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Impact of Addition of Biochar Along with PGPR on Rice Yield, Availability of Nutrients and their Uptake in Alluvial Soil

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A pot experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences B.H.U., Varanasi, India during *kharif* season of 2012 to investigate the efficacy in enhancing uptake of nutrients and yield of rice crop grown in an alluvial soil. The treatments comprised of four levels of rice husk biochar (RHB) (i.e. 0, 1.8, 3.6 and 7.2 g kg^{-1} soil), two levels of plant growth promoting rhizobacteria (PGPR) (uninoculated and inoculated) and two levels of N, P, K and Zn fertilizers (100% and 75% of RDF). One of the important findings of the investigation pointed beneficial effects of RHB could be exploited if it was applied along with PGPR. Combined application of RHB (3.6 g kg^{-1} soil) along with PGPR was produced significantly higher rice yield and uptake of nutrients in rice. Inoculation with PGPR also resulted in significantly higher rice yield, nutrients uptake and their availability in soil over uninoculated conditions.

Keywords: Rice yield; Rice husk biochar; PGPR; Nutrient uptake; Nutrient availability.

After onset of green revolution (1965-66) in India, there has been remarkable increase in production of food grains due to intensive cultivation and enhanced use of agrochemicals mainly fertilizers and pesticides. However, it has resulted in decline in soil organic carbon and deterioration of soil quality at many places. At national and international level there is growing consciousness to protect the environment without compromising the higher productivity of crops. This involves using techniques to achieve high crop yields without harming the natural environment. Now a day's great deal of researchers how to obtain higher productivity of crops with organic sources of nutrients which have low cost of inputs compare to inorganic sources of nutrients.

In this context, biochar and PGPR are good options for maintaining the crop productivity as well as soil quality.Biochar is a carbon rich solid substance which has a condensed aromatic structure which is fairly resistant to microbial decomposition but decomposition varied biochar to biochar. Lehmann *et al.* (2006) estimated that total of 9.5 billion tonnes of carbon could potentially be stored in soils by the year 2100 using a wide variety of biochar application programmes.

Biochar application has been a sustainable technology to recover deeply weathered or degraded tropical soils (Lehmann and Rondon, 2006). Consequence of biochar application on soil health and soil fertility has been different but usually helpful (Blackwell *et al.*, 2009; Major *et al.*, 2010; Steiner *et al.*, 2007 and Asai *et al.*, 2009). Use of biochar to soil can improve growth of plants and physical, chemical and biological properties of soil, all improved properties

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ultimately augment productivity of different crops (Lehmann and Rondon, 2006; Yamato *et al.*, 2006).Currently, biochar has been used as a soil amendment in Europeans countries at large scale, but in India biochar has not been used due to some constraints like lack of research, diverse effect of biochar on soil quality and crop productivity. On the other hand, plant growth promoting rhizobacteria (PGPR) are one of the possible alternatives to chemical fertilizers. The PGPR refers to the rhizobacteria that exert beneficial effect on plant growth. PGPR inoculants promote plant growth through several mechanisms; improved nutrient acquisition by atmospheric nitrogen fixation, nutrients solubilisation, phytohormone production and suppression of plant diseases (Klopper *et al.*, 1989). PGPR stimulate plant growth directly either by synthesizing hormones such as indole acetic acid or by promoting nutrition, by phosphate solubilisation or generally by accelerating mineralization process. They can also stimulate growth indirectly by acting as bio control agent by protecting the plant against soil born fungal pathogens or deleterious bacteria. Some PGPR suppress pathogen by synthesizing antifungal metabolites (Vassilev *et al.*, 2006).

Although PGPR have been extensively documented for their positive impact on plants, but sometimes its results are inconsistent due to some soil factor, climatic factor, and quality of inert material. , biochar not only worked as a soil amendments, but also used as a carrier for PGPR. Possibility of addition of biochar along with PGPR might be helps in augmenting crop productivity and soil quality. Most importantly, the combination of PGPR and biochar would be replenish degraded soils with organic carbon and fosters the growth of soil microbes essential for nutrient absorption.The application of biochar can be a promising approach for managing soil microorganisms. Biochar addition may affect the soil biological community composition as demonstrated for the biochar rich Terra preta soils in the Amazon (Kim *et al.*, 2007;O'Neill*et al.*, 2009; Grossman *et al.*, 2010), and has been shown to increase soil microbial biomass (Liang *et al.*, 2010; O'Neill *et al.*, 2009). Still very little research work has been done to find out possibility of utilization of biochar in combination with PGPR in cereals crops. Hence this study was conducted to investigate the effect of rice husk biochar and PGPR along with chemical fertilizers on yield, nutrient uptake of rice and nutrient availability in an alluvial soil of Varanasi.

