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Soumya Ranjan Dehury
Department of Soil Science & Agricultural Chemistry, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Rajeswari Das
Department of Soil Science & Agricultural Chemistry, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Madhusmita Pradhan
Department of Soil Science & Agricultural Chemistry, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Santanu Mohanty
Department of Soil Science & Agricultural Chemistry, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Correspondence
Rajeswari Das
Department of Soil Science & Agricultural Chemistry, College of Agriculture OUAT, Bhubaneswar, Odisha, India

Growth and chemical properties of rice cultivar *Pyari* as influenced by *Azotobactervinelandii* strain SRIAz3 and N-source

Soumya Ranjan Dehury, Rajeswari Das, Madhusmita Pradhan and Santanu Mohanty

Abstract
The effect of *Azotobacter vinelandii* strain SRIAz3 and N-source on growth and post harvest soil chemical properties of rice was investigated. *A. vinelandii* strain SRIAz3 was isolated from SRI Field (Central Farm, OUAT, Bhubaneswar). The experiment was carried out in a randomised complete block design (RCBD) with 3 replications and 8 treatments namely Control, N1 (50% N), N2 (75% N), N3 (100% N), *A. vinelandii* strain SRIAz3, *A. vinelandii* strain SRIAz3 + N1, *A. vinelandii* strain SRIAz3 + N2 and *A. vinelandii* strain SRIAz3 + N3 from T_1 to T_8 respectively. Result of post-harvest soil chemical properties revealed that soil pH raised from 4.57 to 5.26 being highest with T_7 (5.26) and lowest with T_1 (5.09), soil organic carbon status from 0.68 to 0.78 being highest with T_8 (0.78) and lowest with T_1 (0.42), soil available nitrogen increased from a range of 143.0 - 179.6 to 112.8- 227.6 kg ha^{-1} being highest with T_8 (227.6 Kg ha^{-1}) and lowest with T_1 (112.8 Kg ha^{-1}), soil available phosphorous increased from a range of 7.14 - 9.59 to 10.18-15.80 kg ha-1 being highest with T_5 (15.80 kg ha^{-1}) and lowest with T_1 (10.18 kg ha^{-1}) and soil available potassium increased from a range of 139.21 - 153.32 to 148.96 - 189.84 kg ha^{-1} being highest with T_8 (189.84kg ha-1) and lowest with T_1 (148.96 kg ha-1) likewise result of plant growth parameterslike Chlorophyll content, plant height (cm) and no. of tillers per plant at 45 DAT and 90 DAT revealed that T_7 recorded highest chlorophyll content 47.10 (90 DAT) followed by the treatment T_6(46.94 90 DAT), T_8 recorded highest plant height (88.25cm) followed by T_7 (74.00 cm) at harvest and T7 recorded highest no. of tillers per hill (14.35) at 45 DAT where as T_5 recorded highest no. of tillers per hill (18.25) at harvest. To conclude, use of *Azotobacter vinelandii* strain SRIAz3 isolated from SRI Field (Central Farm, OUAT, Bhubaneswar) as biofertilizer significantly influenced post harvest soil chemical properties, which consequently enhanced the growth parameters of rice cultivar *Pyari*.

Keywords: *Azotobacter vinelandii*, Rice, post-harvest, Chlorophyll

Introduction
The indiscriminate and excessive application of fertilisers in crop production has been generally recognised as damaging to the environment and thus becoming an obstacle to soil productivity and causing irreparable damage to the overall system (Huang, 2000).The use of these agrochemicals can be expected to increase more in face of growing demands for agricultural produce driven by increasing population and continuous decline in land use devoted to agricultural production (Isherwood, 2000). Such scenario can most likely be expected in rice production as it remains the staple food of much of world's population.

While agricultural chemicals arehave negative impacts to the environment, decreased dependence on these chemicals cannot be expected in the near future as it is impossible to sustain high agricultural yields without relying on them. However, effective alternative strategies that can significantly reduce chemicals without compromising yield and quality of agricultural produce can be explored. One such alternative that is worth investigating is the use of biofertilisers (Parr *et al*. 2000). Presently, a huge number of studies has been recorded on the efficiency of several formulation of biofertilisers developed by research institutions on rice growth and production in developing countries like India.

