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Research Paper

CYANOBACTERIA FROM RICE FIELD AND COMPARATIVE STUDY OF THEIR PERFORMANCES AS BIOFERTILIZER ON RICE PLANTS

Md. Abul Hasan

Department of Agronomy and Agricultural Extension,
Rajshahi University, Rajshahi-6205,
Bangladesh.

Abstract

In this research cyanobacteria (Blue-Green Algae) were isolated, identified, multiplied and used as inoculums in pot rice experiment. The heterocystous cyanobacteria were only found in soil samples identified as *Anabaena and Nostoc* with four species of each genus. The germination of rice seeds treated with cyanobacteria both of *Anabaena* and *Nostoc* was faster than control. Again, the germination of rice seeds treated with *Anabaena* inoculants was faster than that of *Nostoc* inoculants. The results of pot experiment due to the inoculants of *Anabaena* were: increase of 51% in plant height; 68% in roots length; 56% in fresh shoot weight; 92% in fresh root weight; 120% in dry shoot weight; 146% in dry root weight; 32% in soil moisture; 30% in soil porosity and a decrease of 9.3% in soil bulk density and 3.9% in soil particle density. On the other hand, the results due to *Nostoc* inoculants were: increase of 47% in plant height; 54.8% in roots length; 50% in fresh shoot weight; 80% in fresh root weight; 100% in dry shoot weight; 115% in dry root weight; 28.6% in soil moisture; 28% in soil porosity and a decrease of 7.1% in soil bulk density and 2.7% in soil particle density. There were significant differences ($P < 0.05$) in pot treated with BGA as compared with control. Significant differences ($P < 0.05$) were also observed in pot treated with *Anabaena* as compared to *Nostoc*. *Anabaena* inoculants showed better performance on rice plant and soil properties in comparison to control treatment as well as the treatment treated with *Nostoc* inoculants by the excess increase of 3.2% in plant height; 8.3% in roots length; 3.7% in fresh shoot weight; 6.7% in fresh root weight; 12.5% in dry shoot weight; 14.3% in dry root weight; 3.2% in soil moisture; 1.6% in soil porosity and a decrease of 1.99% in soil bulk density and 1.1% in soil particle density.

Key words: Cyanobacteria, Biofertilizer, Inoculation, Comparative study, Rice growth.

INTRODUCTION

Cyanobacteria (Blue-Green Algae) are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. Rice crops remove around 16–17 kg N for the production of each ton of rough rice including straw . After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers [1]. But the excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. Moreover, excessive use of chemical fertilizers deteriorates soil texture, structure, fertility and changes the soil physical and chemical properties negatively. These problems can be tackled by the use of biofertilizers [2]. Biofertilizers, more commonly known as microbial inoculants, include bacteria (Azotobacter), algae (Blue-green algae) and mycorrhizal fungi; they are natural, beneficial and ecological, and they provide nutrients for the plants and maintain soil structure [3]. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer [4]. The acts of these algae include: (1) Increase in soil pores with having filamentous structure and production of adhesive substances. (2) Excretion of growth-promoting substances such as hormones (auxine, gibberellins), vitamins, amino acids [5, 6]. (3) Increase in water-holding capacity through their jelly structure [5]. (4) Increase in soil biomass after their death and decomposition. (5) Decrease in soil salinity. (6) Preventing weeds growth. (7) Increase in soil phosphate by excretion of organic acids [7].

Most paddy soilshave a natural population of cyanobacteria whichprovides a potential source of nitrogen fixation at no cost [8]. The paddy field ecosystem provides a favorable environment forthe growth of cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. Culture studies were introduced by [9] and the importance of blue-green algal nitrogen fixation in helping to maintain fertility of rice fields was first recognized by [10]. There after Watanabe [11], Venkataraman (1972) [12] and Roger and Reynaud (1982) [5] studied further on this base. Amongst cereals, rice is one and only staple food in Bangladesh. Rice is cultivated almost all over the cultivated land of this country in three seasons (Aush, Amon and Boro) around the year. But there is no significant research has been done regarding to the role of cyanobacteria and their inoculants on improving soil properties, growing rice plants as well as the enhancing of rice production. The aim and attention of this research is pointing out:

i. Evaluate the role of cyanobacteria as a biofertilizer on rice plants in Bangladesh.

ii. Find out the influence of Cyanobacteria as a biofertilizer on changing the soil properties and fertility.

iii. Carry out the best performer amongst cyanobacteria as a biofertilizer on rice plants and soil in Bangladesh.