MATERIALS AND METHODS

Study area

The experiment was conducted during kharif season of 2012 in net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India in factorial completely randomized design with three replications. The treatment consisted of inorganic fertilizers viz., 100% of recommended doses of fertilizers (RDF) (60:30:30 mg N, $P_2O_5,$ K_2O kg^{-1}) and 75% of RDF (Factor 1) and 0, 1.8, 3.6 and 7.2 g kg^{-1}RHB (Factor 2) and two levels (uninoculated and inoculated) of PGPR

Experimental Soil

Bulk soil sample (0-15cm) was collected from Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University. Varanasi, India. After collecting, it was ground and passed through 5.0-mm sieve and 10 kg of soil filled in the each polythene lined pot. Soil in each pot was puddled manually and 5 seedlings of rice (variety BPT-5204) were transplanted. After establishment, four plants were maintained. The pots were irrigated and 2 cm of standing water was maintained by daily addition of water. The soil used for experimentation was sandy loam with bulk density 1.63 Mgm^{-3}, pH (1:2.5) 7.6, E.C. 0.21 dsm^{-1}, CEC 11.63 cmol (P^+) kg^{-1}, organic carbon 0.34%, available N 135 kg ha^{-1}, available P 22.7 kg ha^{-1} and available K 183 kg ha^{-1}.

Characteristics of Biochar and PGPR used in experiment

The characteristics of rice husk biochar were bulk density 0.40 Mgm^{-3}, particle density 1.40 $Mgm^{-3,}$ pH (1:2.5) 10, porosity 71.42%, water holding capacity 218%, total carbon 45.60%. Scanning electron microscope (Zeiss EVOMA10 Scanning Microscope) was used for image analysis. Surface element (C, O,Si, Mg, Ca, K, and Fe) analysis was conducted simultaneously with the SEM at the same surface locations using energy dispersive X-ray spectroscopy (EDS).

The PGPR used for experimentation included *Pseudomonas* species, *Azotobacter chroococcum and Azospirillum brasilense*. Required quantities of fertilizers for 10 kg soil were calculated and applied in solution form, using urea, KH_2PO_4, KCl and $ZnSO_4.7H_2O$ as source of N, P, K and Zn applied respectively.

Sampling and analysis

Plant samples were dried at 65-70°C and grain and straw yield was recorded at physiological maturity stage. The soil samples were analysed for organic carbon by modified Walkley–Black method (Jackson, 1973); available N by potassium permanganate method (Subbiah and Asija, 1956); available P by the Olsen method (Olsen *et al*, 1954); available K in soil with flame photometer (Hanway and Heidel, 1952); and DTPA extractable Zn and Fe with AAS (Lindsay and Norvell, 1969). Data pertaining to rice yield, availability of nutrients in soil and their uptake were statistically analysed. Uptake of nutrients by grain and straw was calculated by multiplying per cent of the individual nutrient in grain and straw with the corresponding yield.

RESULTS AND DISCUSSION

Effect on rice yield

Application of $RDF_{100\%}$ enhanced the grain and straw yield by 13.36% and 4.77% respectively over $RDF_{75\%}$. The inoculation with PGPR ($PGPR_1$) also showed significantly higher grain and straw yield over uninoculated levels ($PGPR_0$).Sole application of RHB resulted in non-significant effect on grain and straw yield of rice whereas combined application of RHB @ 3.6 g $^{-1}$ kg soil with PGPR produced significantly higher grain and straw yield of rice (40.48 g pot^{-1} and 48.88 g pot^{-1}) over other treatments. Whereas sole application of RHB @ 1.8 g kg^{-1} soil (without PGPR) resulted in lowest value of grain and straw yield (34.25 g pot^{-1} and 44.06 g pot^{-1}). Saxena *et al.* (2013) reported that addition of biochar along with *Bacillus sp.* increased the growth and yield of French beans. Significant positive interaction between RHB and PGPR was probably due to the fact that large porosity of biochar provides surfaces for increasing population and growth of microorganisms in soil (Thies and Rillig, 2009)

Effect on uptake of nutrients by rice

Application of full dose of recommended fertilizers ($RDF_{100\%}$) showed significantly higher N, P and K in grain and straw than $RDF_{75\%}$. RHB applied @ 3.6 g kg^{-1} soil ($RHB_{3.6}$) was found to produce non significant effect on nitrogen and phosphorous uptake whereas potassium uptake by grain and straw registered significant enhancement over RHB_0 (Table 3). The PGPRinoculation also showed significantly higher N, P and K uptake in grain and straw over $PGPR_0$.

