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AZOLLA AND CYANOBACTERIA AS POSSIBLE NITROGEN BIOFERTILIZER SOURCE IN RICE PRODUCTION

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Abstract

A greenhouse experiment was carried out to study the effect of *Azolla* and /or cyanobacteria inoculation each alone or in combination with chemical nitrogen fertilizer (urea) on rice growth and yield production. Slightly higher rice yield observed in cyanobacteria (SBI) inoculated plots were not significantly different from corresponding non- inoculated treatments. Applying 60 kg Nha^{-1} as urea or as *Azolla* had similar effect on grain yield. Highest yield was obtained with the combination of 30 kg Nha^{-1} as urea and 30 kg Nha^{-1} as *Azolla*. This value was not significantly different from the values obtained with 60 kg Nha^{-1} as urea but was significantly higher than that obtained with 60 kg Nha^{-1} as *Azolla*. *Azolla* and / or cyanobacteria did not affect the rice harvest index . The nitrogen use efficiency decreased with increasing nitrogen level. The highest plant nitrogen uptake was recorded when *Azolla* mixed with urea at 30 kg N ha^{-1} each.

Results show that *Azolla* application alone or in combination with urea is more beneficial to rice than inoculation with cyanobacteria. *Azolla* also increased significantly the soil organic carbon content.

Introduction

The success of rice production mainly depends on an efficient and economic supply of N, an element required in the largest quantity in comparison with other essential ones. The use efficiency of N from fertilizer sources in flood rice field is notoriously, low, because of its loss from soils through various chemical and biochemical process. Besides, increasing the application of nitrogenous fertilizers is neither environmentaly friendly (Conway and Pretty 1988) nor economically viable (Cassman and Pingali 1994). It has, therefore, become necessary to look for alternative renewable resources to meet at least a part of N demand of rice crops. Nitrogen fixing blue-green algae (BGA) or and *Azolla,* have been shown to be the most important in maintaining and improving the productivity of rice field (Roger *et al.*, 1993). The role of blue-green algae and *Azolla* in supplying N to rice field is well documented. The beneficial effect of blue-green algae on the growth and yield of rice has been reported earlier by various workers (De and Mandal 1956; Postgate 1978; Ghazal 1980). They pointed out that BGA biofertilizer is definitely effective in rice cultivation and that the average amount of nitrogen contributed by BGA biofertilizer amounts to about 25 kg Nha^{-1}, both in the absence and presence of other fertilizer. However, Alimagno and Yoshida (1975) suggested the possibility of a gradual build-up of a nitrogen reserve in the soil caused by either the native or the introduced nitrogen fixing cyanobacteria, or both. However, they added that algal inoculation did not significantly affect the growth and yield of the rice plant in both greenhouse and field experiments. They attributed this insignificant trend to some reasons such that the dried

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BGA inoculum applied failed to develop from its dried from, as well as grown ones are not able to compete the indigenous algae materials in soil.

Azolla is also used successfully as a biofertilizer to increase the yield of rice in many countries such as Vietnam and China (Lumpkin and Plucknett 1982). *Azolla* is a small water fern harbors the nitrogen fixing cyanobacterium *Anabaena azolla,* as a symbiont in the leaf cavity. The *Anabaena* in the plant apex is undifferentiated and actively divides among the leaf primordia, but lacks a nitrogen fixing activity (Hill 1977). As the leaf matures, *Anabaena* increases its number and heterocyst frequency and become able to fix atmospheric nitrogen symbiotically and supplies the fixed nitrogen to the fern (Maejima *et al.*, 2002). Due to symbioses , *Azolla* has been used extensively and effectively as green manure in rice fields, instead of chemical fertilizers (Wagner 1997).

Both free living cyanobacteria (BGA) and/or *Azolla* (in algal association) bring out directly or indirectly a number of changes in the physical, chemical and biological properties of the soil and soil-water interface in rice field (Mandal *et al.;* 1999), for example, BGA liberate extra cellular or organic compounds and photosynthetic O_2 during their growth while *Azolla* prevents a rise in the pH, reduces water temperature, curbs NH_3 volatilization and suppresses weeds; and both of them contribute biomass. *Azolla* and/or *Aulosira* applied to rice plants before transplanting at the rate of 60 kg Nha^{-1} produced significantly higher grain yield than that produced by either farmyard manure or urea (Satapathy 1999).

