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Physico-chemical Changes in Different Varieties of Rice as Influenced by the Application of Blue-green Algae

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Abstract: The residual effect of a mixture of blue-green algae (*Anabaena variabilis, Aulosira fertilissima, Scytonema* sp., *Calothrix javanica* and *Westiellopsis prolifica*) on the growth and yield of two cultivars of rice (BR-3 and BR-7) grown in two consecutive seasons was studied. Four treatments were considered viz. No- Control, UN- Chemical fertilizer applied 24 hours before transplantation, AN- Algal mixture applied seven days after transplantation and UN + AN- Fertilizer and algal inoculum used together. Results showed positive residual effect in terms of leaf breadth and length, number of grain and yield of straw in t. Aman season. Increase in grain yield over control was recorded in Boro and t. Aman season following only AN treatment. Combined effect of UN + AN was much better over control in first, second and third crop respectively. Highest amount of nitrogen and protein content of rice grain, nitrogen uptake by grain, nitrogen content of plants and uptake of nitrogen by plant were recorded suggesting positive residual effect of algalization. Enhancement effect of nitrogen fixation by blue-green algae on the yield of rice was found to be significant at 5% level.

Key words: Physico-chemical changes, rice, varieties, blue-green algae

Introduction

Rice is one of the most important food for more than half the world's population and 90% of it is grown and consumed in the tropical South and South East Asia. Bangladesh is very important, being the 3rd largest rice growing country in the world. The economy and well being of the people of Bangladesh is intimately bounded up with the rice because it is not only the main food and major energy source but also is one of the main plants that uphold the economic set up in Bangladesh. Rice plant can suit itself to any condition, if the plant is provided with abundant sunshine and sufficient water required for particular variety. But for the increased production of grains per hectare, soil fertility is the most important factor. This fertility mainly depends upon three major nutrients namely nitrogen, phosphorus and potassium and also on other micronutrients. Among all the major elements nitrogen compound is one of the key element for the fertility of soil so to say the increased crop production. Most of the poor, small rice farmers are facing acute problems, as they are unable to provide capital for chemical fertilizers. Any alternative source of nitrogen fertilizer without affecting the productivity would be of great economic importance and it has been proved that biological nitrogen fixation in farming system would be of great help to face this shortage of chemical fertilizers and investment problems in the developing countries (Venkataraman, 1977; Watanabe, 1978). By biological nitrogen fixation, maximum inorganic molecular nitrogen of the atmosphere is fixed and converted into an organic nitrogen compound. Postgate (1975) reported that more than 90% of total terrestrial nitrogen comes from biological nitrogen fixation. *Azolla* and Blue-green algae (BGA) are dominant among the nitrogen-fixing agents. BGA played a vital role in maintaining nutrient status of rice soils from time immemorial (Roger and Kulasooriya, 1980). Jaganathan *et al.* (1978) showed that BGA application provided sufficient inoculum for subsequent crops. In the light of above, the present study was undertaken to determine the physico-chemical changes of different varieties of rice by the application of BGA.

Materials and Methods

During present investigation two high yielding varieties, BR-3 (BRRI-BIPLAB) and BR-7 (BRRI-BALAM) were used as plant materials of rice. Seed were obtained from the Bangladesh Rice Research Institute (BRRI), Joydebpur, Dhaka, Bangladesh and the research was carried out in the laboratory of Microbiology and Biochemistry, Bangladesh Jute Research Institute. A mixture of five BGA spp. (*Anabaena variabilis, Aulosira fertilissima, Scytonema* sp., *Calothrix javanica, Westiellopsis prolifica*) was used as a substitute for nitrogen fertilizer i.e. urea.

