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Response of soil enzymes to applied rice residues and its products

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Abstract
Soil enzymes are considered as ideal indicators of soil quality with their direct involvement in nutrient cycles and thus nutrient transformations. A study was conducted to evaluate the effects of different rice residues (rice straw and husk) and its products (vermicompost and biochar) along with inorganic fertilizers on soil enzyme activity at different stages of rice. Nine treatments were applied of which organic amendments (rice straw, husk, vermicomposted rice straw, vermicomposted rice husk, rice straw biochar, rice husk biochar, and farmyard manure) were applied at the rate of 5t ha^{-1}. Enzyme activity followed an increasing trend upto panicle initiation stage of rice after which it decreased in all treatments. Vermicomposted rice straw application along with inorganic fertilizers stimulated the activities of soil enzymes like dehydrogenase, urease, and acid phosphatase at a higher rate as against biochar and rice residues.

Keywords: rice residues, vermicompost, biochar, dehydrogenase activity, urease activity, acid phosphatase activity

Introduction
Rice is the most important food crop of the developing world, the staple food of more than half of the world's population, and an important residue producing crop in Asia. Straw and husk are the major residues that are generated in copious amounts during rice cultivation and processing. In India, straw and husk production are 168.50 million tonnes and 33.70 million tonnes respectively (FAO, 2017) [4]. Rice residues are good sources of nutrients and primary source of organic matter. Many a times, the crop residues are looked upon as waste materials that calls for disposal, but it has been increasingly realized that they are important organic resources and not wastes. Disposal of the residues in land-fills and burning are equally dangerous. Appropriate management of crop residues for agricultural use is of great significance and relevance. One of the best management options is the recycling of crop residues, which converts the surplus farm waste into useful products that meets nutrient requirement of crops. Impacts of residue management on soil organic matter and long term fertility is becoming more relevant in the context of soil quality, as evident from many of the earlier studies. As far as soil fertility restoration and soil health are concerned, it is ideal to recycle this organic waste through vermicomposting and carbonisation.
Soil is a living system where all biochemical activities proceed through enzymatic processes. Soil enzymes are a group of enzymes that are usual inhabitants in the soil playing a major role in maintaining soil fertility and soil health. These enzymes have been suggested as suitable indicators of soil quality because they are strictly related to the nutrient cycles and transformations. Based on these perspectives, the present study was conducted with the objective to assess the response of soil enzymes to applied rice residues and its products along with soil test based nutrient recommendation in lowland rice.

Materials and Methods
A field experiment was conducted to study the response of soil enzymes on applied rice residues and their products in lowland rice, with rice variety Uma as the test crop. The experiment consisted of nine treatments with three replications *viz*., absolute control (T_1), Adhoc KAU organic POP (T_2), and treatments T_3 to T_9 comprised of soil test based nutrient recommendation along with FYM (T_3), vermicomposted rice husk (T_4), vermicomposted rice straw (T_5), rice husk biochar (T_6), rice straw biochar (T_7), rice husk (T_8), and rice straw (T_9) at 5t ha^{-1}. Fertilizers were given as per the package of practices recommendations of KAU

modified based on soil test results (KAU, 2016) [7]. Five tonnes of FYM and 600 kg neem cake ha^{-1} were applied in T_2 (KAU, 2017) [8]. The effects of treatments on soil enzymes namely dehydrogenase, urease, and acid phosphatase were studied at different stages of crop *viz*., before planting, at tillering, panicle initiation and at harvest.

Enzyme assay

The dehydrogenase activity was determined as per the procedure explained by Casida *et al*. (1964) [2] before and after the incubation. For that, 1g of soil was weighed into an air tight screw capped test tube of 15 mL capacity, to which 0.2 mL of 3 per cent triphenyl tetrazolium chloride solution was added. Then, 0.5 mL of 1per cent glucose solution was added into each tube. Gently tapped the bottom of the tube to drive out the trapped oxygen fully there by forming a water seal above the soil thus ensuring freedom from air bubbles. Tubes were then incubated at 28±0.5° C for 24 h. After incubation, 10 mL methanol was added and the contents were vigorously shaken. Samples were allowed to stand for six hours and the intensity of pink colour was read in spectrophotometer at a wave length of 485 nm.

