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Influence of *Azotobacter* Culture Spray on Improvement of Rice Crop

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Abstract

A field experiment was conducted during *kharif* season (2009) on direct seeded rice to find out the efficacy of *Azotobacter* culture spray on growth and yield of rice cv Vandana. Where effect of *Azotobacter* culture (as foliar application) in different concentration (25, 50, 75 and 100%) was compared with two doses (20 and 40 kg/ha) of nitrogen and one spray of Burk's broth media with three replication. All the growth variables studied, including yield attributing characters and yield , nutrient uptake, soil fertility status and bacterial population of leaf were recorded . Application of nitrogen at 40 kg/ha and *Azotobacter* culture spray at 100% recorded the significantly higher plant height, number of effective tillers and panicle weight. Highest yield of rice grain (2, 333 kg/ha) and straw (2,767 kg/ha) was obtained when nitrogen was applied at 40 kg/ha (T_3). While, rice grain and straw yield recorded by *Azotobacter* culture spray at 100% (T_8) and 75% (T_7) were statistically at par with the respective highest values. Percentage increase in grain yield was observed to be highest 89% with treatment T_3 followed by T_8 (77%) and T_7 (70%) over control . However, in straw yield was highest in treatment T_8 (64%) and T_3 (63%). Spray of *Azotobacter* culture at 100% resulted into significantly higher nitrogen uptake by rice grain and straw (28.03 and 21.44 kg/ha, respectively). Highest phosphorus and potassium uptake was recorded by *Azotobacter* culture spray at 100%, however, the effect of T_3 and T_7 were statistically at par. No significant influence of all treatments on soil fertility. Maximum bacterial population were observed when *Azotobacter* culture sprayed at 100% and the increase recorded after first (30 days), second (45 days) and third (60 days) spray were 226, 293 and 238%, respectively as compared to control.

Key words : *Azotobacter* culture, Spray, Nutrients, Rice, Yield.

The beneficial effect of culture spray may be attributed to meeting nitrogen nutrient of plants through dinitrogen reduction by ectosymbiotic association of bacteria on leaves (1). Production of hormonal substances like auxins, cytokinins and gibberellins by several non-symbiotic nitrogen fixing microorganisms including *Azotobacter Chrococcum, Azotobacter Paspali, Azotobacter Vinelandii, Azotobacter Beijerinckia* and *Azotobacter Brasilense* may also attributed to increase in yield and yield attributing characters (2). Production of hormonal substances may improve the growth of plants directly or indirectly by affecting metabolic processes and ion uptake (3). Soil plays a major role as the source of minerals influencing metabolism of the green plant and the supply of phyllosphere nutrients exuded at the leaf. The intermittent exchanges between leaf and the phyllosphere results in an increase in the population of microorganisms which gradually alters the conditions in the phyllosphere and lead to a slowly changing dynamic equilibrium in the microbial cover. Occurrence of microbial population rich in N-fixing organisms on leaf surface of vegetation may be of decisive importance for nutrition.

Methods

An investigation was conducted during *kharif* season (2009) taking direct seeded rice as the test material in the Department of Soil Science and Agricultural Chemistry, Birsa Agricultural University, Ranchi, Jharkhand. The soil was low in organic carbon (0.42%), available N (245. 0 kg/ha), P_2O_5 (12.4 kg/ha) and K_2O (116.0 kg/ha) content with pH 5.7. The experiment was laid out in rendomized block design with eight treatments in three replications. Vandana rice was sown in 5m × 4m size plot with spacing of 25 × 25 cm on 24 June, 2009. It was carried out to evaluate efficacy of *Azotobacter* culture on growth and yield of crop with different treatments consisted with

Tabel 1. Yield attributing characters and yield of direct seeded rice as influenced by spraying of *Azotobacter* culture.