Biofertilisers are inoculants of live microorganisms capable of fixing atmospheric nitrogen, solubilising phosphate, stimulating plant growth through synthesis of growth promoting substances and adding considerable amount of organic matter to the soil increasing its fertility (Vessey, 2003). Biological nitrogen fixation (BNF) has become important in rice farming systems because this process diminishes the need for expensive chemical fertilizers which have been associated with numerous health and environmental problems. As crop growth and development are closely related to the nature of the soil microflora, especially those in close proximity to plant roots, i.e. the rhizosphere. Therefore control of microorganisms is

essentially required to overcome the limitations of conventional agricultural technologies (Dehury *et al*. 2018) Among different microorganisms *Azotobacter* plays an important role in Biological nitrogen fixation in rice crop. *Azotobacter* are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen which is inaccessible to plants and releasing it in the form of ammonium ions into the soil (nitrogen fixation). In this study, the effect of biofertiliser on the post harvest soil chemical properties and various growth parameters of rice cultivar Pyari was investigated at Agronomy Research Plot of OUAT, Bhubaneswar under irrigated condition.

Materials and Methods

The techniques of investigation followed and the materials used for the experiments are described in this section.

Field experimentation

The Field trial was conducted in the Agronomy Research Plot, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha located at $20^0$26'N latitude and $85^0$80'E longitude with an altitude of 30 m above mean sea level, which is 60 km away from Bay of Bengal. The experimental site experienced high temperature in summer and mild temperature during winter. The annual rainfall was about 1505mm, out of which more than 85 per cent was received between months of July to October. The field experiment was conducted in Randomized complete block design (RCBD) with eight treatment combinations (Table 1) which were replicated thrice.

Medium duration rice cultivar *Pyari* was raised in nursery bed with recommended management practices. At the time of final land preparation vermicompost was applied @ 2 t ha^{-1} to each plot. The test crop received N-P_2O_5-K_2O @ 80-40-40 kg ha^{-1} in the form of urea, DAP and MOP respectively. Thirty (30) days old seedlings were uprooted from the nursery bed on the day of transplanting and were dipped in *Azotobacter* broth (10^{10}cfu ml^{-1}) for 2 hrs and kept under shade. The seedlings were then transplanted @ 1-2 seedlings/hill in the main field with the recommended spacing of 20 cm X 10 cm. The crop was harvested when more than 80% grains turned yellow and were sundried in the field for 3-4 days and threshed. The grains, chaffs and straw yields were recorded after proper sun drying to retain 14% moisture content in grains.

Representative composite soil samples were collected from all the treatments till harvest. The samples were dried under shade, grinded with wooden hammer and sieved through 2 mm sieve. The samples were preserved in polythene bags for analysis. Fresh rhizosphere soils were used immediately for estimating microbial parameters before transplanting, 45 DAT, 90 DAT and at harvest.

Analysis of Soil samples

The soil samples were analysed at initial and post harvest for different parameters. Soil pH was determined in 1: 2.5 soil: water ratio by pH meter as described by Jackson (1973). Organic carbon content of soil was determined by wet digestion procedure of Walkley and Black as outlined by Page *et al*., 1982. Available nitrogen in soil was determined by alkaline KMnO4 method (Subbiah and Asija, 1956). Available phosphorous in the soil was determined by Bray's 1 method as out lined by Page *et al*. 1982. Available potassium was determined by extracting the soil with neutral normal ammonium acetate solution and estimated by flame photometer.

Growth Parameters

Ten hills per plot were selected randomly in the plot and tagged for recording observations for following parameters. Chlorophyll content of the leaves was measured using Spad meter at 45 and 90 DAT (Days after transplanting).The plant height of ten hills were measured from the ground level to tip of the most leaf at early stages, up to the tip of main panicle at maturity and the average height was expressed in centimetre. The observations were recorded at 45, 90 DAT and harvest. The number of tillers from ten hills was counted and the average was worked out. The observations were recorded at 45, 90 DAT and harvest.

Statistical analysis

Statistical analysis were performed by the software R version 3.2.2 and were tested with Duncan's new multiple range test at 5% critical range using the package "agricolae" and the graphs were constructed by using the software Graph Pad Prism 6.0. The values are the means of three replicates.

Results and Discussions

The experimental findings of the field experiment and laboratory analysis undertaken during the period of investigation are reported in this chapter

Effect of bioinoculation and N sources on postharvest soil chemical properties

Post-harvest soil chemical properties viz. pH, organic carbon, available N, P and K were evaluated and the data obtained are presented in table.

