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Effect of biochar, carpet waste, FYM and PGPR on soil biological properties under organically grown rice (*Oryza sativa* L.)

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ABSTRACT

A field experiment was conducted at Agricultural Research farm, BHU, on sandy clay loam soil with rice (var. HUR-105) to find out the influence of biochar, carpet waste, FYM and PGPR on biological properties of alluvial soils of Varanasi. The enzyme dehydrogenase activity ranged from 95.9 to 231.6 µg TPF produced g $^{-1}$soil day^{-1} whereas the enzyme alkaline phosphatase activity ranged from 49.1 to 108.7 µg PNP produced g $^{-1}$ soil hr^{-1}. The activity of both the enzyme was highest at 80 days after transplanting (DAT) of rice in treatment receiving biochar + carpet waste + FYM + PGPR. The microbial population of bacteria, actinomycetes and fungi were ranged 23-48 ×105, 26-12 ×104 and 10.3-20 ×103 cfu g $^{-1}$ of soil respectively. The microbial population was highest at 80 DAT of rice than 40 and 120 DAT of rice crop. The maximum microbial population was found in T $_{10}$ which receive biochar + carpet waste + FYM + PGPR at all the growth events. The biological properties of soil were positively and significantly correlated with organic carbon content of soil.

Key words: *Biochar, carpet waste, dehydrogenase, alkaline phosphatase, bacteria, fungi and actinomycetes*

INTRODUCTION

Intensification of rice cultivation is necessary to meet the food demand of increasing human population, especially in India where approximately 80% of rice is grown and consumed (Roy et al., 2011; Kumar et al., 2016). Submerged rice soil ecosystems are predominantly anaerobic and are different from upland in respect to physico-chemical and biological properties (Adhya and Rao, 2005). The continuous application of agrochemicals such as inorganic fertilizers and pesticides has adversely affected biological composition of the soil biota and their activities while the use of organic fertilizers maintains soil health (Khan et al., 2010). Organic agriculture is a production system which avoids or largely excludes the use of synthetically produced fertilizers, pesticides and growth regulators relying instead on crop rotations, crop residues, animal manures, legumes, green manures, and aspects of biological pest control to maintain soil productivity (Lampkin, 1990). Organic residues including green manure, animal waste and farm yard manure (FYM) are traditionally applied to rice soil in order to maintain the soil organic matter (SOM) status, to increase the levels of plant nutrients and to improve the physical, chemical and biological soil properties that directly and indirectly affect soil fertility (Nayak et al., 2012). Now-a-days the insufficient availability of organic fertilizer like FYM, compost etc. offer a choice of alternate use of biochar, carpet waste as a sources of organic fertilizer.