Treatments		Plant height (cm)	Effective tillers/m	Length of panicle (cm)	Panicle weight (g)	1000 grains wt (g)	Yield (kg/ha) Grain	Yield (kg/ha) Straw	Percent increase in yield over control Grain	Percent increase in yield over control Straw
T_1	Control	105.46	75.33	15.88	1.50	21.41	1233	1700	–	–
T_2	Nitrogen 20 kg/ha	110.36	111.33	16.13	1.69	22.11	1733	2200	41	29
T_3	Nitrogen 40 kg/ha	112.08	124.00	18.11	2.23	23.90	2333	2767	89	63
T_4	Medium (Burk's broth) spray	104.60	108.33	16.60	1.56	22.48	1633	2067	32	22
T_5	*Azotobacter* culture spray 25%	104.73	109.33	16.21	1.54	22.31	1717	2117	39	25
T_6	*Azotobacter* culture spray 50%	105.13	111.33	16.92	1.67	21.84	1750	2367	42	39
T_7	*Azotobacter* culture spray 75%	109.82	117.00	17.39	1.77	21.56	2100	2533	70	49
T_8	*Azotobacter* culture spray 100%	111.60	121.67	17.61	2.16	23.70	2183	2783	77	64
	SE ±	0.853	3.935	0.79	0.11	1.34	80.89	90.28		
	CD (*P*=0.05)	2.59	11.93	NS	0.35	NS	245.37	273.85		
	CV%	14.22	6.21	8.13	14.85	10.34	7.63	6.75		

two levels of nitrogen fertilizer in the form of urea (20 and 40 kg N/ha), one Burk's broth spray (N-free medium) and four levels of *Azotobacter* culture (25, 50, 75 and 100%) spray.

Basal dose of nutrients i.e. 20 kg N, 20 kg P_2O_5 and 20 K_2O kg/ha were applied uniformly in all treatments at the time of sowing. The source of fertilizers used in the experiment was urea, single superphosphate and muriate of potash for N, P, K respectively. After sunset, bacterial culture (*Azotobacter* sp.) suspensions containing 10^8 cells/ml were sprayed three times at fortnightly intervals through hand sprayer. Beginning, from one month after sowing and continuing till a fortnight before harvest. Full dose of bacterial culture was at 500 ml per plot. With the same time of culture spray nitrogen was applied with appropriate ammount in recommended plot. Crop and soil samples were analyzed for uptake and availability of nitrogen, phosphorus and potash as per standard laboratory procedures (4). Bacterial population on leaf surface was enumerated with the help of serial dilution techniques using Burk's nitrogen free medium with following composition (Burk's salt-1.3; Fe-Mo mixture-1.0 ml; sucrose-20.0 g and distilled water-1000 ml).

Results and Discussion

Yield Attributing Characters and Yield

Application of nitrogen at 40 kg/ha and *Azotobacter* culture spray at 100% recorded the significantly higher plant height i.e. 112.08 cm and 111.60 cm, respectively, number of effective tillers 124 and 121.67 , respectively and panicle weight i.e. 2.23 g and 2.16 g, respectively of direct seeded rice (Table 1). However, no significant effect of the treatments on panicle length and 1,000 grain weight was observed. Similarly, in yield of crop table indicate that application of nitrogen in both doses, spray of Burk's broth and spray of *Azotobacter* culture in all concentrations significantly increased grain and straw yield of direct seeded rice. Highest yield of rice grain (2,333 kg/ha) and straw (2,767 kg/ha) was obtained when nitrogen was applied at 40 kg/ha (T_3). However, rice grain and straw yield recorded by *Azotobacter* culture spray at 100% (T_8) and 75% (T_7) were statistically at par with the respective highest values. In treatment (T_4) minimum increase in rice grain (1,633 kg/ha) was observed when Burk's broth medium was sprayed. However, the effect of treatments T_2 (nitrogen at 20 kg/ha), T_5 (25% *Azotobacter* culture) and T_6 (50% *Azotobacter* culture) on grain yield, T_2 and T_5 on straw yield were at par with T_4. Percentage increase in grain yield was observed highest 89% increase with treatment T_3 followed by T_8 (77%) and T_7 (70%) over control . However, in straw yield it was observed highest in treatment T_8 (64%) and T_3 (63%).

The magnitude of increase in rice grain yield

Table 2. Nutrient uptake pattern (kg/ha) in direct seeded rice as influenced by spray of *Azotobacter* culture.

