and Discussion

Soil enzyme activity is an indirect indication on the activities of microbes which is directly correlated with soil microbial dynamics. Enzyme activity in the soil environment is considered to be a major contributor of overall soil microbial activity (Burns *et al.*, 2013) [1]. Due to the effects of external disturbance on their activity, enzymes can serve as sensitive indicators of soil quality (Dick *et al.*, 1994: Nedunchezhiyan *et al.*, 2013) [9]. The data pertaining to the effect of different INM treatments on the enzyme activities and their correlations with organic carbon content were presented in the table 1 and 2.

Urease (µg of NH_4^+-N g^{-1} soil $2h^{-1}$)

Among the soil enzymes, urease has received greater attention. It is an important enzyme in soil mediating the conversion of urea to inorganic nitrogen by the hydrolysis of urea to ammonia. Urease activity ranged from 20.13 to 38.50 µg of NH_4^+ released g^{-1} soil $2h^{-1}$ at harvest of *kharif* rice crop. The highest urease activity was (µg of NH_4^+ released g^{-1} soil $2h^{-1}$) recorded in the treatment of 100 % RD of NPK + FYM @ 5 t ha^{-1} (T_4) followed by T_6 (35.63), T_7 (34.25), T_5 (33.58), T_8 (32.38) and T_3 (31.13). However, all the treatments showed significantly different activity from one another.

Urease activity ranged from 23.28 to 46.24 µg of NH_4^+ released g^{-1} soil $2h^{-1}$ at harvest of *rabi* rice crop. The highest urease activity was recorded in the treatment of100 % RD of NPK + FYM @ 5 t ha^{-1} (T_4) followed by T_6 (50 % RD of NPK + 50 % N through FYM) with 43.58 µg of NH_4^+ released g^{-1} soil $2h^{-1}$ (Table 1). However, T_4 and T_6 were on par with each other. The correlation between urease and organic carbon at harvest stages (r=0.823 and r=0.889, respectively, table 2) were positively significant in both *kharif* and *rabi* seasons. Similar results were reported by Yang *et al.* (2008) [26].

Maestre *et al.* (2011) [5] reported a decrease in the urease activity with addition of inorganic N whereas crop residues and organic manure additions increased its activity. Enzyme activities of soils are usually correlated with their organic carbon. Higher levels of organic carbon stimulate microbial activity, and therefore enzyme synthesis.

Dehydrogenase (µg of TPF g^{-1} soil day^{-1})

Dehydrogenase is an enzyme that occurs in all intact viable microbial cells. These soil enzymes function as a measure of the metabolic state of soil microorganisms by relating it to the presence of viable microorganisms and their oxidative capacity. The results pertaining to dehydrodenase activity revealed that highest activity (570.1 and 596.3 µg of TPF g^{-1} soil day^{-1}) was recorded in the treatment receiving 100 % RD of NPK + FYM @ 5 t ha^{-1} (T_4) at harvest of *kharif* and *rabi* rice crops, respectively. Whereas lowest activity observed in control (231.4 and 284.5 µg of TPF g^{-1} soil day^{-1}) at harvest of *kharif* and *rabi* rice crops, respectively. However, T_3 and T_4 were on par with each other (Table 1). Significant positive correlation between dehydrogenase and organic carbon observed in both *kharif* and *rabi* at harvest (r=0.745, 0.732, respectively, Table 2).

It may be attributed to the fact that organic source of nutrient stimulated the activity of microorganisms to utilize the native pool of organic carbon which acts as a substrate for dehydrogenase. These results suggest that changes in the size of microbial populations and respiratory activity occurred in response to the increase in available substrate. In addition, an increase in available substrate corresponds to more readily available C and N pools, which were most likely disproportionally enhanced as a result of manure addition (Sharma and Subehia, 2014) [15].

Acid and Alkaline Phosphatase (µg p-nitrophenol g^{-1} soil h^{-1})

Phosphatases are a group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. Tabatabai and Eivazi (1977) [22] reported that air drying of the soils increased the activity of acid phosphatase and phosphodiesterase but decrease the activity of alkaline phosphatase. In soil ecosystems, these enzymes play critical roles in P cycles (Speir and Ross, 1978) [16] as evidence showed that they were correlated to P stress and plant growth. The data pertaining to acid phosphatase activity revealed that T_3 (100 % RD of NPK + FYM @ 5 t ha^{-1}) showed highest 85.36 and 87.96 µg p-nitrophenol released g^{-1} soil h^{-1} at

harvest of *kharif* and *rabi* rice crops, respectively, followed by T_6 (80.39 and 82.86 µg p-nitrophenol released g^{-1} soil h^{-1}). The lowest activity 42.27 and 44.04 µg p-nitrophenol released g^{-1} soil h^{-1} was recorded in T_1 (control) at harvest of *kharif* and *rabi* rice crops, respectively (Table 1). Significantly correlated with organic carbon content in *kharif* and *rabi* at harvest stages (r=0.856 and r=0.880, respectively).