Dixit and Gupta (2000) stated that the average increase in rice grain yield due to BGA inoculation was 0.24 t ha^{-1} (7.5 %).

This work is an attempt to evaluate the use of both cyanobacteria (BGA) or *Azolla* as alternative nitrogen biofertilizer source to rice plant.

Materials & Methods

Algae soil based inoculum (SBI):

Blue-green algae (BGA) {cyanobacteria} was prepared using a mixture of nitrogen fixing cyanobacteria strains viz. *Anabaena oryzae, Nostoc muscorum, Aulosira fertilissima* and *Nostoc calcicola* identified by Rippika *et al.*, (1979). These cyanobacteria strains were previously propagated in the laboratory on Watanabe medium modified by El-Nawawy *et al.*, 1958. under continuous illumination (5000 Lux) and temperature of 28-30°C. After three weeks, the considerable cyanobacteria growth (BGA) was collected by filtration and used to produce the soil based algal inoculum (SBI). The soil based inoculum (SBI) was then prepared in a greenhouse according to Venkataraman's method (1981) using shallow galvanized iron trays (1.00 m * 0.23 m) containing 8 – 10 Kg clayey soil, 5-15 cm tap water above the soil, 200 g super-phosphate and 25 g $tray^{-1}$ carbofuran (3% active ingredient) to prevent the insects attack. After the soil has settled, fresh grown cyanobacteria strains were mixed together each in equal portion and then 100 ml of the mixed culture were sprinkled on the surface of the standing water. The trays were kept in the greenhouse under open air conditions and completely exposed to the sun. Two weeks later, the growth of the cyanobacteria will cover the surface of water forming thick mat. Water was then allowed to evaporate completely in the sun. The dry remained cyanobacteria formed mat will crack into flakes which represent the SBI inoculum.

Multiplication of *Azolla*:-

Azolla pinnata strain (established by Lamark, 1783) was grown in plastic containers 35 cm in diameter and 15 cm depth containing 20 g of peat moss in 2 liters tap water. According to the manufacture this material contains (K 220 – 250, Ca 1000 – 1200 , P 80 – 100 mg /kg and N 0.8 – 1%). These containers were Kept in an insect proof greenhouse till *Azolla* covered the entire surface. This material (fresh *Azolla* fronds) was used as an inoculum for rice fertilization in the greenhouse on the basis that *Azolla* contains 95 % moisture and 4% nitrogen on the dry weight basis (FAO/ IAEA, 1986).

Rice greenhouse experiment:-

The effect of algalization and *Azolla* utilization on growth and productivity of rice variety Sakha 101 were studied in plastic pots, 30 cm diameter with 7 kg clayey soil. Five rice seedlings 35 days old were transplanted per pot. Cyanobacteria inoculum (SBI) at the rate of 250 and \or 500 g ha^{-1} as recommended by El-Nawawy *et al.*, (1972) was inoculated 7 days after transplanting *Azolla* and /or urea was incorporated at transplanting and maximum tillering stages. Pots were kept flooded until two weeks before rice harvesting. The experiment involved the following treatments with three replicates in complete randomized design:

1. Control (without any nitrogen application).
2. 30 kg Nha^{-1} urea
3. 60 kg Nha^{-1} urea
4. 60 kg Nha^{-1} as fresh *Azolla*
5. 250 g ha^{-1} SBI
6. 500 gha^{-1} SBI
7. 30 kg Nha^{-1} urea + 250 g ha^{-1} SBI
8. 30 Kg Nha^{-1} urea + 500 g ha^{-1} SBI
9. 60 kg Nha^{-1} urea + 250 g ha^{-1} SBI
10. 60 kg Nha^{-1} urea + 500 g ha^{-1} SBI
11. 30 kg Nha^{-1} urea + 30 kg Nha^{-1} as fresh *Azolla*.

