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Effects of *Azotobacter* Inoculant on the Yield and Nitrogen Uptake by Wheat

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Abstract: A pot experiment was conducted to evaluating the effects of *Azotobacter* inoculant on the yield of wheat (cv. Kanchan). The treatments were T_0 (control), T_1, T_2, T_3, T_4 and T_5. Except 1000 grain weight, all the yield components of wheat viz. plant height, filled spikelets spike^{-1}, spike length and the number of grain spike^{-1} were influenced significantly by the treatments. The highest grain yield of 780 mg plant^{-1} i.e. 84% increase over the control (425 mg plant^{-1}) was obtained due to the treatment of T_5 which did not differ significantly from the yield obtained (687 mg plant^{-1}, 732 mg plant^{-1} and 715 mg plant^{-1}) with the application of T_1, T_3 and T_4, respectively. There was 18% increase in grain yields due to using *Azotobacter* inoculant only over the control, which was not statistically significant. The straw yields showed a similar pattern. *Azotobacter* inoculation also influenced the root growth significantly. Total N uptake in grain, straw and root increased significantly due to different treatments. The highest N uptake (23.17 mg plant^{-1}) was recorded with the treatment T_5 and the lowest with the T_0 (control), (11.03 mg plant^{-1}). The total N uptake was increased by 89, 36, 101, 88 and 109% over the control due to T_1, T_2, T_3, T_4 and T_5, respectively. *Azotobacter* either alone or in combination with urea N had some beneficial effects on the yield of wheat, which amounted to saving about 20% of urea N.

Key words: *Azotobacter*, urea, wheat, N uptake

Introduction

Fertility of Bangladesh soils has been declining due to extensive use of land and chemical fertilizers in quest of producing more food for ever-increasing population. The organic matter content of most soils is below the critical level mainly due to rapid decomposition under high temperature and high humidity prevailing in Bangladesh. In addition, the use of urea accelerates further decomposition of organic matter. As a result yield stagnancy has arisen as a national problem. Since Bangladesh soils are very deficient in nitrogen, but this nutrient is required in larger quantities for obtaining a good crop yield and therefore, urea is being used extensively for all crops. Its extensive use has been inflicting an adverse effect on environment causing pollution of drinking water and damaging beneficial soil flora and fauna. Under such situation, to address the issues of soil fertility and crop productivity, the overall management system of crop culture needs to be improved. Combination of inorganic, organic and biofertilizers are being stressed upon now a days as an approach called the integrated nutrient management In this context biofertilizer may play a vital role to improve crop yields through better nutrient supplies.

Azotobacter is a free living N_2 fixing bacterium. It can successfully grow in the rhizospheric zone of wheat, maize, rice, sorghum, sugarcane, cotton, potato, tomato, brinjal, cabbage and many others and fix 10-20 kg N ha^{-1} cropping season^{-1} (Jadhav *et al.*,1987). Besides N_2 fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pentothenic acid, biotin, heteroauxins, gibrellins etc. which enhance root growth of plants (Rao, 1986). Another important characteristic of *Azotobacter* association with crop improvement is excretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986). *Azotobacter* has the ability to produce antifungal antibiotics and fungistatic compounds against pathogens like *Fusarium, Alternaria, Trichoderma* (Wani *et al.*, 1988). All these factors combined together produce positive effects on crop yield. Under the above circumstances, this study was undertaken to test the effect of *Azotobacter* inoculant on wheat in pot.