Among the different INM treatments, alkaline phosphatase activity showed higher activity in T_3 (100 % RD of NPK + FYM @ 5 t ha^{-1}) with 138.69 and 144.87 µg p-nitrophenol released g^{-1} soil h^{-1} at harvest of both *kharif* and *rabi* rice crops respectively (Table 2). The lowest 99.48 and 102.90 µg p-nitrophenol released g^{-1} soil h^{-1} was registered T_1 (control) at harvest of *kharif* and *rabi* rice crops, respectively. The hypothesis is also supported by the alkaline phosphatase activity and organic carbon highly significant positively correlated both in *kharif* and *rabi* at harvest stages (r=0.812 and r=0.820, respectively) (Table 2). The similar results were reported by Meena *et al*. (2013) [6]

This indicates that organic material significantly increases the enzyme activity in soil. Sriramachandrasekharan and Ravichandran (2011) [8] similar reported that the addition of organic substances to the soil served as a carbon source that enhanced microbial biomass and phosphatase activity, showing that these enzymes are of microbiological origin and crop growth stage also significantly influenced soil enzyme activities. Microbes in the soil may be stimulated to obtain more phosphate under NPK + FYM, which results in higher phosphatase activities (Srinivas *et al*., 2015) [17].

Table 1: Long-term effects of INM on enzyme activities of the soils under rice-rice cropping system at Maruteru

Treatments	OC (%)		Acid phosphatase (µg p-nitrophenol g^{-1} soil h^{-1})		Alkaline phosphatase (µg p-nitrophenol g^{-1} soil h^{-1})		Dehydrogenase (µg of TPF g^{-1} soil day^{-1})		Urease (µg of NH_4^+-N g^{-1} soil $2h^{-1}$)	
	Kharif	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*
T_1 – Control	0.58	0.53	42.27	44.04	99.48	102.90	231.4	284.5	20.13	23.28
T_2 – 100 % RD of NPK + $ZnSO_4$ @ 40 kg ha^{-1}	1.15	1.25	64.04	66.37	111.15	109.34	337.9	344.6	25.25	28.35
T_3 – FYM @ 10 t ha^{-1}	1.69	1.85	70.19	71.83	120.15	126.88	538.8	565.4	31.13	40.29
T_4 – 100 % RD of NPK + FYM @ 5 t ha^{-1}	1.39	1.58	85.36	87.96	138.69	144.87	570.1	596.3	38.50	46.24
T_5 – 50 % RD of NPK + 50 % N through Green leaf manure (*Calotropis* spp)	1.65	1.80	76.15	78.30	137.02	131.62	394.9	402.4	33.58	41.43
T_6 – 50 % RD of NPK + 50 % N through FYM	1.70	1.88	80.39	82.86	134.90	141.45	438.6	475.8	35.63	43.58
T_7 – 50 % RD of NPK + 25 % N through Green leaf manure + 25 % N through FYM	1.62	1.75	78.46	80.90	133.96	139.39	452.2	494.1	34.25	42.25
T_8 – 50 % RD of NPK + *Azospirillum* @ 2.5 kg ha^{-1}	1.40	1.60	73.47	74.86	128.47	130.99	404.8	444.5	32.38	40.31
CD (P=0.05)	0.16	0.17	6.93	9.50	12.26	16.45	38.26	40.34	2.45	3.69
SEm ±	0.05	0.06	2.26	3.10	4.00	5.37	12.50	13.17	0.80	1.20

Table 2: Simple correlations between different organic carbon and soil enzyme activities

Variables			r-values	
			Kharif	*Rabi*
Organic carbon	vs	Acid phosphatase	0.8567	0.8800
Organic carbon	vs	Alkaline phosphatase	0.8189	0.8203
Organic carbon	vs	Dehydrogenase	0.7455	0.7317
Organic carbon	vs	Urease	0.8233	0.8891

Acknowledgements

I gratefully acknowledge the Acharya N.G. Ranga Agricultural University, Government of Andhra Pradesh and Professor Jayashankar Telangana State Agricultural University Government of Telangana for their financial assistance provided in the form of stipend during my Ph.D. programme.

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