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Effects of long term application of inorganic and organic fertilizers on soil biological properties of rice

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Abstract
Long Term Fertilizer Experiments were conducted since kharif, 2000 at Regional Agricultural Research Station, Jagtial, Professor Jayashankar Telangana State Agr4icultural University in rice – rice cropping system on an Inceptisol. The present study was undertaken during kharif, 2016 to estimate the soil biological properties (urease, dehydrogenase and phosphatise activity) at different growth stages of rice. In all the treatments, enzymes exhibited higher activity at flowering stage (60 DAT) and thereafter the activity was decreased towards 90 DAT. Continuous application of FYM along with balnced fertilizer application i.e. 100 % NPK recorded highest urease and dehydrogenase activity during both the stages of crop growth. Super optimal dose of NPK (150 %NPK) recorded significantly higher acid and alkaline phosphatase activity, whereas this activity was comparable with 100 % NPK + FYM treatment. Integrated nutrient management practice including FYM and recommended dose NPK showed as best treatment with respect to increase enzymatic properties.

Keywords: kharif, DAT, FYM, NPK

Introduction
Usage of imbalanced fertilizers badly influences production potential and soil health. Integrated nutrient management will not only sustains the crop production but also be effective in improving soil health and enhancing nutrient use efficiency. Enzyme activities are considered as an index of microbiological activity. A better understanding of the role of these soil enzymes in the ecosystem could provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to the changes in soil management. Enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter, composition and activity of living organisms and intensity of the biological processes. Since rice grows in the interactive ecosystem involving soil – microorganism – rice and atmosphere, rice development consequentially affect soil microorganisms and soil enzymatic activities

Among the various enzymes, phosphatase speeds up soil organic phosphorus decomposition and improves soil phosphorous concentration, which is an important index to assess soil phosphorus bio – availability. Phosphatases are capable of catalysing hydrolysis of esters and hydrides of phosphoric acid. In soil ecosystem, these enzymes are believed to play critical roles in 'P' cycle as evidence shows that they are correlated to 'P' stress and plant growth. Apart from being good indicators of soil fertility, phosphatise enzymes play key role in the soil system. Acid phosphatase provides a potential index of mineralisation of soil organic P. Keeping this in view, an experiment was taken up to study the effect of continuous application of fertilizers on soil enzyme activity at different stages of rice.

Materials and Methods
A field experiment was conducted during *karif,* 2016 at Regional Agricultural Research Station, Jagtial (India) on an ongoing long term (16 years) experiment which was initiated in *kharif,* 2000. The soil of the experimental site is a Typic Ustochrept. The soil was clay in texture, with a bulk density of 1.47 Mg m^{-3} and infiltration rate of 0.6 cm hr^{-1}, slightly saline in reaction (pH of 7.1) with a electrical conductivity of 0.47 dSm^{-1}, high in organic carbon content (0.79 %) and low in available N (107.6 kg ha^{-1}), medium in available P_2O_5 (44.2 kg ha^{-1}) and high in available K_2O (440 kg ha^{-1}). The experiment consisted of 12 treatments (Table 1) which were arranged in a randomised block design with four replications. The dimensions of the experimental plot are 12 m x 9 m. The soil samples were collected at 60 (flowering stage) and 90 DAS and analysed for acid and alkaline phosphatise activity by quantifying the amount of p-nitrophenol released and expressed as µg of pnitrophenol released g-1 soil h^{-1}as

described by Tabatabai and Bremner (1969) [18]. Urease activity was assayed by the quantifying the amount of ammonium released and expressed as mg NH_4^+ released g^{-1} soil h^{-1} described by Tabatabai and Bermner (1979). Dehydrogenase assays based on the 2,3,5-triphenyl tetrazolium chloride (TTC) to the creaming red colored triphenyl formazen (TPF) and it was quantifying the amount TPF released and expressed as of mg TPF produced g^{-1} soil d^{-1} described by Cassida *et al*., 1964 [3].