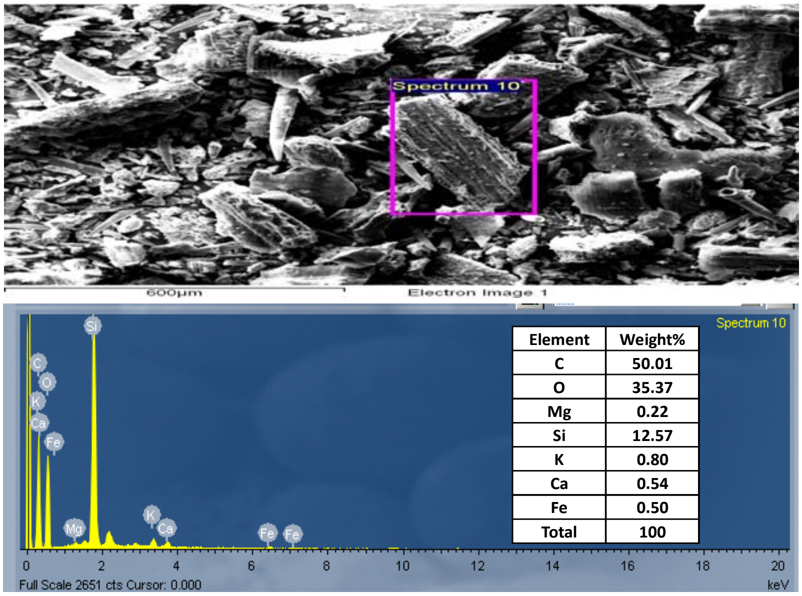

Fig. 1. SEM-EDX of rice husk biochar

Significant positive interaction between RHB and PGPR was found on N, P and K uptake by grain and straw. The highest N, P and K uptake by grain and straw (Table 4) was recorded in treatment $RHB_{3.6}$ x $PGPR_1$ and lowest N and P was found in treatment $RHB_{7.2}$ x $PGPR_0$ whereas, lowest potassium uptake by grain and straw was found in treatment RHB_0 x $PGPR_0$.

Combined application of biochar along with PGPR (RHB×PGPR) was found to produce significantly higher uptake of phosphorus in grain and straw of rice compared to sole application of biochar. It was probably due to Secrete ions of organic acids by PGPR may reduce pH and solubilise phosphorus. Similar results were also reported by Ishii and Kodoya, (1994) who found that intensity of VAM association was increased by biochar addition in mandarin trees which results in increased leaf phosphorus concentration**.** Lifshitz *et al*. (1987) reported that application of PGPR caused phosphate solubilization and production of IAA that contributed to increased root development and thereby increased phosphorous uptake. The highest K uptake by rice plants in present study might be due to high K content in ash of biochar. These findings are in accord with Lehmann *et al*. (2003) who reported that K uptake in plants increased with biochar application.Rondan *et al.* (2007)also reported that potassium concentration in plant significantly increased with biochar applications.The increase in potassium uptake caused by PGPR was probably due to the organic acids like citric, oxalic, tartaric, succinic etc. produced by the PGPR which are able to chelate metals and mobilize potassium containing minerals **(**Abbasi *et al.* 2011).