At harvest all hills were harvested by cutting just above soil surface , dried and cleaned. Plant height, 1000 grains weight, grain and straw yields, {biological yield (total dry matter), harvest index (grain yield / biological yield * 100), nitrogen fertilizer use efficiency (g grain / g nitrogen), Srivastava and Mehrotra, 1982)}, plant nitrogen uptake and N-content of grain and straw (Black *et al.*, 1965) were measured. Carbon content of remained soil were also determined (Walkley and Black, 1934). The obtained data were statistically analyzed as described by Gomez and Gomez (1984).

Results and Discussion

The effect of cyanobacteria inoculation (SBI inoculum) at 250 and 500 g ha^{-1} and / or fresh *Azolla* in the presence or absence of urea on rice and soil carbon under greenhouse conditions are shown in Tables 1 & 2 . SBI inoculation alone had no significant effect on grain yield, straw yield, 1000 grains weight, plant height and straw N content.

Table (1) . Effect of urea, cyanobacteria (SBI) and *Azolla pinnata* on yield components of rice grown under greenhouse conditions and soil organic carbon.

Treatment	Grain Yield (g pot^{-1})	Straw Yield (g pot $^{-1}$)	1000 grains weight (g)	Plant Height (cm)	No. of Panicles (hill $^{-1}$)	Biological yield (g pot^{-1})	Harvest index (%)	Soil carbon (%)
Control	44.8 g	82.4 e	22.4 ab	95 e	5 g	127.2 g	35.2	0.95 i
30 kg Nha^{-1} (urea)	66.8 ef	103.8 cd	21.8 ab	103 cde	6 f	170.6 ef	39.2	0.97 h
60 kg Nha^{-1} (urea)	85.4 ad	166.5 ab	21.7 ab	115 a	11 b	251.9 ad	33.9	0.98 g
60 kg Nha^{-1} (*Azolla*)	83.1 bcd	157.7 b	21.6 ab	113 ab	10 c	240.8 bcd	34.5	1.41 a
250 g ha^{-1} SBI	48.5 g	87.5 e	22.6 a	98 de	7 e	136.0 g	35.7	1.03 f
500 g ha^{-1} SBI	51.3 fg	91.1 de	22.4 ab	100 de	8 d	142.4 g	36.0	1.07 e
30 kg N ha^{-1} + 250 gha^{-1} SBI	69.9 de	104.7 cd	22.1 ab	104 be	9 c	174.6 de	40.0	1.09 c
30 kg N ha^{-1} + 500 g ha^{-1} SBI	71.2 cde	109.7 c	21.8 ab	105 bcd	12 b	180.9 cde	39.4	1.09 c
60 kg N ha^{-1} + 250 g ha^{-1} SBI	86.6 abc	167.8 ab	21.7 ab	110 abc	13 a	254.4 abc	34.0	1.08 d
60 kg N + 500 g ha^{-1} SBI	88.9 ab	170.6 ab	22.2 ab	110 abc	14 a	259.5ab	34.3	1.09 c
30 kg urea –N ha^{-1} + 30 kg *Azolla* -N ha^{-1}	102.01 a	177.9 a	21.4 b	120 af	15 a	279.91a	36.4	1.38 b

In a column , means followed by a common letter are not significantly different at 5% level by DMRT .

Table (2). Effect of urea, cyanobacteria (SBI) and *Azolla pinnata* on nitrogen status of rice grown under greenhouse conditions.

Treatment	Grain-N (%)	Straw-N (%)	N-uptake (g pot $^{-1}$)	N-use efficiency g grain per g nitrogen
Control	1.07 h	0.52 d	0.91	-----
30 kg Nha^{-1} (urea)	1.26 f	0.57 c	1.43	314
60 kg Nha^{-1} (urea)	1.35 bcd	0.66 b	2.25	201
60 kg Nha^{-1} (*Azolla*)	1.33 cde	0.65 b	2.14	195
250 g ha^{-1} SBI	1.10 g	0.53 d	0.99	-----
500 g ha^{-1} SBI	1.11 g	0.53 d	1.05	-----
30 kg Nha^{-1} + 250 g ha^{-1} SBI	1.30 c	0.57 c	1.51	328
30 kg Nha^{-1} + 500 g ha^{-1} SBI	1.32 dc	0.58 c	1.58	334
60 kg Nha^{-1} + 250 g ha^{-1} SBI	1.36 bc	0.68 b	2.32	203
60 kg Nha^{-1} + 500 g ha^{-1} SBI	1.38 ab	0.67 b	2.37	209
30 kg urea -N ha^{-1} + 30 kg *Azolla*- N ha^{-1}	1.41 a	0.71 a	2.70	240

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

The increases in these parameters over the control treatment due to SBI inoculation alone were not significant.