MATERIALS AND METHODS

Soil sample collection: Soil samples were collected from the depth of0–8 cm on a paddy field of Agronomy and Agricultural Extension research field, Rajshahi University, Bangladesh in the second month of each couple months in the year.

Isolation of cyanobacteria. The dilutions of water and soil 10^{-2} and 10^{-3} were prepared. A nitrogen-free media, modified Benecke's solutions medium (Table.1) [13, 14] were prepared and solidified with 15 g agar. 1 ml from 10^{-2} and 10^{-3} dilutions was added to plates. Then plates were incubated at 26–30°C and 12/12 h photoperiod for 30–35 days in incubator set [13, 14]. For purification, identification and multiplication of colonies, a part of colony was removed by a loop and transferred to a new plate. After three weeks, colonies were transferred to liquid media, shaken at 100 rpm [15] at 26–30°C and 12/12 h photoperiod in a shaker for 14 days [12].

1. Table Modified Benecke's solution

Modified Benecke's solution	
Materials	Quantity (g/l)
K_2HPO_4	0.2
$MgSO_4.7H_2O$	0.2
$CaCl_2$	0.1
Agar	15
$FeCl_3$	2 drops
KNO_3	0.2

Seed germination: Three Petridis marked as A, B, and C was used in seed germination. Rice seeds were kept in each Petridis for soaking with water. 0.25 g wet cyanobacteria (*Anabaena* inoculum) @ of 500 hundred seeds and 0.25 g wet cyanobacteria (*Nostoc* inoculum) @ of 500 hundred seeds were added in Petridis B and Petridis C, respectively.

Petridis A was treated as control that means no cyanobacterial inoculum was added to the same. After 20 days, seedlings height and roots length were measured.

Pot culture: Three pots were used in pot culture. They were also marked as A, B, and C. Then 6 seedlings each with the height of 2.5- 6.5 cm were transferred from the Petridis A, B and C to pot A, B and C, respectively. One week before and one week after transferring the seedlings, 1 g of wet algal inoculums (*Anabaena*) was added to the soil of pot B. Similarly, 1 g of wet algal inoculums (*Nostoc*) was added to the soil of pot C. After three weeks, plants height, roots length, fresh and dry weight of leaf and stem and root was measured. Moisture [16], bulk density, particle density and porosity of soil were also recorded.

Statistical analysis: An analysis was performed with independent Samples *t*-test and significant differences were studied at 0.05% level.

RESULTSAND DISCUSSION

Only two genus of heterocystous cyanobacteria were found in soil samples identified as *Anabaena and Nostoc* with four species each: a. *Anabaena spherica*,b. *A. solitaria*, c. *A.variabilis*, d. *A*. *circinalis* (Figure.1) and a. *Nostoc communae*, b. *N. vaucher*, c. *Nostoc linckia*, *d. Nostoc calcicola* (Figure. 2). Species of *Anabaena and Nostoc* were identified by the key of Desikachary [17].

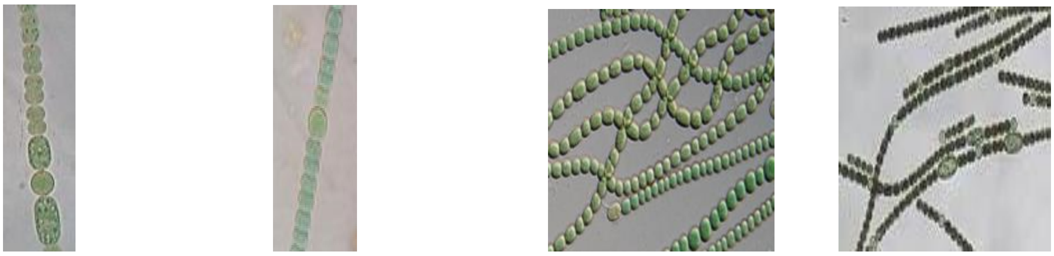

Fig.1 a. ***Anabaena spherica*** **b.** ***Anabaena solitaria*** **c.** ***Anabaena variabilis*** **d.** ***Anabaena circinalis***