The experiment was set in 32 plasticized earthen pots (30 cm in height and 20 cm in diameter) for two varieties viz. BR-3 and BR-7 where 11 Kgs air-dried soil samples, free from large particles of stones and bricks, were placed in each pot. Dilute solution of NaOH was added to the soil to raise the pH to about 7.5-8.0 for promoting the optimum growth of BGA used as biofertilizer. NPK was applied in the ratio of 0.60 g: 0.48 g: 0.36 g/pot calculated on the basis of recommended dose of NPK 45: 36: 27 Kg/ha. The entire amount of triple super-phosphate and muriate of potash along with 50% of urea was applied 24 h prior to transplantation. The other half of urea was applied before panicle initiation. To minimize the loss of nitrogen through volatilization, urea was always applied below the sub-surface soil. After seven days of transplantation 2.0 g of algal inoculum (a mixture of *Anabaena variabilis, Aulosira fertilissima, Scytonema* sp., *Calothrix javanica* and *Westiellopsis prolifica*) was inoculated in dry state as a substitute for urea. The different treatment combinations were designed as follows: No = Control, UN = where source of nitrogenous fertilizer was chemical fertilizer, AN = where source of nitrogenous fertilizer was algal inoculum, UN + AN = where source of nitrogenous fertilizer was urea and algal inoculum together. There were four replications for each treatment of the two cultivars of rice BR-3 and BR-7. These replicates were arranged in a completely randomized block design. Randomization was achieved following a standard random permutation table with 1-20 integers.

The seeds of BR-3 were germinated on the moist filter paper in the laboratory in dark in Boro season for first crop. For second crop the seeds of BR-3 were sown in t. Aman season. The germinated seeds were spot sown in the seedbed, which was provided with sufficient manure and water supply. Similarly the seeds of BR-7 were also sown in t. Aman season. For each variety two healthy seedlings (28 days old) were transplanted into the soil which had been made puddle before transplantation. One of the plants was uprooted at the maximum tillering stage for the purpose of chemical analysis. Various cultural operations were made from time to time such as weeding, water supply, etc.

The height of the plants were recorded at different growth stages and was considered 1.0 cm above the soil surface to the tip of the largest panicles of the plant. Length and breadth of the leaves were recorded before and after initiation of panicles i.e. maximum tillering and flowering stages of both varieties. Samples of straw and grain were collected after harvest during two consecutive seasons (Boro and t. Aman) in case of BR-3 and of BR-7 in t. Aman season. The straw obtained from different treatment combinations were oven dried for 24 h at 60-65°C and then their weights were recorded. These dry samples were ground properly and preserved in polythene bags separately for chemical analysis. Total nitrogen and protein content of plant materials were also analyzed where total nitrogen from straw and grain was estimated from all replicates by micro Kjeldahl method (Jackson, 1958), and total protein content of the grain was determined by multiplying the total nitrogen with the conventional factor 6.25 as outlined by Philipp *et al.* (1947).

Results

Changes in physical properties of BR-3 and BR-7

Leaf length and breadth: In BR-3 variety, the highest leaf lengths 43.35 cm and 53.67 cm were observed in only chemical fertilizer treated plants at flowering stage in Boro and t. Aman seasons, respectively (Table 1). The second highest leaf length was observed in control treatments in both the seasons. In t. Aman season, leaf length was less (48.95 cm) in the plants where algal inoculum was used (AN). In BR-7 as well the highest leaf length (60.90 cm) was observed in the plants treated with only fertilizer in t. Aman season, although it was higher (54.32 cm) in control plants in Boro season. The leaf length of 51.80 cm was observed in only algal treated plants rather than that (44.50-cm) in the treatments where both algal inoculum and fertilizer were added (UN + AN). Curiously enough here the second highest leaf length (56.90-cm) was observed in control treatment.

From Table 1, it was found that, in BR-3 variety, practically no effect or residual effect on the leaf breadth of the Boro crop of BR-3 variety was observed as a result of the application of algal inoculum. The highest value (1.17cm) was observed in both Boro and t. Aman seasons in the plants treated with only algal inoculum. The lowest value of 1.07 cm was observed in both control treatment and in the treatments where algal inoculum and fertilizer were used together. In BR-7 variety, the highest value (1.20 cm) was obtained in the plants treated with only fertilizer and the lowest value (1.0 cm) was found in control and only algal treated plants.

Average number of grains of rice plant: The average number of grains per plant followed by the application of BGA and

Table 1: Leaf length and breadth of rice plant as influenced by the application of blue green algae with or without urea.

