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Effect of Integrated Nutrient Management in Rice on Nitrogen Availability, L-asparaginase and L-glutaminase Activity in Acidic Soil

Abhik Patra[1], V. K. Sharma[1*], T. J. Purakayastha[1], Mandira Barman[1], Sarvendra Kumar[1], D. Chakraborty[2], Kapil A. Chobhe[1] and D. J. Nath[3]

[1]Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India
[2]Division of Agricultural Physics, ICAR-IARI, New Delhi 110 012, India
[3]Department of Soil Science, Assam Agricultural University (AAU), Jorhat, 785 013, Assam, India
**Corresponding author*

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Long term field experiment on rice commenced in *kharif* 2006 at Instructional-cum-Research (ICR) farm of the Assam Agricultural University, Jorhat with integrated nutrient management (INM) treatments for assessing its impact on soil properties and microbial activities in acidic soils. The treatments consisted of T_1; absolute control, T_2;100% recommended doses (RD) of inorganic NPK, T_3; 50% RD of inorganic NP + 100% K + biofertilizers, T_4; 50% RD of inorganic NP + 100% K + 1 tonne enriched compost ha^{-1} and T_5; 25% RD of inorganic NP + 100% K + 2 tonnes enriched compost ha^{-1}. Results indicated that after 10 years of experiment, INM treatments with enriched compost as well as biofertilizers had pronounced influence on improving available nitrogen status as well as microbial enzymatic activity as compared to inorganic treatment under acid soil.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal food crops of India in terms of area, production and consumer preference. India is the second largest producer and consumer of rice in the world. Productivity of rice in the diverse rainfed ecosystems of South and South-East Asia has not shown much improvement due to various factors. Low recovery of N to the extent of 30-40% is frequently encountered due to uncontrolled water accumulation in rice crop (Ghosh and Sharma, 1999). The continued use of inorganic fertilizers over the years in paddy field without application of organic amendments resulted in the change of soil structure as well as decreasing the soil fertility (Sannathimmappa *et al.*, 2015).

One third of the soils of the world are acidic having pH<6.5, and also 50% of the world's potentially cropped lands are acidic (Uexküll and Mutert, 1995). In India, around one-third of the cultivated soils are affected by soil acidity (Mandal, 1997). Most of these soils are from north-eastern region of India, with approximately 65% of area being under extreme soil acidity having pH below 5.5 (Sharma and Singh, 2002). Out of 142 million ha of arable land in India, 49 million ha covered by acid soils and spreading over 24 states of the country (34% of cropped land in India) (Sharma and Singh, 2002 and Maji *et al.,* 2012). Productivity of such soils are mainly constrained due to iron (Fe) and aluminium (Al) toxicity, phosphorus (P) deficit, impaired biological activity, low base saturation and other acidity-induced soil plant nutritional and fertility problems (Kumar *et al.,* 2012).So, soil acidity negatively impacted on the production of staple food crops. This indicate the importance of soil acidity management and crop productivity improvement on such soils for enhancing food security regionally and globally.

Integrated nutrient management through use of inorganic fertilizers along with organic sources are applied to soil for increasing the status of plant available nutrients and improving the physico-chemical and biological properties of soil which directly affect soil fertility (Sannathimmappa *et al.,* 2015). In contrast to nutrients in organic fertilisers, this required microbial metabolism to make most of them available to plants. So, inorganic fertilisers can directly affect crop growth and yields. Organic and inorganic fertilizer amendments are used primarily to increase nutrient availability to plants, but they can also affect soil microorganisms. Besides, the presence of these inorganic and organic substances in the soil is related with an increase in nutrient contents of soil and with their subsequent effects on soil properties such as microbial activity, the humus fraction, soil structure and cation exchange capacity (Kunc, 1988 and Kirchner *et al.,* 1993). Therefore, INM may be a feasible approach to ensure the sustained availability of nitrogen and enhancing its use efficiency as well as microbial activity. Effect of integration of organic and inorganic inputs on important soil properties and microbial activities has been assessed in the present investigation.