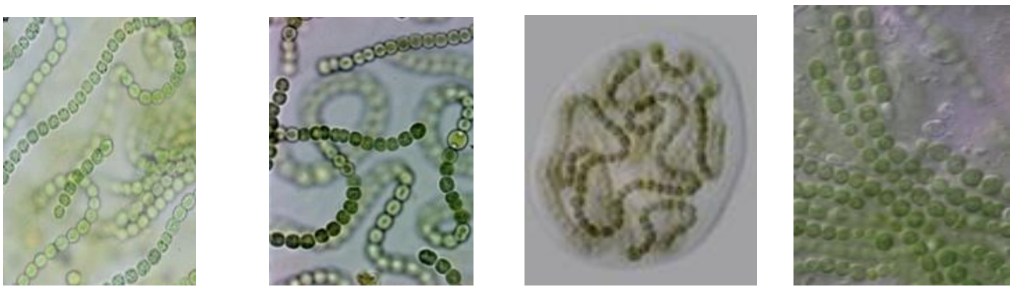

Fig.2 a. ***Nostoc communae*** **b.** ***N. vaucher*** **c.** ***Nostoc linckia*** **d.** ***Nostoc calcicola***

Seeds soaked with water and cyanobacteria (*Anabaen*a inoculants), the starting of germination was observed after 2 days and the seedlings height of 6.5 cm and roots length of 3.2 cm were recorded after 10 days. Similarly, in case of (*Nostoc* inoculants), the starting of germination was observed after 3 days and seedlings height of 5.5 cm and roots length of 3 cm were recorded after 12 days. And in control, however, germination was observed after 5 days and seedlings height of 2.5 cm and roots length of 1.5 cm were recorded after 20 days (Table 2, Figure 3).

Table 2. Effect of cyanobacteria on germination of rice seeds

Sample/parameter	Control	Treatment (T_A)	Treatment (T_N)
Seedlings height (cm)	2.5	6.5	5.5
Root length (cm)	1.5	3.2	3
Period of germination time (days)	20	10	12
Germination rate (%)	46	95	91

T_A = Treatment by *Anabaena*; T_N = Treatment by *Nostoc*

Fig. 3: A. B. C.

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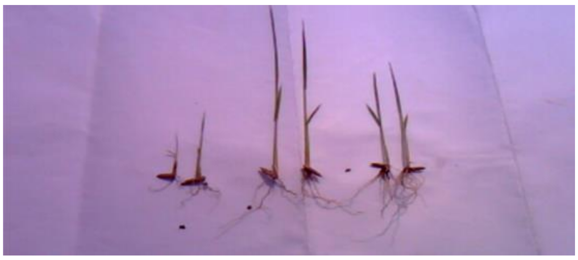

Fig. 3: A. B. C.

Figure. 3:A. Rice seeds soaked with only water (left), **B.** Rice seeds soaked with water and cyanobacterial inoculum *Anabaena* (middle), **C.** Rice seeds soaked with water and cyanobacterial inoculum *Nostoc* (right).

Venkataraman and Neelakantan (1967) [18] noticed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant yield. This evidences that hormonal effects have come from treatments of rice seedlings with algae. The result of pot experiment due to the inoculants of *Anabaena* were: increase of 51% in plant height; 68% in roots length; 56% in fresh leaf and stem (shoot) weight; 92% in fresh root weight; 120% in dry leaf and stem (shoot) weight; 146% in dry root weight; 32% in soil moisture; 30% in soil porosity and a decrease of 9.3% in soil bulk density and 3.9% in soil particle density determined in comparison to control. On the other hand, the result of pot experiment with *Nostoc* inoculants were: increase of 47% in plant height; 54.8% in roots length; 50% in fresh leaf and stem (shoot) weight; 80% in fresh root weight; 100% in dry leaf and stem (shoot) weight; 115% in dry root weight; 28.6% in soil moisture; 28% in soil porosity and a decrease of 7.1% in soil bulk density and 2.7% in soil particle density. *Anabaena* inoculants showed better performance on rice plant and soil properties in comparison to control treatment as well as the treatment treated with *Nostoc* inoculants by the excess increase of 3.2% in plant height; 8.3% in roots length; 3.7% in fresh leaf and stem weight; 6.7% in fresh root weight; 12.5% in dry leaf and stem weight; 14.3% in dry root weight; 3.2% in soil moisture; 1.6% in soil porosity and a decrease of 1.99% in soil bulk density and 1.1% in soil particle density (Table. 4). Significant differences were observed in treatments treated with inoculants as compared to control (Tables. 3 & 4; Figs.3 & 4).

Table 3. Effects of cyanobacteria on rice plant and soil. Analysis was performed with independent Samples *t*-test.