Biochar is a high carbon, fine-grained residue that today is produced through modern pyrolysis processes, which is the direct thermal decomposition of biomass in the absence of oxygen. Biochar in croplands had been projected worldwide as a potential option to increase soil organic carbon (SOC) stock while improving soil fertility and ecosystem functioning (Sohi et al., 2010). The large porosity of biochar provides surfaces for absorption of inorganic nutrients as well as organic substances and gases might provide ideal environments for soil microbes to colonize and grow (Thies and Rillig, 2009). Biochar as a component of compost can have synergistic benefits and increase microbial activity and reduce nutrient losses during composting, undergoing composting helps to charge the biochar with nutrients without breaking down the biochar substance in the process. Biochar has been shown to act as an absorber of NH_3 and water-soluble NH_4^+ and might therefore reduce losses of N during composting of manure. Wool industry fibrous solid wastes have been shown to a have content of some important plant nutrients content and good water-holding capacity, which can potentially benefit their uses in agriculture. Wool carpet waste contains nutrient elements that can provide fertilizing benefit over and above that of non-biodegradable mulch or simple organic mulch. The wool in carpet contains significant amounts of nitrogen and sulphur, and the latex backing is predominantly calcium carbonate filler. Overall, the range of major elements reported for carpet waste in the literature are 5.5 - 17% nitrogen, 1.2 - 3.5% sulphur and 10.8% calcium (McNeil et al., 2007; Mesman et al., 2007). Recent analyses of UK wool rich carpet waste have shown that wool-rich carpet waste has a content of N and S comparable to farm yard manures, but low contents of phosphate, potash and Mg. Wool carpet being largely biodegradable and containing plant nutrients as well as possessing the physical properties to provide weed suppression, moisture retention, moderation of soil temperature and soil stabilization thus opening the opportunity for environmentally friendly disposal options as alternatives to land filling and incineration (Mesman et al., 2006). Soil fertility and microbial functional diversity (Nannipieri et al., 2002; Maurya et al., 2011) can be evaluated by soil enzyme activities which are necessary for the life processes of soil micro-organisms, decomposition of organic wastes, formation of organic matter and cycling of different nutrients (Tabatabai, 1994). Inoculation of Egyptian clover and gram with Rhizobium increased the amount of both acid and alkaline phosphatase in the unsterilized as well as sterilized soil Chhonkar and Tarafdar (1981). Burns et al. (2013) reported that hydrolytic enzyme activity responded to different forms of fertilizers. Organic restitutions stimulate hydrolytic enzymatic soil activity. In contrast, mineral fertilization can inhibit or slow down the synthesis of these enzymes. Microbiological and biochemical conditions of a soil can serve as a marker of the soil status and is closely linked to its natural soil fertility (Watts et al., 2010). Addition of the organic fraction stimulates the natural soil micro organisms and reactivates the biogeochemical cycles, whereas application of farm yard manure (FYM) increased total population of soil microbes (Chandrayan et al., 1980).

Rice (*Oryza sativa* L.) is the most important cereal crop and nitrogen is the most frequent limiting nutrient for rice production, which requires 1 kg of nitrogen to produce 15-20 kg of grain (Ladha and Reddy, 2003). Consequently, rice production currently depends on the large-scale use of chemical fertilizers, which pose an environmental hazard for rice producing areas (Wartiainen et al., 2008; Kumar et al., 2016). However, agricultural systems have changed to improve environmental quality and avoid environmental degradation (Roesch et al., 2007). One popular approach of integrated plant nutrient management systems is the addition of Plant growth-promoting rhizobacteria (PGPR) may have a potential role in the development of sustainable systems for crop production (Shoebitz et al., 2009). PGPR are free-living bacteria which actively colonize plant roots and can stimulate plant growth by direct or indirect mechanisms. Direct mechanisms of plant growth-promotion include biofertilization, stimulation of root growth, rhizoremediation, and plant stress control, while mechanisms of biological control include reducing the level of disease, antibiosis, induction of systemic resistance, and competition for nutrients (Lugtenberg et al., 2009). Exclusive use of only fertilizers have been found to cause a significant reduction in microbial biomass C (Wallenstein et al., 2006 and Wang et al., 2008) as well as reductions in soil respiration (SR) and dehydrogenase (DH), acid phosphatase (AcP), and b-glucosidase (bG) activities (Dinesh et al., 2012). We hypothesized that use of PGPR along with FYM, biochar and carpet waste would attenuate such negative or insignificant effects of chemical fertilizer application on important soil microbial population and enzyme activity. Hence, the primary objective of the study was to determine the effects of biochar, carpet waste, FYM and PGPR on soil microbial population and enzyme activity under paddy field condition.