Soil samples collected at harvest from different treatments were analyzed for soil reaction and data recorded in Table 2 revealed that soil reaction (pH) in treated plot ranged between 4.92- 5.26. At harvest the highest soil pH (5.26) was recorded in the plot receiving treatment T_7while the lowest was recorded in treatment T_4 (N3 - 100% N). The initial soil pH ranged between 4.53 – 4.57.

Soil samples from different treatments collected at harvest were analyzed for soil organic carbon status and data presented in Table 2 revealed that post soil O.C. in treated plots ranged between 0.42- 0.78%. At harvest the highest soil organic carbon (0.78 percent) was recorded in the plot receiving treatment T_8 while the lowest (0.42 per cent) was recorded in T_1. The initial soil organic carbon in the experimental plots ranged between 0.62 - 0.68.

Soil analysis data recorded in Table 2 revealed that postharvest soil available N in different treated plots ranged between 112.8- 227.6 kg ha^{-1}. Highest available N (227.6 Kg ha^{-1}) was recorded in the treatment T_8 and the lowest available N (112.8 Kg ha^{-1}) in T_1. The initial soil available N in the experimental plots ranged between 143.0 - 179.6 Kg ha^{-1}

Postharvest soil available P tabulated in Table 2 revealed that it ranged between 10.18-15.80 kg ha-1. Highest available P (15.80 kg ha^{-1}) was obtained in treatment T_5 and the lowest (10.18 kg ha^{-1}) in T_1. The initial soil available P in the experimental plots ranged between 7.14 - 9.59 kg ha^{-1}.

Soil analysis data recorded in Table 2 revealed that postharvest soil available K in different treated plots ranged between 148.96 - 189.84 kg ha^{-1}. Highest available K (189.84kg ha^{-1}) was recorded in the treatment $T_8$and the lowest available K (148.96 kg ha^{-1}) in T_1. The initial soil available K in the experimental plots ranged between 139.21 - 153.32 kg ha^{-1}.

Post harvest soil pH values were increased as compared to initial. However, plots treated with only mineral N recorded

lower pH, indicating higher soil acidity in urea applied plots which is in agreement with findings of Bouman *et al*. 1993. Significant differences were obtained among the treatments with respect to soil organic carbon, available N and K, whereas no significant difference was recorded in postharvest soil available P, which was low to medium indicating the acidic pH (<5.50). Postharvest soil available N and pH showed significantly higher value with *A. vinelandii* strain SRIAz3 and 100%N over all other treatments. Abbasi *et al*., (2011) also reported enhanced available N with combined application of biofertilizer and inorganic N in wheat rhizosphere.

Table 1: Treatment details

T_1	Control
T_2	N_1 (50% N)
T_3	N_2 (75% N)
T_4	N_3 (100% N)
T_5	*A. vinelandii* strain SRIAz3
T_6	*A. vinelandii* strain SRIAz3 + N_1
T_7	*A. vinelandii* strain SRIAz3 + N_2
T_8	*A. vinelandii* strain SRIAz3 + N_3

Table 2: Effect of bioinoculation and N sources on post harvest soil chemical properties

Treatments	pH	O. C (%)	Available N (kg ha-1)	Available P (kg ha-1)	Available K (kg ha-1)
T_1	5.09	0.42e	112.8d	10.18b	148.96c
T_2	5.11	0.45e	145.6cd	14.26a	185.36a
T_3	4.95	0.54d	169.2bc	15.64a	159.04bc
T_4	4.92	0.58cd	191.6b	15.00a	175.28ab
T_5	5.13	0.62c	165.4bc	15.80a	157.28bc
T_6	5.16	0.62c	162.0bc	14.38a	189.84a
T_7	5.26	0.70b	176.8bc	15.36a	185.92a
T_8	5.03	0.78a	227.6a	14.46a	165.76abc
CV%		6.31	11.65	10.67	8.19
Intial	4.53-4.57	0.62-0.68	143.0-179.6	7.14-9.59	139.2-153.3

*Means averaged over three replicates represented by the same letter in columns are not significantly different (p<0.05)

Effect of bioinoculation and N sources on growth parameters

Data on chlorophyll content of leaves at 45 DAT and 90 DAT were presented in Table 3. The treatment T_7 recorded highest chlorophyll content 42.20, 47.10 at 45 and 90 DAT respectively followed by the treatment T_8. T_1 plots recorded the lowest chlorophyll content i.e. 36.32 and 40.28 at 45 and 90 DAT respectively.