Materials and Methods

This was a pot study carried out in the net house of the Department of Soil Sciences, BAU during 30 November 1999 to 16 March 2000 to study the effects of *Azotobacter* inoculation on wheat (cv. Kanchan). The experiment was laid out in a completely randomized design with 6 treatments. The treatments were: T_0 (control), T_1 (240 kg N ha^{-1} as urea), T_2 (*Azotobacter*), T_3 (192 kg N ha^{-1} as urea + *Azotobacter*), T_4 (168 kg N ha^{-1} as urea + Cowdung) and T_5 (168 kg N ha^{-1} as urea + Cowdung + *Azotobacter*). Seven kg soils were used in each pot and the total number of pots were 24. The experimental soil was collected from BAU farm, which belongs to Sonatola series of Old Brahmapurta Floodplain (AEZ 9). The soil was silt loam in texture having a pH of 7.5 and 1.67% organic matter. Mechanical analysis of soil was done by hydrometer method (Buoyoucos, 1926) and the textural class was determined following "Marshall's Triangular Coordinates" using USDA system. The pH of the soil was determined with the help of a glass electrode pH meter using the soil : water ratio of 1:2.5 (Jackson, 1962).Wet oxidation method was followed to determine the percentage of organic carbon according to Page *et al.* (1989) and then the organic matter content was calculated by multiplying the percent organic carbon with the Van Bemmelen factor, 1.73 (Piper, 1950). Phosphorus @ 34 kg P ha^{-1} as triple super phosphate, potassium @ 240 kg K ha^{-1} as muriate of potash, sulphur @ 24 kg S ha^{-1} as gypsum and boron @ 2.25 kg B ha^{-1} as borax were applied as basal to all experimental pots. Nitrogen @ 240 kg N ha^{-1} as urea was applied in 2 splits - $^2/_3$rd as basal and 1/3 at crown root initiation stage (21 DAS). Cowdung @ 5 t ha^{-1} was applied during the preparation of soil. Wheat seeds were coated with *Azotobacter* inoculant prior to sowing in case of treatments T_2 and T $_a$ The population of the *Azotobacter* inoculant was 10^9 cells g^{-1} inoculant Count of *Azotobacter* was done following the Most Probable Number (MPN) method revised by Clark (1965). Fifteen seeds were sown in each pot and finally 10 plants plot^{-1} were maintained for recording yields and yield components. The crop was harvested at maturity. The grain and straw weights were recorded and those samples were dried in an oven at 60°C for about 48 hours and then ground. The samples were digested and then analyzed for the determination of N contents. Total N content in seed, straw and root samples were determined by following Kjeldahl method. An amount of 100mg oven dry, ground sample was taken in a 100ml Kjeldahl flask. Into the flask, 1.1g catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: Se = 10 : 1 : 0.1), 2ml 30% H_2O_2 and 3ml conc. H_2SO_4 were added. The flask was swirled and allowed to stand for about 10 minutes followed by heating to boiling at about 200°C. Heating was continued until the digest was clear and colourless. After cooling, the contents were taken into a 100ml volumetric flask, and the volume was made with distilled water. A reagent blank was prepared in a similar manner. The concentration of N in the digest was determined by distillation with 40% NaOH followed by titration of the distillate trapped in H_3BO_3 with 0.01 N H_2SO_4 (Page *et al.*, 1989). Data were analyzed following analysis of variance technique (ANOVA) and the mean differences were adjudged by Duncan's multiple range test (DMRT). (Gomez and Gomez, 1984).

Results and Discussion

Yield and yield contributing characters: All the yield components of wheat except 1000 grain weight were significantly influenced by the treatments (Table 1). The highest plant height (cm), spikelets spike^{-1}, grains spike^{-1} and spike length (cm) were recorded with treatment T_5 (168 kg N ha^{-1}+ CD + *Azotobacter*) and the lowest in the control (T_0) treatment (Table 1). The better growth due to the treatment T_5 may be due to inclusion of *Azotobacter* combined with urea. Badawy and Amer (1977) found 24% increased in plant height by *Azotobacter* inoculation over untreated control.

Grain yield of the crop varied from 425 to 7240 mg plant^{-1}(Table 2). The highest yield was obtained with the treatment T_5 (168 kg N ha^{-1}+ CD + *Azotobacter*) and this yield was statistically similar to those of T_1(240 kg N ha^{-1}), T_3 (192 kg N ha^{-1} + *Azotobacter*) and T_4 (168 kg N ha^{-1} + CD) treatments. The lowest yield was observed in T_0 (control) and it was statistically identical to that of T_2(*Azotobacter*) treatment. The yields were increased by 62, 18, 72, 68 and 84% over control due to T_1 (240 kg N ha^{-1}), T_2 (*Azotobacter*), T_3 (192 kg Nha^{-1}+ *Azotobacter*),T_4 (168 kg N ha^{-1} + CD) and T_5 (168 kg N ha^{-1} + CD+ *Azotobacter*) treatments, respectively. The straw yields showed a similar pattern and the corresponding increases in straw yields were 58, 16, 78, 62 and 74%, respectively. Grain yields were found positively correlated with plant height, spikelets spike^{-1}, grains spike^{-1} and straw yield. Such increase in yields due to *Azotobacter* inoculation have been attributed to N_2-fixation, development of better root system, production of plant growth hormones, enhancement in uptake of NO_3^-, NH_4^+, H_2PO_4, K and Fe, improvement of plant water status and increase in nitrate reductase activity (Wani *et al.*, 1988).