Sole application of RHB resulted in non-significant effect on Fe and Zn uptake in grain and straw of rice whereas combined application of RHB @ 3.6 g kg^{-1} soil with PGPR produced significantly higher Zn uptake in grain (0.662 mg kg^{-1}) and straw (1.266 mg kg^{-1}) of rice. Lowest Zn uptake in grain (0.504 mg kg^{-1}) was found where RHB was applied @ 1.8 g kg^{-1} soil without PGPR, and in case of straw, lowest value of Zn uptake (1.010 mg kg^{-1}) was obtained where no application of RHB and PGPR was made. The inoculation with PGPR showed significantly higher zinc and iron uptake

Table 1. Effect of different levels of fertilizers, rice husk biochar and PGPR on rice yield and nutrient availability in soil

Treatment	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	N (mg kg^{-1})	OC (g kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
RDF_{75}	34.26	44.99	97.1	3.7	13.3	117.4
RDF_{100}	38.84	47.13	97.4	3.8	13.3	117.6
SEm ±	0.278	0.209	0.194	0.021	0.067	0.076
CD (0.05)	0.803	0.603	NS	NS	NS	NS
RHB_0	35.80	45.45	97.0	3.6	13.4	117.3
$RHB_{1.8}$	36.60	45.99	97.1	3.7	13.3	117.6
$RHB_{3.6}$	37.38	46.60	97.4	3.8	13.2	117.7
$RHB_{7.2}$	36.43	46.19	97.5	3.9	13.3	117.6
SEm ±	0.393	0.295	0.274	0.030	0.095	0.107
CD (0.05)	NS	NS	NS	0.087	NS	NS
$PGPR_0$	34.43	44.26	95.5	3.7	12.4	117.4
$PGPR_1$	38.68	47.85	99.0	3.8	14.2	117.6
SEm ±	0.278	0.209	0.194	0.021	0.067	0.076
CD (0.05)	6.421	4.825	4.478	NS	1.550	NS
Interaction RHB× PGPR	S	S	NS		NS NS	NS

RDF 75 means Fertilizer application 75% of recommended dose of fertilizers, RDF 100 means Fertilizer application 100% of recommended dose of fertilizers, PGPR0 means rice seedlings were not inoculated with PGPR, PGPR1 means rice seedlings were inoculated with PGPR, RHB0 means application of biochar @ 0 g/kg of soil, RHB1.8 means application of biochar @ 1.8 g/kg of soil, RHB3.6 means application of biochar @ 3.6 g/kg of soil, RHB7.2 means application of biochar @ 7.2 g/kg of soil in grain and straw of rice when compared to the treatments which were deprived of PGPR. Significant positive interaction between RHB and PGPR on iron and zinc uptake was probably due to ability of PGPR to solubilise iron and zinc present in biochar.Elkoca *et al.* (2010) reported that PGPR significantly increased zinc uptake in the common bean and Cakmakci *et al.* (2001) stated that treatments of PGPR had a higher iron uptake than that of control in barley. Sharma *et al.* (2013) also reported that PGPR increased the iron content in grain of rice over uninoculated treatments.

Table 2. interaction effect of rice husk biochar × PGPR on grain yield and straw yield of rice

(RHB ×PGPR)	Grain yield (g pot $^{-1}$)		Straw yield (g pot $^{-1}$)	
	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$
RHB_0	34.62	36.98	44.46	46.45
$RHB_{1.8}$	34.25	38.95	44.06	47.92
$RHB_{3.6}$	34.28	40.48	44.31	48.88
$RHB_{7.2}$	34.57	38.3	44.22	48.16
SEm ±	0.556	0.418		
CD (0.05)	1.605	1.206		

Availability of nutrients in soil

The increasing levels of biochar from 0 to 7.2 g kg-1 soil significantly increased organic carbon content in soil. The inoculation with PGPR also increased the organic carbon content of soil but increment was statistically non-significant over uninoculated levels. The increase in soil organic carbon content with application of rice husk biochar might have been due to biochar has high amount

Table 3. Nutrient uptake in grain and straw of rice as affected by different levels of fertilizers, rice husk biochar and PGPR