Table 1: Details of treatments and source of nutrient

Treatment No	Treatment	kg N-P_2O_5-K_2O ha^{-1}	
T_1	50%NPK	60-30-20	-
T_2	100%NPK	120-60-40	-
T_3	150%NPK	180-90-60	-
T_4	100% NPK +HW	120-60-40	Only hand weeding
T_5	100% NPK+ $ZnSO_4$	120-60-40	10 kg ha^{-1}(in *kharif*)
T_6	100% NP	120-60-0	-
T_7	100% N alone	120-0-0	-
T_8	100% NPK+FYM	120-60-40	10 t ha^{-1}(in each *kharif*)
T_9	100% NPK-S	120-60-40	P through DAP
T_{10}	FYM	-	10 t of FYM each in *kharif* and *rabi* per ha
T_{11}	Control	-	No fertilizers, No manures
T_{12}	Fallow	-	No crop, No fertilizers

Results and Discussion

Acid and alkaline phosphatase activity (µg PNP released g^{-1}soil h^{-1}) in soil

Soil enzyme activities increased with increasing rate of NPK application. Significantly high acid phosphatase activity of 126.87 and 113.55 µg*p* nitrophenol (PNP) released g^{-1} soil hr^{-1} was recorded at 60 and 90 DAT respectively during *kharif*, 2016 in the treatment T_3 (150% NPK). Acid phosphatase activity in T_3 was statistically on par with the treatments of T_8 (100% NPK+FYM) and T_2 (100 % NPK) which were recorded 109.49 and 106.86 µgPNP released g^{-1} soil hr^{-1}during 90 DAT (Table 2) The acid phosphatase activity of 80.81 and 69.61 µgPNP released g^{-1} soil hr^{-1} were recorded due to the application of 100 % N (T_7) during 60 and 90 DAT respectively and it was lowest when compared to other treatments at both the stages of crop growth. It is evident that acid phosphatase activity in T_7 (100 % N) treatment was on par with the treatments of T_6 (100% NP) and control (T_{11}) at 60 DAT. It is also evident that comparable acid phosphatase activity with T_7 (100% N) was observed in T_6 (100 % NP) treatment during 90 DAT of rice.

Application of FYM @ 10 t ha^{-1} significantly increased the acid phosphatase activity by 17.1 percent as against control during 90 DAT indicates that application of organic manure increased the activity of acid phosphatase. This might be due to added quantity of organic matter which inturn increased the organic carbon and nitrogen (Kadlag *et al.,* 2008) [6]. Similarly, the organic acids produced during decomposition of farmyard manure might have resulted in enhanced enzyme activity. Similar results were reported by Benitez *et al.,* (2000) [2], Sheng *et al.,* (2005) [15], Bhattacharya *et al.,* (2005) [1] and Reddy and Reddy (2012) [14].

Alkaline phosphatase activity are presented in Table 2 and depicted in Fig. 4.13. The alkaline phosphatase activity was highest when the crop received 150 % NPK (T_3) and the activities were being 133.56 and 124.61 µg PNP released g^{-1} soil h^{-1} during 60 and 90 DAT respectively. The treatment which received 100% NPK+FYM recorded alkaline phosphatase activity of 127.37 and 106.66 µg PNP released g^{-1} soil h^{-1}at 60 and 90 DAT, respectively during *kharif*, 2016.The lowest alkaline phosphatase activity of 91.85 and 83.00 µg PNP released g^{-1} soil h^{-1} were registered with the application of 100 % N (T_7) at 60 and 90 DAT respectively and it was on par with control treatment during both the stages of crop growth. Application of FYM @ 10 t ha^{-1} significantly increased the alkaline phosphatase activity by 21.5 and 15.2 percent during 60 and 90 DAT, respectively over their control. Application of 100 % NPK + FYM (T_8) significantly increased the alkaline phosphatase activity by 15.0 percent as against over 100 % NPK treatment during 60 DAT, however the significance difference was not found between 100 % NPK and 100 % NPK + FYM treatments at 90 DAT.

In all the treatments, the acid and alkaline phosphatase activity exhibited highest activity at 60 DAT and there after the activity decreased gradually to 90 DAT. Similar results were also reported by Reddy and Reddy, (2012) [14] and Rai and Yadav (2011) [12].

Urease activity (mg NH_4^+ released g^{-1} soil h^{-1}) in soil

In all the treatments, urease enzyme exhibited higher activity at flowering stage (60 DAT) and thereafter the activity decreased towards 90 DAT (Table 3). Significantly highest urease activity of 9.34 and 4.26 mg NH_4^+ released g^{-1} soil h^{-1} was recorded at 60 and 90 DAT respectively due to the application of 100 % NPK + FYM (T_8) followed by 8.17 and 3.92 mg NH_4^+ released g^{-1} soil h^{-1} at 60 and 90 DAT by the application of 150 % NPK (T_3). The higher urease activity in 150% NPK treatment compared to lower levels of NPK could be due to the luxurial growth of crop leaving a large number of stubbles in soil which on decomposition becomes a source of carbon and energy and results in production of diverse extracellular enzymes. Kanchikerinath and Singh (2001) [7] found that urease activity increased significantly with balanced application of inorganic fertilizers.