Parameters	Treatments	BR-3 Variety				BR-7 Variety	
		Boro Season		t. Aman Season		t. Aman Season	
		Maximum tillering stage	Tillering stage	Maximum tillering stage	flowering stage	Maximum tillering stage	Flowering stage
Leaf length	NO	39.50	40.57	41.90	49.42	54.32	56.90
	UN	39.48	43.35	43.95	56.67	51.32	60.90
	AN	39.48	39.68	40.90	48.95	47.97	51.80
	UN + AN	33.75	36.70	39.87	47.35	41.28	44.50
LSD at 5% level			3.70		1.22		5.58
Leaf breath	NO	0.87	1.15	1.0	1.07	0.82	1.0
	UN	0.80	1.10	1.0	1.15	0.82	1.2
	AN	0.92	1.17	1.13	1.17	0.88	1.0
	UN + AN	0.85	1.12	1.0	1.07	0.82	1.17
LSD at 5% level			n.s		0.55		0.104

NO = Control, UN = Chemical fertilizer, AN = Algal mixture, UN + AN = Fertilizer and algal inoculum.

Table 2: Ratio of grains and straw of rice plant as influenced by the application of blue green algae with or without urea.

Treatments	BR-3 Variety						BR-7 Variety		
	Boro Season			t. Aman Season			t. Aman Season		
	Grain	Straw	Ratio	Grain	Straw	Ratio	Grain	Straw	Ratio
NO	18.52	19.75	0.94	23.42	23.49	1.00	26.28	29.10	0.90
UN	24.18	26.37	0.92	34.47	30.20	1.30	32.64	43.20	0.96
AN	27.14	26.90	1.00	43.10	43.10	1.40	32.51	29.80	1.09
UN + AN	25.93	29.25	1.23	50.33	32.25	1.56	43.10	36.26	1.19
LSD at 5% level	9.33	5.39		0.63	2.96		9.43	n.s.	

NO = Control, UN = Chemical fertilizer, AN = Algal mixture, UN + AN = Fertilizer and algal inoculum.

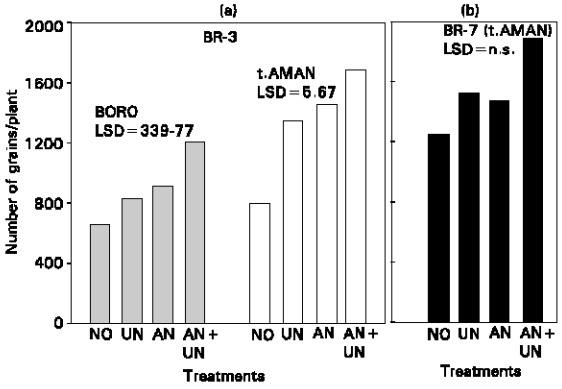

Fig. 1: Average number of grains per plant as influenced by the application of blue-green algae with or without urea.

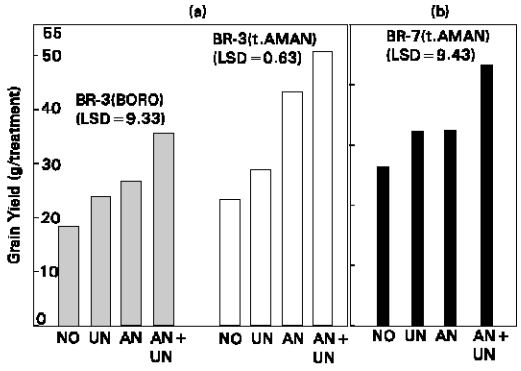

Fig. 2: Yield of rice grains as influenced by the application of blue-green algae with or without urea.

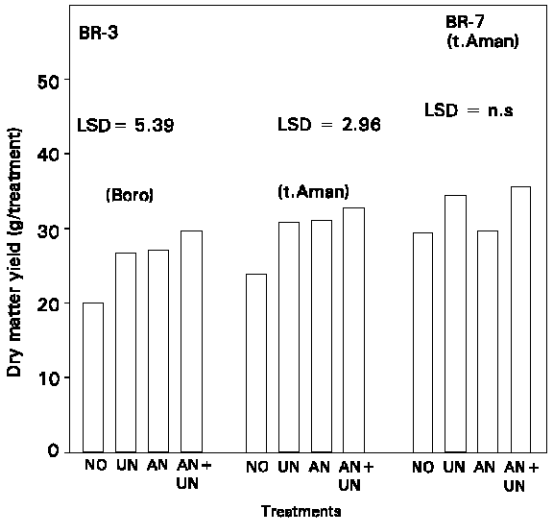

Fig. 3: Yield of rice straw as influenced by the application of blue-green algae with or without urea.