Sample	Control	Treatment (T_A)	Ratio Control/T_A (%)	Treatment (T_N)	Ratio Control/T_N (%)	Ratio T_N /T_A (%)
Plant height (cm)	12.9*	19.5*	66	18.9*	68	97
Root length (cm)	3.1*	5.2*	60	4.8*	65	92
Weight of fresh shoot (g)	0.18*	0.28*	64	0.27*	67	96
Weight of dry shoot (g)	0.05*	0.11*	45	0.10*	50	91
Weight of fresh root (g)	0.25*	0.48*	52	0.45	56	94
Weight of dry root (g)	0.065*	0.16*	40	0.14*	46	88
Moisture (%)	24.1*	32.0*	75	31*	78	97
Bulk density (g/ml)	1.65*	1.51*	109	1.54*	107	102
Particle density (g/ml)	1.88*	1.81*	104	1.83*	103	101
Porosity (%)	14.6*	19*	77	18.7*	78	98

***significant difference at 0.05% level (*P* < 0.05); T_A = Treatment by *Anabeana* Inoculants; T_N = Treatment by *Noctoc* Inoculants**

Table 4 . Comparative study of the effects of cyanobacterial inoculants on rice plant and soil. Analysis was performed with independent Samples *t*-test

Sample	Control	Treatment (T_A)	% of Increase or Decrease by T_A compare to Control	Treatment (T_N)	% of Increase or Decrease by T_N compare to Control	% of Increase or Decrease by T_A compare to T_N
Plant height (cm)	12.9*	19.5*	51	18.9*	47	3.2
Root length (cm)	3.1*	5.2*	68	4.8*	54.8	8.3
Weight of fresh shoot (g)	0.18*	0.28*	56	0.27*	50	3.7
Weight of dry shoot (g)	0.05*	0.11*	120	0.10*	100	12.5
Weight of fresh root (g)	0.25*	0.48*	92	0.45	80	6.7
Weight of dry root (g)	0.065*	0.16*	146	0.14*	115	14.3
Moisture (%)	24.1*	32.0*	32	31*	28.6	3.2
Bulk density (g/ml)	1.65*	1.51*	(-) 9.3	1.54*	(-) 7.1	(-) 1.99
Particle density (g/ml)	1.88*	1.81*	(-) 3.9	1.83*	(-) 2.7	(-) 1.1
Porosity (%)	14.6*	19*	30	18.7*	28	1.6

***significant difference at 0.05% level ($P < 0.05$); T_A = Treatment by *Anabeana* Inoculants; T_N = Treatment by *Noctoc* Inoculants; (-) = Decrease**

Fig. 4: C. B. A.

(15 DAT)

Fig. 4: A. B C.
(55 DAT)

Fig. 4: A. B. C.

(95 DAT; Ripening Stage)

Figure 4. A. Control pot (without Cyanobacteria); **B.** Treatment pot (with Cyanobacteria: *Anabaena*);

C. Treatment pot (with Cyanobacteria: *Nostoc*).

Venkataraman and Neelakantan (1967) [18] also observed 89.5% increase in root dry weight of rice inoculated with algae over control after 30 days of rice root inoculation. In addition, Sankaram (1967) [19] studied the soil physical properties as influenced by algal inoculation. Later on, Aiyer *et al*. (1972) [20] investigated the soil chemical properties as influenced by algal inoculation. They reported the positive effects of algae on soil.

CONCLUSION

In the present study, rice inoculation with heterocystous cyanobacteria isolated from Bangladesh rice field showed that BGA have positive effects simultaneously on rice plant and soil properties. Sao *et al.*, (2015) [21] made similar results in their study. Amongst cyanobacterial inoculants, *anabaena* inoculants exhibited better performance over control and *nostoc* inoculants in relation to improving rice growth and positive changes of soil properties. Chemical fertilizers extensively used throughout most of agricultural Asia including Bangladesh results in causes huge ecological and environmental problems. Biofertilizers, organic fertilizers and other microbial products can overcome this problems because of they are viable components of a healthy and pleasant ecosystem. Cyanobacterial inoculants can play a crucial role in enhancing and repairing of soil fertility as biofertilizer and enhance the growth and yield of crops particularly rice. In today's world, considerable progress has been made in the development of cyanobacteria-based biofertilizer technology although Bangladesh stands behind regarding this issue. It was demonstrated that this technology can be a powerful means of enriching the soil fertility and increasing rice crop yields. However, the technology needs to be improved further for better exploitation under sustainable agricultural systems. Efficient high quality inoculants production and extensive field studies should be given priority in this regard.

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