Treatments		N uptake (kg/ha)		P uptake (kg/ha)		P uptake (kg/ha)	
		Grain	Straw	Grain	Straw	Grain	Straw
T_1	Control	13.03	10.47	1.20	1.07	10.44	13.87
T_2	Nitrogen 20 kg/ha	21.63	16.20	2.93	2.14	15.44	19.34
T_3	Nitrogen 40 kg/ha	27.69	20.85	5.23	3.88	23.09	26.90
T_4	Medium (Burk's broth) spray	20.04	12.61	2.83	1.49	14.29	18.39
T_5	*Azotobacter* culture spray 25%	19.64	16.20	3.16	1.90	15.32	19.46
T_6	*Azotobacter* culture spray 50%	2.64	15.77	3.95	2.66	15.89	23.15
T_7	*Azotobacter* culture spray 75%	26.38	20.57	5.11	3.46	20.37	24.45
T_8	*Azotobacter* culture spray 100%	28.03	21.44	5.28	4.08	21.33	27.70
	SE ±	0.60	0.41	0.24	0.21	0.65	0.56
	CD (*P*=0.05)	1.82	1.26	0.74	0.63	1.99	1.71
	CV %	7.81	6.25	7.80	8.00	9.77	7.43

with spray of *Azotobacter* culture with full concentration (100%) was 950 kg/ha, however, it could not exceed the increase recorded by nitrogen application at 40 kg/ha (1,100 kg/ha). These two values being statistically at par. While, the increase in grain yield by *Azotobacter* spray at 100% was significantly higher than the increase obtained with applied nitrogen at 20 kg/ha. Similar trend in case of straw yield and yield attributing characters like plant height, number of effective tillers and panicle weight were observed. Favorable role of *Azotobacter* culture spray on leaf surface of different crops were earlier documented (5) reported that spray of nitrogen fixing bacteria significantly increased the grain yield of high and low yielding rice cultivars. Similar findings were reported earlier while working on rice and wheat. Our results are also in agreement with Samanta and Sen (7) who worked on cereals.

Nutrient Uptake Pattern

All treatments applied in field experiment showed significant positive influence on nitrogen uptake by rice grain and straw (Table 2). Spray of *Azotobacter* culture at 100% (T_8) resulted into significantly highest nitrogen uptake by rice grain and straw (28.03 and 21.44 kg/ha, respectively). However, nitrogen uptake by rice grain (27.69 kg/ha) and straw (20.85 kg/ha) under urea application at 100% (T_3) and that under *Azotobacter* culture spray at 75% (T_7) were statistically same with the highest values. In phosphorus and potassium uptake by rice grain and straw similar trend as observed in nitrogen uptake was noted. Highest phosphorus uptake was recorded by *Azotobacter* culture spray at100% viz., 5.28 kg/ha by rice grain and 4.28 kg/ha by straw; however, the effect of urea at 100% (T_3) and spray of *Azotobacter* culture at 75% (T_7) were statistically at par with spray of *Azotobacter* culture at 100% (T_8). Same with phosphorus , spray of *Azotobacter* culture at 100% resulted in to highest uptake of potassium by rice grain and straw to the tune of 22.12 and 19.97% respectively over control. However, effect of this treatment (T_8) is statistically same with T_3 (urea at 100%) and T_7 (*Azotobacter* culture at 75%).

It was earlier reported that the stimulation supplied by the extra nitrogen that have been obtained may well developed a better root system allowing extra nitrogen and other nutrients to be obtained from the soil (8). Although the leaves of crop plants, which benefit appreciably from association with N_2-fixing organisms on the leaf surface, leach appreciable amount of organic compounds (9). Ursolic, acid, a major component of leaf, is also actively metabolized by *Pseudomonas*. *Acenetobacter* sp. can degrade the C_{44}—polythelene oligomer, n—tetraconate. The production of growth promoting compounds 3—acetic acid by *Pseudomonas polymyxa* have been suggested to be growth stimulants of crested wheatgrass. Besides, greater root development and proliferation of plants in response to bacterial activities enhances the water and nutrient uptake (10). A close association between microorganisms and green plants occurs in the phyllosphere. Here it is in the transpiring

Table 3. Effect of different treatments on pH, OC and available NPK in soil after harvest of direct seeded rice.