MATERIALS AND METHODS

The study was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi (25^0 18' N latitude, 83^0 03' E longitude and 75.7 m MSL). The soil is inceptisol with sandy clay loam and physicochemical and biological properties of the experimental plot are presented in Table 1. The field was ploughed thoroughly, flooded 2-3 days before transplanting for puddling and leveling. The experiment was laid out in a randomized block design with three replications each. The ten treatment combinations were (T_1) control, (T_2) biochar + carpet waste (1+1) t ha^{-1}, (T_3) biochar + carpet waste (2+1) t ha^{-1}, (T_4) biochar + carpet waste+ FYM (1+1+1) t ha^{-1}, (T_5) biochar + carpet waste + FYM (2+1+1) t ha^{-1}, (T_6) PGPR consortium, (T_7) biochar + carpet waste (1+1) t ha^{-1} + PGPR (T_8) biochar + carpet waste (2+1) t ha^{-1} + PGPR, (T_9) biochar + carpet waste+ FYM (1+1+1) t ha^{-1} + PGPR, (T_{10}) biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR). Recommended doses of biochar, FYM and carpet waste was collected from local area and applied as basal doses before transplanting of rice crop. The physico-chemical properties such as pH, N, P, K and organic carbon content of biochar, were 8.4, .6%, 1.4%, 1.1% and 42.6%, respectively. The organic carbon content of carpet waste and FYM were 56.6% and 59.8%, respectively. Rice seedlings were dipped in PGPR consortium before transplanting. PGPR consortium consists of *Azospirillum brasilensis*, *Pseudomonas aurigenosa*, *Pseudomonas fluorscences* and *Azotobacter chroococcum* was collected from microbiology laboratory of department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU. Rice plants (22 days old seedlings, variety HUR-105) were transplanted as a spacing of 20 cm × 10 cm with two to three seedlings per hill in the field plots. Water was maintained at 2 cm depth during vegetative and 5 cm depth during reproductive stage of the crop until maturity and was drained 10 days before harvest.

Table 1. Physico-chemical and Biological properties of initial soil sample

Sl. No.	Soil properties	value
1	Texture	Sandy clay loam
2	pH	8.4
3	EC(dSm^{-1})	0.2
4	Organic Carbon (%)	0.47
5	Bacteria (10^5 cfu g^{-1} soil)	20
6	Actinomycetes (104 cfu g^{-1} soil)	14
7	Fungi (10^3 cfu g^{-1} soil)	10
8	Dehydrogenase (μg TPF g^{-1} soil day^{-1})	52
9	Alkaline Phophatase (μg PNP g^{-1} soil h^{-1})	37

Soil samples were collected at three different interval *viz.*, 40 DAT, 80DAT and after harvesting (120 DAT) of rice crop from the surface layer at a depth of 0-15 cm from the soil surface in this individual replicated plots. Immediately after sampling, excess water was allowed to drain off, visible root fragments and stubbles were removed manually and transferred to laboratory. Soil samples were taken in plastic bags and kept in a freeze at 4^0 C temperature for a few days to stabilize the microbiological activity. Then it was analyzed for microbial population and enzyme activity. The microbial population and enzyme activity was determined at different interval from collected soil sample. The standard procedure of Rolf and Bakken (1987) was followed for estimation of total bacteria, fungi and actinomycetes population. The dehydrogenase and alkaline phosphatase activity was measured by Tabatabai (1982) and Tabatabai and Bremner (1986), respectively.

RESULTS AND DISCUSSION

The dehydrogenase activity at three stages of rice crop *i.e.*, 40DAT, 80DAT and 120DAT (after harvesting) are presented in Table 2. At 40 DAT the enzyme activity was maximum (156.55 µg TPF produced g^{-1} soil day^{-1}) in treatment (T_{10}) which received Biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR) which was 24.7% more over control and at par with T_9. This may be due to increase in organic matter content (Wlodarczyk et al., 2002) through the application of biochar, carpet waste and FYM. The similar trend was also observed at 80DAT and maximum activity was register with T_{10} followed by T_9 and T_5. The higher dehydrogenase activity at 80DAT is due to high organic matter content at this stage. It is evident that soil enzymatic activity is strongly connected with soil OM content. The higher OM level can provide enough substrate to support higher microbial biomass; hence higher enzyme production (Yuan and Yue, 2012). The