Data on plant height of rice recorded at 45 DAT, 90 DAT and harvest were tabulated in Table 4. Among all the treatments, plant heights of the crop ranged between 32.56- 46.50 cm at 45 DAT, 64.50- 80.25 cm at 90 DAT and 74.00- 88.25 cm at harvest. The treatment T_8 recorded the highest plant height46.50, 80.25 and 88.25cm at 45DAT, 90 DAT and at harvest respectively, closely followed by T_7which were 45.60, 79.63 and 84.88 cm respectively at 45DAT, 90 DAT and at harvest. T_1 plot maintained lowest plant heights of 32.56, 64.50 and 74.00 cm at 45DAT, 90 DAT and at harvest respectively.

Data on no. of tillers per plant recorded at 45 DAT, 90 DAT and harvest were tabulated in Table 5. No. of tillers per plant in different treatments ranged from 9.50-14.35at 45 DAT, 15.15- 18.25 at 90 DAT and 14.00-17.00 at harvest. The treatment T_7 recorded highest no. of tillers per hill (14.35) at 45 DAT where as T_5 recorded highest no. of tillers per hill (18.25 and17.00) at 90 DAT and at harvest respectively. T_1 plot maintained lowest no. of tillers per hill i.e. 9.50, 15.15 and 14.00 at 45DAT, 90 DAT and at harvest respectively.

Plant growth promoting microorganisms exert a positive effect on the growth, development and yield of plants through various mechanisms such as biological N_2 fixation, production of vitamins and growth substances, providing plants with available phosphorus, increasing the amount of nutrients in rhizosphere (Dobbelaere *et al*. 2003; Berg, 2009). The results reveal that the plots receiving combinedly *Azotobacter* and 100% N significantly influenced the plant height at 45 DAT, 90 DAT and at harvest over the rest and control being the lowest while *Azotobacter* inoculation along with N @100% maintained significantly higher no. of tillers per hill towards harvest.

According to Wani *et al*., (2013) *Azotobacter* genus synthesizes auxins, cytokinins, and GA–like substances and these growth materials are the primary substances controlling the enhanced growth. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants. Major yield attributing characters like chlorophyll content, panicle length, no. of grains per panicle and 1000 grain weight were significantly higher in plots receiving *A. vinelandii* strain SRIAz3 inoculation and 75% N whereas root length and root volumes were significantly influenced by combined application of *A. vinelandii* strain SRIAz3 and 100% N.Yadav *et al*., (2013) revealed that *Azotobacter* produces growth promoting substances which improve seed germination and growth of extended root system. Application of 100% recommended dose of nitrogen(RDN) through organic manures along with biofertilizers significantly increased the chlorophyll content in rice.

Table 3: Effect of bioinoculation and N sources on chlorophyll content of leaves

Treatments	45 DAT	90 DAT
T_1	36.32	40.28
T_2	38.64	42.44
T_3	40.16	43.88
T_4	41.14	44.58
T_5	40.58	44.90
T_6	42.04	46.28
T_7	42.20	47.10
T_8	41.98	46.94

Table 4: Effect of bioinoculation and N sources on Plant height (cm)

Treatments	45 DAT	90 DAT	HARVEST
T_1	32.56	64.50	74.00
T_2	36.00	71.00	80.00
T_3	38.50	76.50	85.00
T_4	41.66	78.50	84.75
T_5	37.66	71.50	80.75
T_6	43.60	76.75	85.75
T_7	45.60	79.63	84.88
T_8	46.50	80.25	88.25

Table 5: Effect of bioinoculation and N sources on no. of tillers per hill

Treatments	45 DAT	90 DAT	HARVEST
T_1	9.50c	15.15c	14.00c
T_2	11.85b	16.45abc	15.33abc
T_3	12.35ab	17.10ab	15.66abc
T_4	13.10ab	18.20a	17.00a
T_5	11.60b	18.25a	17.00a
T_6	11.95b	17.45ab	16.50ab
T_7	14.35a	17.20ab	16.36ab
T_8	13.20ab	16.15bc	15.00bc
CV (%)	9.30	6.14	6.49

Conclusion

Use of *A. vinelandii* strain SRIAz3 isolated from SRI field (OUAT, Bhubaneswar) as biofertilizer significantly influenced the post-harvest soil chemical properties and various growth parameters (Chlorophyll content, plant height and no. of tillers per hill) in rice cv. *Pyari*.

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