Treatment	Nitrogen uptake (g pot^{-1})		Phosphorus uptake (g pot^{-1})		Potassium uptake (g pot^{-1})		Zn uptake (mg pot^{-1})		Fe uptake (mg pot^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
RDF_{75}	0.483	0.402	0.111	0.093	0.184	0.530	0.520	1.098	1.566	3.462
RDF_{100}	0.576	0.458	0.131	0.101	0.225	0.571	0.614	1.226	1.780	3.636
SEm ±	0.004	0.002	0.001	0.001	0.002	0.003	0.009	0.007	0.014	0.019
CD (0.05)	0.013	0.006	0.004	0.003	0.006	0.008	0.026	0.020	0.041	0.055
RHB_0	0.518	0.427	0.118	0.094	0.196	0.538	0.540	1.141	1.632	3.496
$RHB_{1.8}$	0.532	0.431	0.122	0.096	0.203	0.545	0.572	1.159	1.674	3.564
$RHB_{3.6}$	0.543	0.434	0.125	0.099	0.211	0.561	0.593	1.178	1.706	3.589
$RHB_{7.2}$	0.526	0.428	0.119	0.097	0.207	0.557	0.564	1.170	1.678	3.548
SEm ±	0.006	0.003	0.002	0.001	0.003	0.004	0.013	0.010	0.020	0.027
CD (0.05)	NS	NS	NS	NS	0.008	0.011	NS	NS	NS	NS
$PGPR_0$	0.481	0.394	0.109	0.086	0.191	0.526	0.514	1.094	1.563	3.379
$PGPR_1$	0.578	0.466	0.133	0.107	0.218	0.575	0.620	1.230	1.783	3.720
SEm ±	0.004	0.002	0.001	0.001	0.002	0.003	0.009	0.007	0.014	0.019
CD (0.05)	0.101	0.050	0.034	0.022	0.046	0.065	0.207	0.158	0.331	0.439
Interaction (RHB×PGPR)	S	S	S	S	S	S	S	S	NS	NS

Table 4. interaction effect of rice husk biochar × PGPR on N, P, K and Zn uptake in grain of rice

(RHB×PGPR)	N uptake in grain (g kg^{-1})		P uptake in grain (g kg^{-1})		K uptake in grain (g kg^{-1})		Zn uptake in grain (mg kg^{-1})	
	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$
RHB_0	0.488	0.548	0.110	0.125	0.188	0.203	0.521	0.560
$RHB_{1.8}$	0.479	0.585	0.109	0.134	0.188	0.219	0.508	0.636
$RHB_{3.6}$	0.478	0.608	0.109	0.142	0.191	0.230	0.523	0.662
$RHB_{7.2}$	0.479	0.573	0.108	0.129	0.195	0.219	0.504	0.623
SEm±	0.009	0.003	0.004	0.018				
CD (0.05)	0.025	0.009	0.011	0.052				

Table 5. interaction effect of rice husk biochar × PGPR on N, P, K and Zn uptake in straw of rice

(RHB×PGPR)	N uptake in straw ($g\ kg^{-1}$)		P uptake in straw ($g\ kg^{-1}$)		K uptake in straw ($g\ kg^{-1}$)		Zn uptake in straw ($mg\ kg^{-1}$)	
	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$
RHB0	0.403	0.451	0.090	0.099	0.524	0.552	1.010	1.183
RHB1.8	0.396	0.467	0.085	0.108	0.519	0.571	1.095	1.224
RHB3.6	0.388	0.480	0.085	0.114	0.530	0.592	1.091	1.266
RHB7.2	0.388	0.467	0.085	0.109	0.530	0.583	1.090	1.249
SEm±	0.004		0.002		0.006		0.018	
CD (0.05)	0.013		0.005		0.016		0.052	

of carbon content, and study was conducted under controlled condition (pot experiment). Utomo (2010) also reported that soil carbon increased significantly over control due to biochar application.The availability of nitrogen, phosphorus and potassium in soil did not vary significantly due to application of different levels of RHB. The increments in available N content (3.58%) and phosphorus content (14.97%) brought about by PGPR ($PGPR_1$) when compared with uninoculated levels ($PGPR_0$) were significant. The interaction effects among the various treatments were also found to be non-significant during investigation. Similar results were reported by Rondan *et al.* (2007). Mittal *et al*. (2008) reported that application of PGPR in chick pea can increase available phosphorus after the harvest of crop up to 26%.

CONCLUSION

A significant increase in grain and straw yield of rice could be achieved by application of RHB and PGPR. Sole application of RHB has no immediate significant effect on grain yield, straw yield, nutrient uptake (except potassium) of rice and availability of nutrients in soils. However, combined application of RHB and PGPR resulted in significantly improved yield and nutrient uptake of rice. Among the various treatments tested, the highest yield and nutrient uptake was obtained with combined application of 3.6 g kg^{-1} soil of RHB and PGPR. Inoculation with PGPR also increased yield, nutrient uptake as well as available N and P over uninoculated treatments. but, there are no significant effect on nitrogen, phosphorus and potassium availability in soil.

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