On other hand, SBI had a significant positive effect on the number of panicles/hill and the nitrogen content of the grains (Table 2) either in the presence or absence of the fertilizers. Increasing SBI inoculant level from 250 to 500 g ha^{-1}in combination with 30 kg urea-N did not affect significantly both grain and straw nitrogen contents. Mixing *Azolla*-N (30 kg N ha^{-1}) and urea-N (30 kg N ha^{-1}) gave the highest nitrogen contents of 1.41 and 0.71 % for grain and straw, respectively.

All the treatments increased the nitrogen plant uptake (Table 2) over the control treatment without nitrogen. The highest nitrogen plant uptake value was 2.7 g pot $^{-1}$ followed with 2.32, 2.25 and 2.14 g pot $^{-1}$ for 30 kg urea-N + 30 kg *Azolla*-N ha^{-1}, 60 kg urea-N ha-1 and 60 kg *Azolla*-N ha^{-1}, respectively. The least plant nitrogen uptake value (0.91 g pot $^{-1}$) was recorded by the control treatment.

The nitrogen use efficiency (Table 2) was maximal (334 g grains / g nitrogen) with 30 kg urea-N ha^{-1} + 500 g SBI treatment followed with 328 g grains / g nitrogen for 30 kg urea-N ha^{-1} + 500 g SBI treatment and then decreased with increasing the amount of applied nitrogen. However, SBI inoculation had recorded the highest N-use efficiency, indicating the capability of this inoculum to compensate some of nitrogen fertilizer demands for rice cultivation (Yanni, 1991).

Applying 60 kg N as urea or as *Azolla* had a similar effect on grain and straw yield, 1000 grain weight, plant height, grain N content & straw N content, while the application of 60 kg N as *Azolla* increased significantly the number of panicles hill^{-1}.

The highest grain yield (102.01 g pot^{-1}) was obtained with the combination of (30 kg N as urea and 30 kg N as *Azolla*) ha^{-1}. This value was not significantly different from the value obtained by 60 kg N ha^{-1} as urea but was significantly higher than that recorded by 60 kg N ha^{-1} as *Azolla* alone.

These result show that the application of *Azolla* individually or in combination with nitrogen fertilizers is more beneficial than the blue-green algae (cyanobacteria).

The recorded values of the biological yield (Table 1) for all treatments were significantly higher than that of the control treatment except those inoculated with SBI alone. The highest biological yield of 279.91 g pot^{-1} was achieved with the combination of *Azolla* - N and urea – N both at the rate of 30 kg ha^{-1}. This high biological value was significantly higher than those received urea or *Azolla* each alone and those inoculated with SBI alone or combined with urea.

The harvest index per cent (Table 1) had fluctuated within relatively narrow range, indicating no definite trend effects for mineral nitrogen and / or biofertilizer nitrogen application.

Concerning the soil organic carbon content as influenced by SBI or *Azolla* in the presence or absence of urea (Table 1), results indicate significant increases when compared to control without nitrogen. The higher value (1.41%) was noticed for 60 kg Nha^{-1} as *Azolla* and the least value (0.97%) was for 30 kg Nha^{-1} as urea. Addition of SBI either alone or in combination with urea at the two levels resulted in progressive increases. No response exhibited by increasing the levels of SBI from 250 to 500 gha^{-1} in combination with either 30 or 60 kgNha^{-1}. Generally, The combination between *Azolla* and nitrogen was higher than that of all SBI treatments and *Azolla* in single use.