The root weight of the crop ranged from 140 to 279 mg plant^{-1} and the highest root weight was found in the treatment T_5 (168 kg N ha^{-1}+ CD + *Azotobacter*) which was statistically similar to those of T_2 (*Azotobacter*), T_3 (192 kg N ha^{-1} + *Azotobacter*) and T_4 (168 kg N ha^{-1} + CD) treatments. They were significantly different from T_1 treatment (240 kg N ha^{-1}) and the control (To). Rao (1986) explained that root growth was enhanced by due to secretion of different growth regulators like B vitamins, nicotinic acid, pentothenic acid, biotin, heteroauxin, gibbrelins etc. by *Azotobacter*.

Nitrogen concentration and uptake: The N concentration in wheat grain was influenced significantly by the treatments. Grain N concentration varied from 1.79 to 2.29%. The highest grain N concentration was found with the treatment T_1 (240 kg N ha^{-1}) and the lowest in the control treatment. The N concentrations in grain due to T_1 (240 kg N ha^{-1}), T_3 (192 kg N ha^{-1} + *Azotobacter*), T_4 (168 kg N ha^{-1} + CD) and T_5 (168 kg N ha^{-1}+ CD + *Azotobacter*) remained statistically similar. Wadad and Vlassak (1988) reported higher N concentration in grain due to *Azotobacter* inoculation. Like grain N concentration, root N concentration also increased significantly (Table 3). The root N content ranged from 1.04 to 1.18%. The highest root N concentration of 1.18% was recorded with the treatment T_3 (192kg N ha^{-1}+ *Azotobacter*) which did not differ significantly

Table1: Effects of *Azotobacter* inoculation on plant height, filled spikelets spike^{-1}, grains spike^{-1}, spike length and 1000 grain weight of wheat

Treatments	Plant height (cm)	Filled spikelets/spike	Spike length (cm)	Grain/spike	1000 grain weight (g)
T_0 : (Control)	43.94e	6.54c	4.10c	9.40b	44.42
T_1 : (240 kg N ha^{-1})	47.34cd	8.13ab	5.51ab	14.90a	46.10
T_2 : (*Azotobacter*)	45.67de	7.34bc	5.12b	10.99b	45.76
T_3: (192 kg N ha^{-1} + *Azotobacter*)	51.27ab	8.48ab	5.88ab	15.09a	48.52
T_4 : (168 kg N ha^{-1} + CD)	49.70bc	8.20ab	5.74ab	14.168a	49.12
T_5 : (168 kg N ha^{-1} + *Azotobacter* + CD)	53.74a	8.93a	6.31a	15.10a	51.72
SE (±)	0.992	0.434	0.318	0.943	NS
CV (%)	4.08	10.89	11.71	14.14	7.97

Table 2: Effects of *Azotobacter* inoculation on biomass components (grain, straw and root) of wheat

Treatments	Grain yield		Straw yield		Root weight		Total dry matter	
	mg plant^{-1}	Increase over control (%)	mg plant^{-1}	Increase over control (%)	mg plant^{-1}	Increase over control (%)	mg plant^{-1}	Increase over control (%)
T_0 : (Control)	425b	-	311b	-	140c	-	876d	-
T_1 : (240kg N ha^{-1})	687a	62	493a	58	201b	44	13240b	58
T_2 : (*Azotobacter*)	503b	18	3168b	16	263a	88	1127c	29
T_3: (192kg N ha^{-1} + *Azotobacter*)	732a	72	554a	78	257a	84	1543a	76
T_4: (168kg N ha^{-1} + CD)	715a	68	503a	62	235ab	68	1453ab	66
T_5: (168kg N ha^{-1} + *Azotobacter*+CD)	7240a	84	540a	74	279a	99	1599a	83
SE (±)	46.1	-	31.6	-	14.2	-	54.9	-
CV (%)	14.40	-	18.20	-	12.40	-	8.27	-