urea are presented in Fig. 1a and b. The maximum number (grains/treatment) of 1197.70 and 1666.50 was

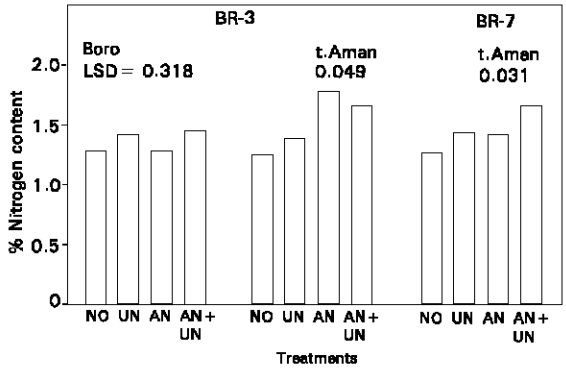

Fig. 4: Nitrogen content of rice grain as influenced by the application of blue-green algae with or without urea.

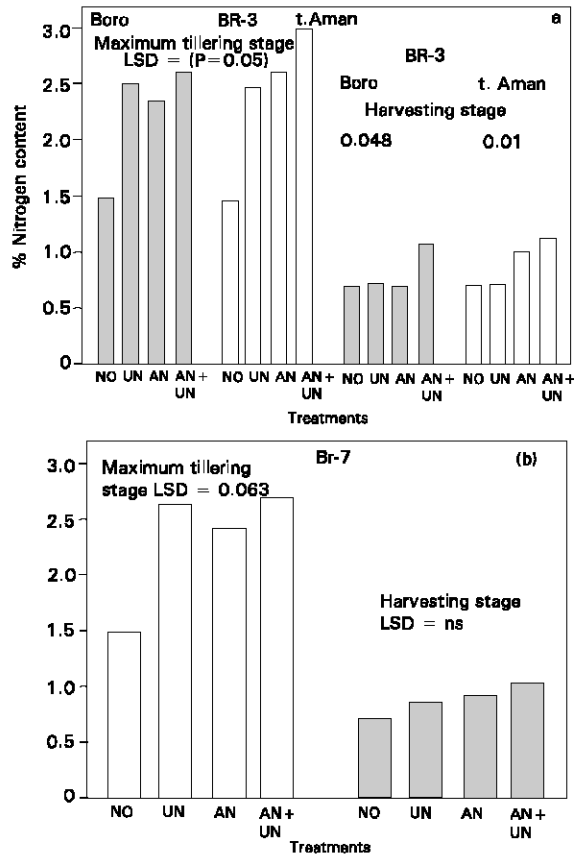

Fig. 5: Nitrogen content of rice at different stage of growth as influenced by the application of blue-green algae with or without urea.

observed in the variety BR-3 in Boro and t. Aman season, respectively following the application of algal inoculum in combination with urea. As expected the lowest number of filled grains of 623.85 and 786.00 was observed in the control treatments in Boro and t. Aman season, respectively. In BR-7 (t. Aman season) the highest and the lowest value

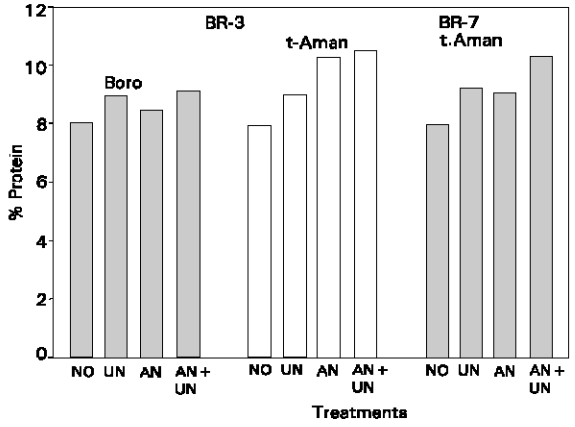

Fig. 6: Protein content of rice grains as influenced by the application of blue-green algae with or without urea.