The lowest urease activity of 5.11 and 2.11 mg NH_4^+ released g^{-1} soil h^{-1}at 60 and 90 DAT respectively in control (T_{11}) plot. The lowest urease activity in control plot was statistically on par with 100 % N (2.68 mg NH_4^+ released g^{-1} soil h^{-1}) and 50 % NPK (2.94 mg NH_4^+ released g^{-1} soil h^{-1}) at 60 DAT, whereas this was not observed at 30 DAT. The mineral fertilizer application in treatments receiving 100% N and control plots recorded lower urease activity and this could be attributed to lack of sufficient substrate *i.e.,* organic carbon which acts as a source of energy for proliferating microorganisms. Similar results were also reported by Ramalakshmi (2011) [13].

Application of FYM @ 10 t ha^{-1} (T_{10}) significantly increased

the urease activity to 7.24 and 3.19 mg NH_4^+ released g^{-1} soil h^{-1} at 60 and 90 DAT over their respective control treatments. The increased enzyme activity with 100% NPK+FYM and FYM treatments may be ascribed to the fact that the organic matter added to rice soil (with water more than saturation capacity) enhances microbial fermentation of the organic compounds producing compounds which are subjected to reduction and oxidation. A number of fermentation products like ethanol, acetate, lactate act as rich energy sources for proliferating microorganisms and the microorganisms release these enzymes into the soil for the reactions necessary to release energy (Bhattacharya *et al*., 2005) [1].

The results of the present study indicate that profile of activities of urease showed highest activity at flowering stage and after that a decreasing trend was observed. Similar results were reported at different growth stages of rice by Vajantha *et a.,* (2010) [19] and in different rice growing soils of Karnataka by Nayak and Manjappa (2010) and in rice crop in different cropping systems under integrated nutrient management systems by Ramalakshmi *et al.* (2012).

Table 2: Effect of long term fertilizer and manure application on acid and alkaline phosphatise activity

Treatments	Acid phosphatise activity (µg p-nitrophenol released g^{-1} soil h^{-1})		Alkaline phosphatise activity (µg p-nitrophenol released g^{-1} soil h^{-1})	
	60 DAT	90 DAT	60 DAT	90 DAT
T_1 - 50% NPK	93.03	92.87	97.92	97.20
T_2 - 100% NPK	104.70	106.86	108.62	101.02
T_3 - 150% NPK	126.87	113.55	133.56	124.61
T_4 - 100% NPK + HW	108.81	99.58	104.89	103.28
T_5 - 100% NPK + Zn	103.63	94.91	101.71	101.7
T_6 - 100% NP	85.96	77.65	92.38	85.27
T_7 - 100% N	80.81	69.61	91.85	83.00
T_8 - 100% NPK + FYM	111.95	109.49	127.37	106.66
T_9 -100% NPK –S	108.31	93.54	106.70	102.45
T_{10}-FYM	99.85	99.64	118.74	105.30
T_{11}- Control	90.45	82.60	93.20	89.25
T_{12} –Fallow	98.79	97.63	111.71	107.05
S.E(m)	5.57	5.53	3.74	6.18
CD(0.05)	11.37	11.29	7.64	12.62
CV (%)	7.78	8.27	4.95	8.74

Table 3: Effect of long term fertilizer and manure application on urease and dehydrogenase acivity