Table 2. Effect of biochar, carpet waste, FYM and PGPR consortium on soil enzyme activity at different interval during field experiment

Treatment	Dehydrogenase (µg TPF produced g^{-1} soil day^{-1})			Alkaline Phosphatase (µg PNP produced g^{-1} soil hr^{-1})		
	Days after transplanting			Days after transplanting		
	40	80	120	40	80	120
T_1	122.88	108.40	95.92	49.07	76.5	62.41
T_2	126.66	182.52	101.63	53.37	79.17	65
T_3	131.76	185.50	105.89	59.47	82.67	67.22
T_4	138.69	198.23	111.69	63.60	88.58	78.52
T_5	155.44	218.38	125.70	73.73	101.0	88.89
T_6	135.59	182.90	109.10	50.80	78.33	65.19
T_7	141.37	200.60	113.94	57.60	84.25	68.69
T_8	146.01	215.60	117.82	64.13	91.33	70.93
T_9	150.15	228.07	121.28	70.67	94.75	81.85
T_{10}	156.55	231.56	126.63	82.80	108.67	97.78
SEm±	2.45	0.52	2.12	0.15	0.85	0.07
CD(P=0.05)	7.84	1.69	6.79	0.49	2.73	0.25

similar finding was also reported by Watts et al. (2010). At 120 DAT lower dehydrogenase enzyme activity in comparison to 80 DAT and highest dehydrogenase activity recorded with T_{10} and at par with T_5 and T_9 the increase in activity during 80 DAT compared with 120 DAT suggests that greater microbial biomass occurred with a change in growth stage. 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. After harvest the decrease in enzyme activity is due to the increase in oxidation status of soil as the soil moisture content decreases. Water availability strongly affects on soil microbial activity, community composition (Geisseler et al., 2011), and consequently on soil enzymatic activities. As soils dry, the water potential increases, and dehydrogenase activity slows down (Geisseler et al., 2011).

Alkaline phosphatase is an enzyme which has great importance in transformation of organic phosphorus compound to inorganic phosphorous which are easily taken by plant (Maestre et al., 2011). Alkaline phosphatase was significantly different among the days after sowing of rice. Alkaline phosphatase was found more at 80 DAT (Table 2). Alkaline phosphatase enzyme was found in the order of 80 DAT > 120 DAT > 40 DAT. The alkaline phosphatase activity was lowest in control (49.07 µg PNP produced g^{-1} soil hr^{-1}) and highest (108.67 µg PNP produced g^{-1} soil hr^{-1}) in T_{10} (biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR) in all DAT. The table reveals that at 40 DAT there was no significant difference between T_1 , T_6 , T_2 and T_7. The treatments followed the order $T_{10}>T_5>T_9>T_8>T_4>T_3>T_7>T_2>T_6>T_1$. There was an increase in phosphatase activity at 80 DAT mainly due to increase in microbial population and organic matter through the application of biochar, carpet waste FYM and PGPR. Sriramachandrasekharan and Ravichandran (2011) also 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 (Bohem et al., 2005).

The experimental results revealed that the population of bacteria, actinomycetes and fungi were found to be increased due to the application of biochar, carpet waste, FYM and PGPR in comparison to absolute control (Table 3).The bacteria population was maximum (48.0 × 105 cfu g^{-1} of soil) at 80 DAT and lowest (23.0 × 105 cfu g^{-1} of soil) at 40DAT. The maximum bacteria population was observed at 80 DAT in treatment T_{10} which received the treatment combination of Biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR followed by T_9. At this stage there is no significant difference between T_5 and T_8. This may be due to application of PGPR. The decrease in bacteria

Table 3. Effect of biochar, carpet waste, FYM and PGPR consortium on microbial population of soil at different interval during field experiment