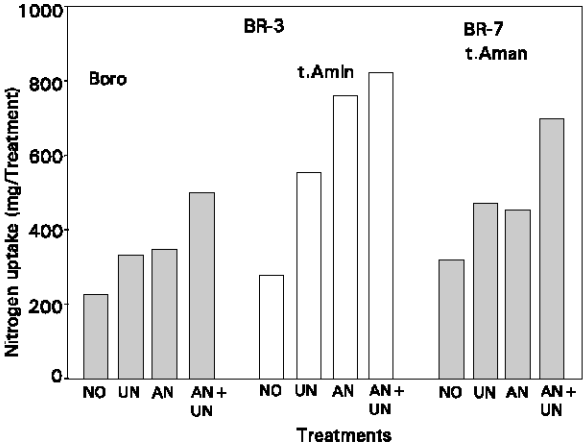

Fig. 7: Uptake of nitrogen by rice grains as influenced by the application of blue-green algae with or without urea.

were 2082.50 and 1314.00 following the application of algal inoculum and urea together and control treatments, respectively. In BR-3 variety in Boro season the number of grains was found to show 46.54% and 94.00% increase over control following the application of only algal inoculum or in combination with urea respectively and in t. Aman season as well positive effect showing 84.03% and 114.90% increase over control following the application of algal inoculum alone or in combination with urea respectively was observed. In BR-7, the number of grains was found to show 24.20% and 64.00% over control following only the fertilizer treatment and fertilizer in combination with algal inoculum, respectively. In contrast to BR-3, the percent increase over control following only algal inoculum was less (23.70% over control) than that of only fertilizer (24.20% over control) treatment.

Yield of grain: The average grain weights of rice as influenced by the application of BGA with or without combination of fertilizer are presented in Fig. 2a and b. In Boro season the highest grain yield of 35.93 g was found in the treatment, where algal mixture in combination with urea was applied. As expected the lowest value (18.52 g) was in control. In t. Aman season as well the highest value of 50.33 g was observed in treatments, where algal inoculum and chemical fertilizer together were used. In BR-7 variety (t. Aman) also the yield was found to be significantly higher (43.10 g), where algal mixture in combination with urea was applied.

Straw yield of plants: The average yield of straw as affected by fertilizer and BGA is presented in Fig. 3. In BR-3 variety, highest yield of 29.25 and 32.25 g were recorded in plants treated with algal mixture and fertilizer together in Boro and t. Aman season respectively. In case of fertilizer treated plants the straw yield was found to be 26.37 g in Boro season and 30.20 g in t. Aman season. The lowest values of 19.75 g and 23.49 g of straw weight were obtained in control treatments in Boro and t. Aman seasons respectively. In BR-7 variety, the highest and lowest values of 36.26 g and 29.10 g were obtained in the plants treated with algal inoculum in combination with fertilizer and control treatments respectively.

Ratio of grain and straw: In BR-3 variety, in Boro and t. Aman season the highest ratio of 1.23 and 1.56 were obtained respectively in the treatments where algal inoculum in combination with fertilizer (urea) was applied (Table 2). In BR-7 variety, the treatment of dual combination showed the maximum ratio of 1.19 in t. Aman season. There was little variation among only algal, only fertilizer and control treatments, the ratio being 1.09, 0.96 and 0.90 respectively.

Changes of chemical properties of BR-3 and BR-7

Nitrogen content of rice grain: Significant changes were observed at 0.1% level in the grain of both the varieties as influenced by fertilizer in combination with BGA (Fig. 4). In BR-3, in Boro season the highest nitrogen content (1.44%) was obtained in the grains as a result of application of algal mixture and fertilizer together. The lowest value, 1.26% was obtained in the grains of control treatments. In BR-7, also the grain treated with fertilizer and algal inoculum together showed the maximum value of nitrogen content (1.64%). The lowest value (1.26-%) was found to be in control grains.

Nitrogen content of rice plant: The average nitrogen content of rice plants at all stages of growth showed (Fig. 5a and b) significant changes. In Boro season, at maximum tillering stage of BR-3 showed highest value (2.60%) of nitrogen in treatment where algal inoculum and fertilizer were added together and the lowest value (1.47%) was observed in control treatments. Algal inoculum treated plants showed less amount of nitrogen (2.32%) than the fertilizer treated plants (2.49%). At harvesting stage of measurement, it was found that percentage of nitrogen decreased in every treatment. But some increment was observed in the treatments of dual combination of algal mixture and fertilizer, where the value was 1.03%. The lowest percentage of nitrogen (0.67%) was found to be in control. In BR-7 variety also the highest value of nitrogen content (2.52%) was observed in treatment, where algal inoculum in combination with fertilizer was added and the lowest value (1.40%) was found to be in control treatments.