Materials and Methods

General information about experimental site

The experiment started in 2006. The experimental site is located at Instructional-cum-Research (ICR) farm of the Assam Agricultural University, Jorhat. The latitude of the location $26^0 43'$N and longitude $94^o 11'$ E. The research site has a typical subtropical climate with a mean annual rainfall of 184.24 cm during the crop growing period, of which maximum mean annual rainfall was observed during July (381.70 cm). The minimum monthly mean temperature was 10.03°C in January and a maximum monthly mean temperature was 32.34°C in August. The experimental soil was Inceptisol with clay loam texture (28.6% clay) and is classified as an Aeric Endoaquept, with dominant kaolintic minerals.

Experimental details

The experiment was laid down in randomized block design and replicated four times with five treatments. The plot size was 8 x 5 m^2. The treatments consisted of T_1; absolute control, T_2;100% recommended doses (RD) of inorganic NPK, T_3; 50% RD of inorganic NP + 100% K + biofertilizers, T_4; 50% RD of inorganic NP + 100% K + 1 tonne enriched compost ha^{-1} and T_5; 25% RD of inorganic NP + 100% K + 2 tonnes enriched compost ha^{-1}. The recommended dose of inorganic fertilizers were 40:20:20 (N: P_2O_5: K_2O kg ha^{-1}) for rice. The sources of fertilizer for N, P and K were urea, single superphosphate (SSP) and muriate of potash, respectively. The inorganic fertilizers were applied as per recommended package of practices.

Biofertilizers *viz., Azospirillum* and phosphate solubilising bacteria (PSB) were applied as seedling root dip treatments which were kept for overnight. The enriched compost (EC) [primed with *Azospirillum* and PSB @ 1% broth each containing 10^8 – 10^9cfu. ml^{-1} and adjusted with 1% rock phosphate (RP contained 19.46% P_2O_5) and cured for 1 month] was used in the experiment prepared from rice biomass. The rice variety *Ranjit* was transplanted on the second week of July 2015 with a row spacing of 20 x 20 cm.

Soil processing and analysis

The soil samples were collected after harvesting of rice during *kharif* 2015. The samples were separated into two parts; one part was processed for chemical analysis and the other part was preserved in refrigerator at 4^oC for enzymatic analysis. The samples for chemical analysis were dried in shade, ground in wooden pestle-mortar, and sieved to pass through a 2 mm sieve. The pH of acid soil in 1:2 (soil: KCl) suspension was determined by digital pH meter using combined electrode. Organic carbon content in soil was determined by wet oxidation method (Walkley and Black, 1934). Organic matter was calculated by using formula as given below:

Organic matter (g kg^{-1}) = %Organic carbon × 1.723 × 10

Available nitrogen in soil was determined by alkaline potassium permanganate ($KMnO_4$) method (Subbiah and Asija, 1956).Total nitrogen in soil was determined following the Kjeldahl method. The L-glutaminase activity and L-asparaginase activity in soil was measured by the method described by Kanazawa and Kiyota (1995) and extracted NH_4^+ from solution was measured by the modified Nessler method (Omura *et al.,* 1987). Statistical analysis of data was done using SAS-9.3.

Results and Discussion

pH $_{(KCl)}$ and organic matter

As soil pH is one of the most important factors which governs the availability of plant nutrient in soil, the effect of application of enriched compost on soil $pH_{(KCl)}$ was studied (Table 1). There was no significant effect of organic, inorganic and integrated treatments on pH of the soil, at any soil depth. Though some numeric increase was observed in different treatments as compared to control

Result indicated that increasing levels of enriched compost application increases the organic matter content significantly in all three soil depth (Table 1). Among all the treatments, organic matter content of soil ranged from 14.9 to 19.5, 12.5 to 17.4 and 11.8 to 16.7 g kg^{-1} soil at three different soil depths *i.e*., 0-5cm, 5-15 cm and 15-30 cm, respectively after harvesting of rice crop. Highest organic matter content (19.5 g kg^{-1}) was recorded with 25% RD of NP + 100% K + enriched compost @ 2 t ha^{-1} treatment at 0-5 cm soil depth. Organic matter content decreases gradually as soil depth increases under all treatments. Majumder *et al.,* (2008) reported 24.3 % higher SOC under NPK + FYM treatments as compared to control. Ros *et al.,* (2003) and Sharma *et al.,* (2015), also found the similar results. The higher organic matter under INM treatments might be due to the direct application of carbon input, which could be enhanced further through root exudates, root residue of rice and biofertilizers application (Banswasi and Bajpai, 2006).