Treatment	Bacteria (cfu ×10^5 g^{-1} soil)			Actinomycetes (cfu ×10^4 g^{-1} soil)			Fungi (cfu ×10^3 g^{-1} soil)		
	Days after transplanting			Days after transplanting			Days after transplanting		
	40	80	120	40	80	120	40	80	120
T_1	23.00	33.00	25.67	12.33	17.00	13.33	10.33	13.00	11.33
T_2	24.00	35.00	28.00	13.00	19.33	14.67	11.33	14.33	12.67
T_3	26.00	37.00	31.33	15.00	21.00	15.33	12.00	15.67	15.00
T_4	28.00	40.00	33.00	17.00	22.33	16.00	12.33	17.00	16.33
T_5	30.00	43.00	35.00	20.00	24.00	17.33	14.00	18.33	17.33
T_6	24.00	36.00	27.00	14.00	20.00	16.00	11.00	14.67	12.00
T_7	26.00	40.00	32.00	16.66	21.00	17.33	12.00	15.33	14.00
T_8	27.00	42.00	36.33	17.00	22.00	19.67	13.33	17.00	15.33
T_9	29.00	45.00	38.00	18.67	24.00	20.33	14.00	18.67	17.33
T_{10}	30.00	48.00	41.00	21.33	26.00	22.00	16.00	20.00	19.33
SEm±	0.29	0.29	1.20	0.57	0.33	0.47	0.48	0.31	0.47
CD(P=0.05)	0.94	0.94	3.84	1.85	1.07	1.52	1.54	1.01	1.51

population after harvest is due to decrease in organic carbon as a substrate. The similar finding was reported by Watts et al. (2010). Thus it may be concluded that microbial population significantly increases with increase in organic material. So soil microbial biomass which depends on organic carbon content of soil, can been used as a index of soil fertility (Krishnakumar et al., 2005).

The similar trend was also observed in case of actinomycetes. The highest actinomycetes (26 × 104 cfu g^{-1} of soil) was found in T_{10} (biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR) and the lowest (12 × 10^4 cfu g^{-1} of soil) was found in control. At 40 DAT highest population of actinomycetes was observed in T_{10}, which is at par with T_5 and lowest population in T_1, which is at par with T_2. At 80DAT significantly higher actinomycetes population was recorded under biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR. Initially the rate of decomposition of added treatment was slow, so the availability of organic carbon was limited but at intermediate stage the rate of decomposition was high due high microbial population and finally reduced due to shortage of substrate. The actinomycetes population after harvesting of rice crop was lower than 80 DAT which is due to decrease in organic matter content of soil. This finding is in accordance to the finding of Zak et al. (2011).

The highest population of fungi (20 × 10^3 cfu g^{-1} of soil) was observed in T_{10} followed by T_9 and lowest (10.33× 10^3 cfu g^{-1} of soil) was found in control. The rate of increase of fungi population was not so high as compared to bacteria and actinomycetes due to the high alkalinity condition of soil. The maximum fungi population was observed at 80 DAT. An increase in fungi as a percentage of the microbial community could be significant to ecosystem function, as fungi play important roles in organic matter degradation, nutrient cycling, and the formation of soil aggregates. Belowground microbial processes play an essential role in nutrient cycling and organic matter turnover, and influence the growth of the plants by competing for nutrients (Das et al., 2014). Fungal population was found to decrease after harvesting of rice crop due to lake of availability nutrient and organic matter content. The similar result was also reported by Nedunchezhiyan et al. (2013)

CONCLUSION

From the present investigation it could be inferred that integrated application of biochar + carpet waste + FYM (2+1+1) t ha^{-1} + PGPR resulted in maximum microbial population and activities of various enzymes in soil as compared to biochar + carpet waste + FYM (2+1+1) t ha^{-1} at different stages of crop growth. Similarly application of biochar, carpet waste and FYM showed good result as compared to application of biochar and carpet waste. Soil enzymes were strongly associated with microorganisms. Further studies are needed at a broad-scale with regards to establishing the most effective application rates and, the dynamics of nutrient release in order to synchronise the complex interaction of nutrient availability and plant demand.

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