Protein content of rice grain: In BR-3, in Boro season the highest percentage of crude protein (9.0%) and the lowest percent (7.87%) were in the grains of plants treated with algal mixture and fertilizer together and control treatments respectively. In t. Aman season as well the combination of algal mixture and fertilizer was found to show positive effect in protein content (10.31%). In BR-7 variety (t. Aman) as well

the highest percentage of protein (10.25%) was observed in the grains of plants, where BGA and fertilizer were applied together, and the lowest value (7.87%) was recorded in control treatments (Fig. 6).

Uptake of nitrogen in rice grain: From Fig. 7, it is clear that in BR-3, in Boro season the highest concentration of nitrogen (508.00 mg) was in the grains treated with algal nitrogen (BGA inoculum) and fertilizer together whereas in t. Aman season rice grains showed the maximum uptake of nitrogen of 830.00 mg in the treatments where both algal nitrogen and inorganic soluble nitrogen (urea) were applied. The lowest uptake was found to be in control treatments. In BR-7 varieties treatments of dual combinations (algal inoculum + urea) also showed the highest uptake of nitrogen of 708.00 mg. As expected the lowest uptake (330.00 mg) was in control treatments.

Uptake of nitrogen of rice plant: It is clearly revealed that at harvesting stage of BR-3 in Boro season the highest (300.00 mg) concentration of nitrogen in rice plants was in the treatments where algal inoculum and fertilizer were used together. In algae treated plants uptake of nitrogen was 170.00 mg and the lowest amount of nitrogen uptake of 130.00 mg by rice plants was in control treatments. In t. Aman season as well BR-3 crop showed the maximum uptake of nitrogen (340.00 mg) in the treatment of dual combination where both algal mixture and fertilizer were added and the lowest concentration (150.00 mg) was obtained in control treatments. In t. Aman season in BR-7 variety, also BGA and fertilizer treated plants showed the maximum concentration (340.00-mg) of nitrogen and as expected the lowest concentration of nitrogen (190.00 mg) was obtained in the plants of control treatments.

Discussion

It seems that with few exceptions effect of algalization on the leaf length and breadth of the variety BR-3 was negligible (Table 1). The values of leaf length and breath of the variety BR-7 were found to be significant at 0.1 and 5.0% level respectively. In this variety urea nitrogen produced significant leaf length at 10% level over control and 0.1% over only algal inoculum and in combination with algal inoculum. The reason of this negative reaction of algal nitrogen either alone or in combination with urea fertilizer might be due to the initial tie up of the inorganic soluble nitrogen by the algae itself leaving inadequate nitrogen for the growing plants. Pantastico and Gonzales (1976) and Nowawy *et al.* (1958) also showed some positive effects of the addition of algal inoculum on leaf length. Watanabe *et al.* (1951) showed some positive effects of three blue-green algae on the length of the leaves of pot culture rice plant. In case of yield of plant, the various yield components were found to be affected by blue-green algal inoculum and fertilizer together and to a lesser extent by blue-green algae alone. When the data on number of grains were analyzed statistically, the overall effect of treatment showed highly significant impact on BR-3 variety at 5% and 0.1% level in Boro and t. Aman seasons respectively and BR-7 showed non significant impact of grains number. The results showed a wide variation between and among the various treatment combinations. Algal nitrogen alone or in combination with urea showed an increase in grain number in BR-3 as compared to fertilizer nitrogen urea. Subrahmanyan *et al.* (1965) reported an increase in yield by 19.13%, 14.19% and 30.29% in field where only lime, only blue-green algae, and combination of blue-green algae and lime, respectively were added. In pot experiment the same authors showed increased rice yield by 15.72%, 15.72% and 81.25% over the control following either only algal treatment or only lime treatment and combination of two treatments respectively. According to the authors this might be due to the fact that lime application enhanced the nutrition of blue-green algae and raised the pH of soil water medium which in turn helped in developing blue-green algal mass and application of some nutrients other than nitrogen. Shtina (1965) also reported a 13-20% increase in rice yields due to the application of *Nostoc punctiformae* in USSR. Aiyer *et al.* (1972) as well showed an increase in the grain yield following algal supplementation. Begum and Islam (1982) showed that with NPK fertilizers the yield of rice was about 180% over control, and the yield was about 239-260% higher over control where different blue-green algae namely *Cylindrospermum* sp., *Scytonema* sp. and *Calothrix marchica* were used together.