Table 3: Effects of *Azotobacter* inoculation on nitrogen uptake in grain, straw and root of wheat

Treatments	N content in grain %	N content in straw %	N content in root (%)	N uptake in grain (mg plant^{-1})	N uptake in straw (mg plant^{-1})	N uptake in root (mg plant^{-1})	Total N uptake (mg plant^{-1})
T_0 : (Control)	1.79c	0.63a	1.04b	7.61b	1.96b	1.46c	11.03c
T_1 : (240kg N ha^{-1})	2.29a	0.59a	1.16a	15.63a	2.90a	2.33b	20.86a
T_2 : (*Azotobacter*)	1.95bc	0.63a	1.09ab	9.82b	2.27ab	2.87ab	14.96b
T_3: (192kg N ha^{-1} + *Azotobacter*)	2.18ab	0.57a	1.18a	15.93a	3.15a	3.03ab	22.12a
T_4 : (168kg N ha^{-1} + CD)	2.12ab	0.59a	1.11ab	15.16a	2.96a	2.61ab	20.73a
T_5 : (168kg N ha^{-1} + *Azotobacter*+CD)	2.20ab	0.51b	1.18a	17.12a	2.75ab	3.29a	23.17a
SE (±)	0.078	0.018	0.032	1.592	0.263	0.208	1.635
CV (%)	6.58	3.98	4.90	20.35	17.08	13.85	15.5

from that of T_1 (240kg N ha^{-1}), T_2 (*Azotobacter*) and T_4 (168kg N ha^{-1} + CD). Straw N concentration was also influenced 0.63%. Maximum straw N concentration was registered by the plants of T_0 (Control) and the minimum by the plants of T_5 significantly by different treatments showing a range of 0.51 to treatment (168kg N ha^{-1} + CD + *Azotobacter*).

The use of *Azotobacter* inoculation alone or in combination with urea and/or CD significantly increased N uptake in grain, straw and root of wheat (Table 3). The highest N uptake in grain (17.12 mg $plant^{-1}$) was observed in the treatment T_5 (168 kg N ha^{-1} + CD + *Azotobacter*) which did not differ statistically with the N uptake due to T_1 (240 kg N ha^{-1}), T_3 (192 kg N ha^{-1} + *Azotobacter*) and T_4 (168 kg N ha^{-1} + CD) treatments. Increase of straw N uptake in all the treatments but control was statistically identical. Root N uptake also showed similar trend except T_1 (240 kg N ha^{-1}) treatment. It is statistically different and inferior to T_2 (*Azotobacter*), T_3 (192 kg N ha^{-1} + *Azotobacter*) and T_4 (168 kg N ha^{-1} + CD) treatments. All these treatments differed significantly with T_2 (*Azotobacter* only) and T_0 (Control). Root N uptake also showed a similar trend. Total N uptake was the highest (23.17 mg N $plant^{-1}$) for the treatment T_5 (240 kg N ha^{-1} + *Azotobacter* + CD) which was closely followed by T_3 (192 kg N ha^{-1} + *Azotobacter*), T_1 (240 kg N ha^{-1}) and T_4 (168 kg N ha^{-1} + CD) treatments. They were statistically similar but differed significantly from T_2 (*Azotobacter*) and To (the control). The treatment T_2 (*Azotobacter*) was statistically superior to the control. The increases in N uptake were 89, 36, 101, 88 and 109% over the control (T_0) due to T_1 (240kg N ha^{-1}), T_2 (*Azotobacter*), T_3 (192kg N ha^{-1} + *Azotobacter*), T_4 (168kg N ha^{-1} + CD) and T_5 (168kg N ha^{-1} + *Azotobacter* + CD) treatments, respectively.

From the above discussion it is clear that *Azotobacter* inoculant may be used as biofertilizer for wheat and it may help reduce the use of urea N by about 20% approximately.

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