The urease activity of the field samples were estimated according to the method outlined by Tabatabai and Bremner, 1972 [11]. To 5g soil in a 50 mL volumetric flask, 0.2 mL toluene, and 9 mL of THAM buffer (Tris (hydroxymethyl) amino methane) were added and shaken for few seconds to mix the contents properly. Then, 0.2M urea (1 mL) solution was added and again swirled for few seconds and kept for incubation at 37° C for 2h. After that, 35 mL of KCl-Ag_2SO_4 solution was added. Then the contents were mixed properly and allowed to stand until the contents got cooled to room temperature. Final volume was made up to 50 mL by the addition of KCl-Ag_2SO_4 solution. 20 mL of aliquot of the suspension was pipetted into a 100 mL distillation flask and determined the ammoniacal nitrogen released by steam distillation of this aliquot with 0.2 g of MgO for four minutes. Controls were performed as per the same procedure described for assay of urease activity. But 1 mL of 0.2M urea solution was added after the addition of 35 mL of KCl-Ag_2SO_4 solution. Phosphatase activity was estimated as per the procedure defined by Tabatabai and Bremner, 1969 [10]. For each sample, two sets of 1 g soil were weighed in 50 mL volumetric flasks. Among the two, one was kept as control. Toluene (0.2 mL) and MUB buffer at pH 6.5 (4 mL) were added to samples. After that for one set of samples, 1 mL of p- nitro phenyl phosphate solution was added. Contents were mixed properly and kept for 1h incubation. After that, 1 mL of $CaCl_2$ and 4 mL of 0.5 M NaOH were added. The contents were swirled for few seconds for mixing. In control, para nitro phenyl phosphate solution was added after incubation. All the suspensions were filtered quickly through Whatman No. 2 filter paper. The yellow colour intensity of the filtrate was measured using spectrophotometer at a wave length of 440 nm.

Result and Discussion

Dehydrogenase activity

Data on dehydrogenase activity at different growth stages of rice after treatment application are shown in Table 1. Dehydrogenase activity was found to increase in all the treatments upto panicle initiation stage followed by a decrease at harvest.

Before planting, the treatments T_5 (soil test based nutrient recommendation+ vermicomposted rice straw) and T_3 (soil test based nutrient recommendation + FYM) recorded statistically higher dehydrogenase activity than the other treatments. This might be due to the presence of easily degradable organic substrates in these treatments. Application of organic amendments significantly enhanced dehydrogenase activity possibly due to the utilization of energy in the form of carbon by microorganisms from the organic materials (Sarma *et al*., 2017) [9].

Table 1: Effect of treatments on dehydrogenase activity

Treatments	Dehydrogenase activity (µg TPF $g^{-1}day^{-1}$)			
	Before planting	At tillering	Panicle initiation	Harvest
T_1	38.57^{e}	47.43^{g}	52.36^{g}	36.42^{f}
T_2	55.44^{b}	73.57^{c}	82.64^{c}	61.48^{c}
T_3	57.92^{a}	76.78^{b}	86.21^{b}	64.21^{b}
T_4	55.18^{b}	71.30^{d}	79.36^{d}	60.55^{c}
T_5	59.66^{a}	79.78^{a}	90.84^{a}	66.36^{a}
T_6	47.75^{c}	60.67^{e}	67.13^{e}	52.05^{d}
T_7	48.02^{c}	60.94^{e}	67.40^{e}	52.33^{d}
T_8	40.83^{d}	53.55^{f}	59.91^{f}	45.07^{e}
T_9	47.28^{c}	60.00^{e}	66.36^{e}	51.54^{d}

At tillering, panicle initiation and harvest, T_5 recorded highest dehydrogenase activity. Lower dehydrogenase activity was registered by T_1 (absolute control) compared to other treatments because of lack of any external inputs in these plots.

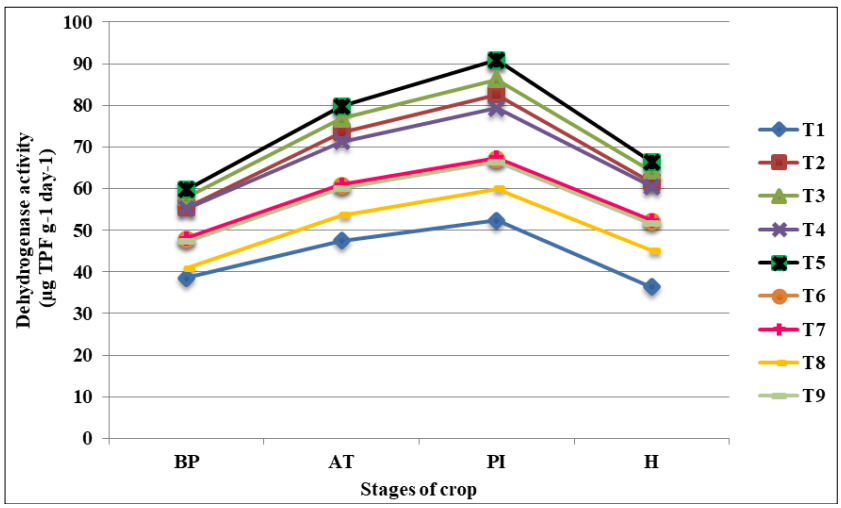

Fig 1: Effect of treatments on dehydrogenase activity at different stages of crop

Dehydrogenase activity was found to increase upto panicle initiation (Figure 1) and this might be due to the decomposition of organic materials and also the increased root activity. Lower dehydrogenase activity at harvest was mainly due to the dry condition as well as reduced root activity that prevailed at harvest of rice. Dehydrogenase activity is strongly affected by soil moisture. When soil becomes dry, the water potential increases affecting negatively the microbial activity and thus the enzyme activity (Geisseler *et al*., 2011) [5].