Such results have been confirmed by (Nazeer and Prassad, 1984; Mian, 1984; Singh and Singh, 1986; Goyal, 1987; Jain and Kaushik, 1989; Sisworo, 1990 and Mishra *et al*., 1998). They claimed that *Azolla* as biofertilizer when combined with urea in rice cultivation gave significantly higher grain yield than cyanobacteria combined with urea. They also added that the highest plant nitrogen uptake was recorded with *Azolla* + urea application other than the utilization of cyanobacteria + urea. Mishra *et al*., (1998), explained this trend by confirming the poor performance of the dried cyanobacteria with N- requirements during critical period of rice growth in comparison with fresh *Azolla*. The dried cyanobacteria need more time to overcome the dormancy phase, while fresh *Azolla* can rapidly decompose and release 78 % of its nitrogen within one week (Ghazal *et al*., 1997). Sisworo (1990) found that *Azolla* was equally as urea on rice when both were applied at the rate of 30 kg N ha^{-1} at transplanting and maximum tillering.

Azolla, cyanobacteria and urea did not exhibit any definite trend on harvest index (Yanni, 1991 and Ghazal *et al.*, 1997). Cyanobacteria and /or *Azolla* application in rice field may improve the available soil nutrients and also soil fertility which in turn affect the plant growth and productivity. Therefore, either *Azolla* or cyanobacteria can compensate partially some of the nitrogen required for rice crop production. However, it is evident that *Azolla* application is more beneficial in rice farming than cyanobacteria (Mishra *et al.*, 1998)

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الملخص العربى

إمكانية استخدام الازولا والسيانو بكتيريا كسماد حيوي ومصدر للنيتروجين لإنتاج الأرز

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أجريت تجربة استخدمت فيها معدلات تلقيح من لقاح الطحالب الخضراء المزرقة (السيانو بكتيريا) والذي تم تحضيره بالمعمل من خلط نسب متساوية من سلالات *Nostoc muscorum, Anabaena oryzae, Nostoc calcicola, Aulosira fertilissima* ولقد استخدم اللقاح بمعدلات مختلفة هي ٢٥٠ و ٥٠٠ جم / هكتار .

وكذلك استخدم لقاح الازولا من سلالة *Azolla pinnata* بمعدل ٦٠ كجم نيتروجين / هكتار ، ٣٠ كجم نيتروجين يوريا على أساس أن الازولا تحتوى على ٤% من وزنها الجاف نيتروجين . وقد تم في هذه التجربة دراسة اثر التلقيح بأي من الطحالب أو الازولا سواء أي منهما منفردا أو مخلوطا مع سماد اليوريا على نمو وإنتاجية نبات الأرز وكذلك على محتوى التربة من المادة العضوية – ولقد أوضحت النتائج ما يلي :-

١ – أن التلقيح بالطحالب أدى إلى زيادة محصول الأرز زيادة طفيفة وذلك عند إضافتها مع اليوريا .
٢ – ليس هناك فرق معنوي في محصول الأرز عند التلقيح بالطحالب بمعدل ٢٥٠ جم أو ٥٠٠ جم / هكتار
٣ – بالنسبة للكربون العضوي بالتربة لم يتأثر بإضافة الطحالب .
٤ – أدى التلقيح بالازولا منفردا أو مع إضافة اليوريا إلى زيادة محصول الأرز وكذا الكربون العضوي بالتربة إذا ما قورنت بمعاملة المقارنة .
٥ – لوحظ أن أعلى محصول للأرز أمكن الحصول عليه مع المعاملة ٣٠ كجم نيتروجين / هكتار (يوريا) + ٣٠ كجم نيتروجين / هكتار (ازولا) وكانت هذه المعاملة مساوية تقريبا للمعاملة ٦٠ كجم نيتروجين / هكتار (يوريا) وأعلى معنويا من المعاملة ٦٠ كجم نيتروجين / هكتار (ازولا) .
٦ - لم يكن للتلقيح بأى من السيانوبكتريا أو الازولا أى تأثير محدد على دليل الجصاد لمحصول الأرز.
٧ – استخدام الازولا كسماد نيتروجينى حيوى فى زراعة الأرز أكثر كفاءة من السيانوبكتريا.