	Treatments	pH (1 : 2.5)	OC (%)	Available nutrient (kg/ha) N	P	K
T_1	Control	5.5	0.45	272.63	13.25	146.63
T_2	Nitrogen 20 kg/ha	5.5	0.46	273.53	13.50	147.50
T_3	Nitrogen 40 kg/ha	5.6	0.46	270.90	13.53	144.13
T_4	Medium (Burk's broth) spray	5.6	0.46	279.53	13.28	147.77
T_5	*Azotobacter* culture spray 25%	5.5	0.47	273.90	13.16	147.80
T_6	*Azotobacter* culture spray 50%	5.5	0.47	273.73	13.39	144.87
T_7	*Azotobacter* culture spray 75%	5.5	0.47	279.73	13.74	147.63
T_8	*Azotobacter* culture spray 100%	5.6	0.49	279.87	14.24	145.17
	SE ±	0.09	0.01	6.40	0.22	3.19
	CD (*P*=0.05)	NS	NS	NS	NS	NS
	CV%	3.05	5.08	4.02	10.02	3.75

leaf which supplies the necessary organic and mineral nutrients for the development of phyllosphere population. Free water as a major factor in this environment enables the phyllosphere population to use the leaf exudates and allows an exchange of materials to take place between the leaf surface and the phyllosphere. This treatment had no significant influence on phosphorus and potassium content in rice grain and straw, beneficial effect of the treatments on rice grain and straw yield might be contributed for increased uptake of these nutrients.

Nutrient Status of Post Harvest Soil

Table 3 indicates the effect of treatments on post harvest nutrient status of soil. No significant influence of all treatments on pH (1 : 2.5), organic carbon (%), available nitrogen, phosphorus and potassium (kg/ha) was recorded.

Bacterial Population on Rice Leaf Surface

Table 4 indicate the effect of *Azotobacter* culture spray on phyllospheric bacterial count at 30, 45 and 60 days after sowing of rice. A significant increase in bacterial population was observed with each spray of *Azotobacter* culture in all concentrations and Burk's broth medium. However, application of both doses of urea did not show any significant influence on bacterial count. Maximum bacterial population were observed when *Azotobacter* culture was sprayed at 100% and the increase recorded after first (30 days),

Table 4. Survival record of bacteria on leaf surface before and after spraying of *Azotobacter* culture on direct seeded rice. *Bacterial population at $\times 10^3/cm^2$ on the nitrogen free medium.

	Treatments	Bacterial population* on days 30 Before	30 After	45 Before	45 After	60 Before	60 After
T_1	Control	3.67	6.33	7.67	9.33	10.33	12.00
T_2	Nitrogen 20 kg/ha	3.67	6.67	7.33	8.67	9.67	11.33
T_3	Nitrogen 40 kg/ha	4.00	7.00	7.67	9.33	10.00	12.33
T_4	Medium (Burk's broth) spray	3.33	9.67	10.00	11.67	13.67	15.67
T_5	*Azotobacter culture* spray 25%	3.33	13.67	11.33	20.67	19.33	22.67
T_6	*Azotobacter* culture spray 50%	4.00	16.00	14.67	24.33	22.67	26.67
T_7	*Azotobacter* culture spray 75%	3.67	18.33	16.33	29.67	27.67	32.00
T_8	*Azotobacter* culture spray 100%	3.33	20.67	18.67	36.67	34.67	40.67
	SE ±	0.30	0.36	0.39	0.61	0.67	0.67
	CD (*P*=0.05)	NS	1.09	1.18	1.86	2.02	2.04
	CV%	14.28	5.06	5.24	5.64	6.24	5.36

second (45 days) and third (60 days) spray were 226, 293 and 238%, respectively as compared to control. Thus *Azotobacter* culture spray in rest of concentrations and medium spray had considerable significant positive impact on bacterial population as compared to control and urea application in both doses. The order of increase in bacterial count observed were as follow Burk's broth medium spray>*Azotobacter* culture at 25%>*Azotobacter* culture at 50%>*Azotobacter* culture at 75%.

The increase in bacterial population after culture and medium spray might be due to supply of necessary organic and mineral nutrients by transpiring leaves. Free water as a major factor in this environment enables the phyllosphere population to use the leaf exudates and allows an exchange of materials to take place between the leaf surface and the phyllosphere. In this exchange proximity to the site of photosynthetic activity may prove to be highly advantageous. Results observed during present investigation may be supported by the work carried out by (11). They sprayed nitrogen fixing bacteria on wheat leaves and noticed a significant increase in phyllospheric bacterial count.

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