Urease activity

Urease enzyme plays an important role in nitrogen cycling and is widely distributed in soil. Urease activity at different stages of crop growth after the application of treatments are given in Table 2. Before planting, treatment T_5 (soil test based nutrient recommendation +vermicomposted rice straw) registered higher urease activity followed by T_3 (soil test based nutrient recommendation+ FYM).

Table 2: Effect of treatments on urease activity

Treatments	Urease activity (µg N-NH_4 g^{-1} hr^{-1})			
	Before planting	At tillering	Panicle initiation	Harvest
T_1	32.54^{h}	37.47^{f}	44.93^{g}	29.57^{f}
T_2	60.86^{d}	89.93^{b}	99.48^{c}	72.01^{b}
T_3	77.53^{b}	96.96^{a}	106.67^{b}	84.01^{a}
T_4	70.36^{c}	88.29^{b}	97.32^{c}	71.94^{b}
T_5	81.63^{a}	100.69^{a}	110.68^{a}	89.59^{a}
T_6	48.28^{ef}	64.75^{cd}	72.98^{de}	53.77^{cd}
T_7	50.84^{e}	67.32^{c}	75.67^{d}	56.33^{c}
T_8	40.83^{g}	56.90^{e}	64.96^{f}	46.17^{e}
T_9	46.25^{f}	62.61^{d}	70.79^{e}	48.96^{de}

At tillering, urease activity in T_5 and T_3 were on par and higher than that in other treatments. This might be due to the greater availability of nitrogen resources from the added organic and inorganic materials in the treatment. Treatment T_5 recorded higher activity at panicle initiation stage as well as at harvest. Urease activity is crucial in the transformation of urea, therefore, with the conjoint application of inorganic fertilizer (urea) and vermicomposted rice straw in T_5 its activity increased. As always, absolute control registered lowest urease activity. This may be due to the lack of substrates in T_1 (absolute control). Guan (1987) [6] reported that more availability of nitrogen resource for soil microorganisms resulted in higher soil urease activity because of substrate availability. Urease activity is preferably higher in paddy soil with abundant organic matter and higher levels of total nitrogen content (Zeng Lu- Sheng *et al*., 2005) [12]. The lower urease activity in biochar amended plots compared to vermicompost applied plots might be due to the highly recalcitrant nature of biochar.

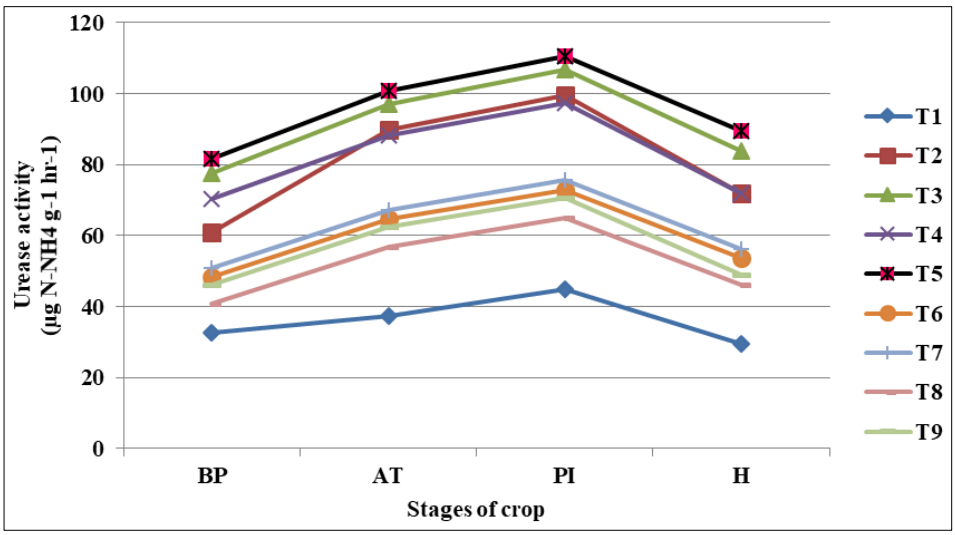

Fig 2: Effect of treatments on urease activity at different stages of crop

Urease activity increased upto panicle initiation followed by a decrease at harvest (Figure 2). Reduction in urease activity at harvest might be due to dry condition of soil and reduced root activity with the crop reaching late maturity stage.