Available nitrogen and total nitrogen

Available and total nitrogen as influenced by manuring and fertilization is presented in Table 2. Results showed that maximum available nitrogen content of 251 kg ha^{-1} in post-harvest soil was recorded under 25% NP

+ 100% K + enriched compost @ 2 t ha^{-1} treatment at 0-5 cm soil depth and minimum (157 kg ha^{-1}) in control at 15-30 cm soil depth. Available nitrogen content increased significantly with manuring and fertilization treatments in all three soil depths over control. Highest available nitrogen content 257 kg ha^{-1} was found with 25% RD of NP + 100% K + enriched compost @ 2 t $ha^{-1}$and which was at par with the treatments consisting of 100% RD of NPK in all three soil depths. Total nitrogen content increased from 1.45 g kg^{-1} in control (15-30 cm) to 1.72 g kg^{-1} in 25% NP + 100% K + enriched compost @ 2 t ha^{-1} treatment at 0-5 cm soil depth. Total nitrogen content in all three soil depths *i.e.*, 0 – 5,5 – 15 and 15 – 30 cm was found 1.72, 1.65 and 1.62 g kg^{-1}, respectively with the application of 25% NP + 100% K + enriched compost @ 2 t $ha^{-1,}$ which was at par with 50% NP + 100% K + enriched compost @ 1 t ha^{-1} and 50% NP + 100% K + biofertilizer and it was found to be similar with 100% NPK (1.66, 1.62 and 1.54 g kg^{-1} at 0-5, 5-15 and 15-30 cm soil depth, respectively).

Table.1 Effect of integrated nutrient management on pH_{KCl} and Organic matter of post-harvest soil

Treatments	pH_{KCl}			Organic matter (g kg^{-1})		
	0 – 5 cm	5 – 15 cm	15 – 30 cm	0 – 5 cm	5 – 15 cm	15 – 30 cm
Control	3.70	3.85	4.05	14.9^{C}	12.5^{C}	11.8^{C}
100% RD of NPK	3.75	3.92	4.16	17.6^{AB}	15.3^{AB}	14.2^{B}
50% RD of NP + 100% K + Biofertilizers	3.81	3.98	4.22	16.1^{BC}	14.7^{BC}	13.6^{BC}
50% RD of NP + 100% K + Enriched compost @ 1 t ha^{-1}	3.89	4.11	4.28	18.8^{A}	16.4^{AB}	15.5^{AB}
25% RD of NP + 100% K + Enriched compost @ 2 t ha^{-1}	3.94	4.25	4.34	19.5^{A}	17.4^{A}	16.7^{A}
Mean	3.82	4.02	4.21	17.4	15.3	14.4
LSD @ 5%	NS	NS	NS	2.70	2.19	2.19

Table.2 Effect of integrated nutrient management on available and Total nitrogen of post-harvest soil

Treatments	Available Nitrogen (kg ha^{-1})			Total Nitrogen (g kg^{-1})		
	0 – 5 cm	5 – 15 cm	15 – 30 cm	0 – 5 cm	5 – 15 cm	15 – 30 cm
Control	180^{D}	173^{D}	157^{B}	1.50	1.49	1.45
100% RD of NPK	242^{AB}	221^{AB}	179^{A}	1.66	1.62	1.54
50% RD of NP + 100% K + Biofertilizers	223^{C}	196^{C}	162^{B}	1.59	1.56	1.48
50% RD of NP + 100% K + Enriched compost @ 1 t ha^{-1}	230^{BC}	211^{BC}	171^{AB}	1.60	1.57	1.51
25% RD of NP + 100% K + Enriched compost @ 2 t ha^{-1}	251^{A}	234^{A}	184^{A}	1.72	1.65	1.62
Mean	225	207	171	1.61	1.58	1.52
LSD @ 5%	18.0	17.0	15.9	NS	NS	NS

Table.3 Effect of integrated nutrient management of L-asparaginase and L-glutaminase activity in post-harvest soil