Treatments	Urease activity (mg NH_4^+ released g^{-1} soil h^{-1})		Dehydrogenase activity (mg TPF produced g^{-1} soil d^{-1})	
	60 DAT	**90 DAT**	**60 DAT**	**90 DAT**
T_1 - 50% NPK	6.94	2.94	3.49	2.57
T_2 - 100% NPK	7.93	3.52	4.16	2.75
T_3 - 150% NPK	8.17	3.92	4.21	3.29
T_4 - 100% NPK + HW	7.57	3.32	3.83	2.77
T_5 - 100% NPK + Zn	7.31	3.35	3.70	3.15
T_6 - 100% NP	7.09	3.09	3.36	2.78
T_7 - 100% N	6.68	2.68	3.29	2.21
T_8 - 100% NPK + FYM	9.34	4.26	4.85	3.77
T_9 -100% NPK –S	7.10	3.24	3.56	3.08
T_{10}-FYM	7.24	3.19	3.70	3.20
T_{11}- Control	5.11	2.11	2.63	2.03
T_{12} –Fallow	7.20	3.20	3.48	3.6.5
S.E(m)	0.48	0.43	28.9	0.23
CD(0.05)	0.98	0.88	0.41	0.47
CV (%)	9.32	18.64	7.55	11.25

Dehydrogenase (mg TPF produced g^{-1} soil d^{-1}) in soil

Soil dehydrogenase activity is an indicator of total soil microbial activity, it reflects the fertility status of soil.

Similar to urease activity the dehydrogenase activity also decreased after flowering i.e., from 60 DAT to 90 DAT (Table 3). In all the treatment the highest activity was observed at flowering when compared to 90 DAS. The highest dehydrogenase activity of 4.85 and 3.77 mg TPF produced g^{-1} soil d^{-1} were recorded at 60 and 90 DAT due to the application of 100 % NPK + FYM (T_8) fallowed by 4.21 and 3.29 mg TPF produced g^{-1} soil d^{-1} were recorded at 60 and 90 DAT in 150 % NPK (T_3) treatment. This significant increase in dehydrogenase activity in NPK+FYM and FYM might be due to addition of organic matter through FYM which increased the microbial activity and microbial biomass. Similar results were also reported by several workers (Prakash*et al.,* 2002, Sheng *et al.,* 2005 [15], Tejada and Gonzalez, 2009 and Ramalakshmi *et al.,* 2012).

The lowest dehydrogenase activity of 2.63 and 2.03 mg TPF produced g^{-1} soil d^{-1} were recorded at 60 and 90 DAT respectively in control (T_{11}) plot. The activity in control plot was statistically on pat with the activity with the application 100 % N at 90 DAT, whereas significant effect was observed in between control and 100 % N treatments. The treatment 100% N recorded significantly lower dehydrogenase activity than other treatments except control. The lower activity of dehydrogenase under 100% N showed the direct inhibitory effect of imbalanced fertilization in making the carbon less available, increase in the retention of carbon and partial sterilization affect from the increased osmotic potential of soil solution due to the fertilizer salts. These conditions decreased the activity of dehydrogenase. Kaur and Brar (2008) reported

similar results with respect to dehydrogenase activity.

The dehydrogenase activity was increased with increasing graded levels of NPK i.e., from control to 150 % NPK and the dehydrogenase activity was increased from 2.63 (control) to 4.21 mg TPF produced g^{-1} soil d^{-1} at 60 DAT and from 2.03 to 3.29 mg TPF produced g^{-1} soil d^{-1} at 60 DAT. This confirms that application of balanced fertilizers NPK either alone or in combination with organic manures maintained active pools of C and N in the soil surface layer due to increased plant biomass addition in these treatments. This indicated that organic pools of carbon associated nutrients, particularly nitrogen, could be maintained in rhizosphere zone for maintaining soil organic matter and enzyme activity there by sustaining soil quality in these treatments. Similar findings were also reported by Bharati *et al.,* (2011).

Conclusions

From the foregoing discussion, it is clear that the in all the treatments soil enzymes (Urease, Dehydrogenase and Phosphatase) exhibited more activity at flowering stage (60 DAT), there after activity was reduced to 90 DAT of rice. Integrated application of fertilizers along with organic manures (100 % NPK + FYM) registered significantly highest urease activity of 9.34 and 4.26 mg NH_4^+ released g^{-1} soil h^{-1} and dehydrogenase activity of 4.85 and 3.77 mg TPF produced g^{-1} soil d^{-1} at 60 and 90 DAT of rice. Application of 150 % NPK registered an acid phosphatase activity of 126.87 and 113.55 µg *p* nitrophenol (PNP) released g^{-1} soil hr^{-1} and an alkaline phosphatase activity of 133.56 and 124.61 µg PNP released g^{-1} soil h^{-1} at 60 and 90 DAT of rice. With respect to all the enzyme the lowest activity was recorded due to the application of 100 % N at both the stages of crop growth.

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