In BR-7 not much difference in the grain yield following only algal treatment or only fertilizer treatment was observed (Fig. 3), however highest yield was obtained following the application of blue-green algal inoculum and fertilizer together. Alimagno and Yoshida (1975) also showed non significant increase in grain yield following algal application. This less amount of grains following only algal treatment might be due to competition for nutrients between the algal inoculum and some of the endogenous algae, which showed maximum growth during this period. According to many authors if a good blue-green flora can be established in a soil, it would be a perpetual source of enrichment of soil, where occasional application of nutrient mixture is enough to promote algal growth and application of some rich nutrients other than nitrogen. Mudholkar *et al.* (1973) in their experiment clearly indicated that the benefit of algal inoculation occurred over the years. It appears that in BR-3, the highest amount of straw was recorded in the pot treated with algal inoculum and fertilizer together in the third crop indicating residual effect; as expected the highest value was in algae treated straw showing again some residual effects (Fig. 3). Residual effect was more evident in case of grain straw ratio following the use of algal inoculum and fertilizer together and algal inoculum alone; where it was more pronounced in the former treatments than the later (Table 2). This might be due the fact that nitrogen requirement was fulfilled by the increased supply through the combined effect of algal inoculum and fertilizer. In Boro season of BR-3 variety, algae alone increased the nitrogen content in grain significant at 1% level over control. The same with urea produced better effect raising the significant value at 0.1% level over control. A similar result was obtained in t. Aman season when algae alone or in combination with urea were applied. In BR-7 (t. Aman) algal inoculum alone or in conjunction with urea produced significant effect (0.1% level) over most of the treatments.

Percent of nitrogen and protein in rice grain presented in Fig. 6 & 7 show that although the nitrogen content of the grain of BR-3 was less in Boro seasons (second crop) but it was highest in t. Aman season following only algal treatments, this result shows a positive residual effect of the application of algal inoculum into the soil. In this variety protein content was also found to show some residual effect (Fig. 7) following either the application of only algal inoculum or in combination with urea. In BR-7 variety, the highest percent of nitrogen (1.64%) in the grain where algal inoculum and fertilizer were used together (Fig. 6) which was significant at 0.1% level. Shukla and Gupta (1967) reported that BGA extracts improved growth and development of rice plants and also protein content of the grain. Mollah (1981) showed highest percent

of nitrogen in the grains of the plants BGA and urea were used together instead of only BGA treated grain. From the Fig. 7, it is evident that in case of BR-3 highest amount of nitrogen uptake was made by the grain treatment with both algal inoculum and fertilizer in Boro and t. Aman seasons and highest nitrogen uptake (830.00 mg) was made in t. Aman season showing a positive residual effect. Grain treatment with only algal inoculum also showed higher nitrogen uptake than those treated with only fertilizer and control. In BR-7 as well the highest amount of nitrogen the grains treated with algal inoculum and fertilizer together made uptake. This result of higher uptake might be due to increased supply of nitrogen through biological nitrogen fixation at maturing stage of rice grains. Alimagno and Yoshida (1975) also showed that uptake of nitrogen by grain was increased due to the inoculation of *Aulosira fertilissima* and *Anabaena spiroides* and *Nostoc* sp. The increment of nitrogen content of rice plant (Fig. 5a and 5b) in third crop might be due to the release of nitrogen either from extra-cellular products of BGA added or by the decomposition of the later (BGA). At the harvesting stage of measurement of plants nitrogen content was found to be less than that in maximum tillering stage in both Boro and t Aman seasons following all the treatments. This might be due to the fact that nutrients were used up for the development of plants and grains. Altogether better increase in nitrogen content was found in algal supplementation over control and fertilizer alone in both varieties.

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