Treatments	L-asparaginase (μg NH_4^+ g^{-1} soil h^{-1})			L-glutaminase (μg NH_4^+ g^{-1} soil h^{-1})		
	0 – 5 cm	5 – 15 cm	15 – 30 cm	0 – 5 cm	5 – 15 cm	15 – 30 cm
Control	35.4^{C}	30.3^{B}	27.9^{B}	117^{C}	108^{C}	94.8^{B}
100% RD of NPK	42.2^{AB}	37.5^{A}	35.1^{A}	129^{A}	121^{AB}	107^{A}
50% RD of NP + 100% K + Biofertilizers	38.9^{BC}	35.6^{A}	34.0^{A}	121^{BC}	117^{B}	103^{A}
50% RD of NP + 100% K + Enriched compost @ 1 t ha^{-1}	41.0^{B}	35.4^{A}	33.2^{A}	126^{AB}	119^{AB}	106^{A}
25% RD of NP + 100% K + Enriched compost @ 2 t ha^{-1}	45.3^{A}	37.8^{A}	35.8^{A}	132^{A}	124^{A}	109^{A}
Mean	40.5	35.3	33.2	125	118	104
LSD @ 5%	3.58	3.68	2.98	7.68	5.95	7.15

Application of Farm Yard Manure (FYM) alone and integration with inorganic fertilizers play a vital role in exploiting high yield potential through its beneficial effect on nutrients supply and chemical and biological properties (Sharma *et al.,* 2016).

Increase in available N both in surface and sub-surface soils might be ascribed to the fact that addition of mineral N along with organic source narrowed the C: N ratio of organic manure and this enhanced the rate of mineralization resulting in rapid conversion of organically bound N to inorganic forms and helped in release of nutrients from the organic matter.

This finding was in agreement with the reports of Singh *et al.,* (2006).The positive balance in available soil N is likely attributed to the positive balance of total SOM and might have been partially due to a slow release of N from enriched compost products.

The present results are in agreement with other workers (Bhandari *et al.,* 2002).

Microbial activities

The L-asparaginase and L-glutaminase activity of post-harvest soil as influenced by manuring and fertilization is presented in (Table 3). The results showed significant enhancement in activity of L-asparaginase in post-harvest soil from 35.4, 30.3 and 27.9µg NH_4^+ g^{-1} soil h^{-1} in control to 45.3, 37.8 and 35.8µg NH_4^+ g^{-1} soil h^{-1} in 25% NP + 100% K + enriched compost @ 2 t ha^{-1} at 0-5, 5-15 and 15-30 cm soil depth, respectively. At 0-5 soil depth L-asparaginase activity (45.3 µg NH_4^+ g^{-1} soil h^{-1}) in treatment 25% NP + 100% K + enriched compost @ 2 t $ha^{-1}$was recorded significantly higher over rest of other treatments. But, in 5-15 and 15-30 cm soil depths any kind of integrated treatment effectively increased the L-asparaginase activity over control. L-glutaminase activity was significantly increased while application of 2 tonne enriched compost ha^{-1} with reduce doses of NP (132, 124 and 109 µg NH_4^+ g^{-1} soil h^{-1} at 0-5, 5-15 and 15-30 cm soil depth, respectively) over control and this treatment was at per with 100% RD of NPK.

L-glutamine and L-asparagine plays an important role in the nitrogen cycle in soils. L-glutamine is decomposed to L-glutamic acid and ammonia by L-glutaminase. L-asparagine is also decomposed to L-aspartic acid and ammonia by L-asparaginase (Ladd and Jackson 1982; Omura *et al.,* 1987). Integrated use of chemical fertilizers and enriched compost brings in more enzyme activities in soil compared to only chemical fertilizers treated plot. Kumawat *et al.,* 2017 also reported that crop residue retention as an organic source enhanced the microbial activities in rhizospheric soils. Fertilizers may meet the demand of mineral nutrition required by the microbes but not that of carbon, which is a major constituent of microbial cells (Ghosal and Singh, 1994). Integrated application of organic and inorganic materials provides a balanced supply of mineral nutrients as well as carbon. This is reflected in terms of increased levels of enzyme activities in soil receiving 25% NP + 100% K + enriched compost @ 2 t ha^{-1}.

From investigation, it is concluded that the integrated treatments involving both inorganic fertilizers and organic sources had pronounced influence in improving available nitrogen status as well as microbial enzymatic activity as compared to recommended dose of fertilizers.

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