Acid phosphatase activity

Acid phosphatase is contributed both by the plant roots as well as soil inhabiting microbes (Chhonkar *et al*., 2007) [3] and it cleaves the phosphate from organic substrates and is involved in the phosphorus cycle in soil. The effects of various treatments on acid phosphatase activity are given in Table 3. The conjoint application of soil test based nutrient recommendation and vermicomposted rice straw (T_5) registered higher acid phosphatase activity at all stages. This might be due to the phosphorus status in T_5 contributed by inorganic fertilizer and vermicomposted rice straw.

Table 3: Effect of treatments on acid phosphatase activity

Treatments	Acid phosphatase activity (µg PNP $g^{-1}hr^{-1}$)			
	Before planting	At tillering	Panicle initiation	Harvest
T_1	37.62^{f}	42.86^{g}	45.48^{g}	34.18^{g}
T_2	68.31^{c}	85.38^{c}	93.91^{c}	72.50^{c}
T_3	80.62^{b}	98.05^{b}	106.76^{b}	84.98^{b}
T_4	79.37^{b}	95.43^{b}	103.42^{b}	83.38^{b}
T_5	86.85^{a}	105.25^{a}	113.88^{a}	91.35^{a}
T_6	58.24^{d}	72.70^{d}	79.93^{d}	61.85^{d}
T_7	58.27^{d}	72.75^{d}	79.99^{d}	61.87^{d}
T_8	48.17^{e}	60.25^{f}	66.28^{f}	51.18^{f}
T_9	55.16^{d}	68.52^{e}	75.20^{e}	58.50^{e}

As expected, lowest enzyme activity was found in T_1 and this might be due to the lack of external inputs. Sarma *et al*. (2017) [9] reported that the conjoint application of organic amendments and inorganic fertilizers significantly increased phosphatase activity than lone application of organic amendments and inorganic fertilizers. They also pointed out that among the organic amendments, vermicompost followed by FYM stimulated phosphatase activity at a higher rate than biochar.

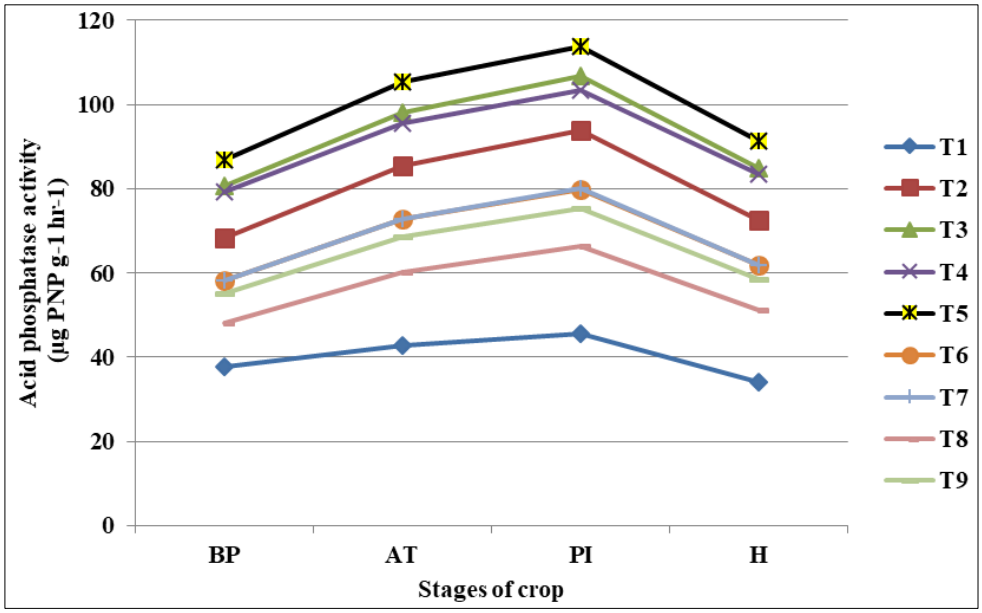

Fig 3: Effect of treatments on acid phosphatase activity at different stages of crop

Acid phosphatase activity followed an increasing trend upto panicle initiation and thereafter it decreased in all treatments (Figure 3). Decrease in acid phosphatase activity at harvest is mainly due to the dry soil condition that prevailed during harvest of rice and reduced root activity of rice at its later maturity stage. Aparna (2000) [1] claimed that decrease in enzyme activity was mainly due to the lack of optimum conditions *viz*., availability of moisture, substrates and nutrients at harvest.

The higher activities of dehydrogenase, urease, and acid phosphatase were recorded in vermicomposted rice straw amended soil than that of biochar and residue amended soil.

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