J. Agric. Sci. Mansoura Univ., 31 (8): 5313 - 5321, 2006

AZOLLA AND CYANOBACTERIA AS NITROGEN SOURCE SUBSTITUTE MINERAL NITROGEN IN RICE CULTIVATION

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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 different levels of chemical nitrogen fertilizer (urea) on rice growth and yield production. The slight higher rice yield increases observed in cyanobacteria (CSBI) inoculated pots were not significantly different from corresponding non- inoculated treatments. Applying 60 kg N fed^{-1} as urea and/ or as *Azolla* had similar effect on grain yield. The highest grain yield was obtained with the combination of 30 kg N fed^{-1} as urea and 30 kg N fed^{-1} as *Azolla*. This value was not significantly different from those obtained with 60 kg N fed^{-1} as urea but was significantly higher than that obtained by the use of 60 kg N fed^{-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 fed^{-1} each.

Results show that *Azolla* application alone or in combination with urea are 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 nitrogen, an element required in the largest quantity in comparison with other essential ones. The use efficiency of N from fertilizer sources in flooded rice fields is notoriously, low, because of its loss from soils through various chemical and biochemical processes. Besides, increasing the application of nitrogenous fertilizers is neither eco-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, cyanobacteria (BGA) and/or *Azolla,* have been shown to be the most important in maintaining and improving the productivity of rice field (Roger *et al.*, 1993 and El-Zeky *et al.*, 2005). The role of cyanobacteria and/or *Azolla* in supplying N to rice fields is well documented. The beneficial effect of blue-green algae on the growth and yield of rice has reported earlier by various workers (De and Mandal, 1956; Postgate, 1978; Ghazal, 1980 and Mussa *et al.*, 2002). They pointed out that cya nobacteria as biofertilizer is definitely effective in rice cultivation and that the average amount of nitrogen contributed by BGA biofertilizer amounts to about 25 kg Nfed^{-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 cyanobacteria inoculum applied failed to develop from its dried from, as well as the grown ones are not able to compete the indigenous cyanobacteria materials inhabited the 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 azollae,* 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 and Elzeky *et al.*, 2005).

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 revealed that cyanobacteria 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 $Nfed^{-1}$ produced significantly higher grain yield than that produced by either farmyard manure or urea (Satapathy, 1999 and Mussa *et al.*, 2002).

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

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

MATERIALS AND METHODS

Cyanobacteria soil based inoculum (CSBI):

Cyanobacteria formally called Blue-green algae (BGA) were prepared using a mixture of nitrogen fixing cyanobacteria strains, namely *Anabaena oryzae*, *Nostoc muscorum*, *Aulosira fertilissima* and *Nostoc calcicola*, identified according to 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 (CSBI). The cyanobacterial soil based inoculum (CSBI) was then prepared in a greenhouse according to Venkataraman's method (1981) using shallow galvanized iron trays (1.00 m x 0.60 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 (previously prepared in the laboratory) 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 daily sun light. Two weeks later, the growth of the cyanobacteria will cover the surface of water forming a thick mat. Water was then allowed to evaporate completely in the sun. The dry remained cyanobacteria formed mat will be cracked into flakes which represent the CSBI 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, peat moss 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 then collected to be 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 reference (FAO/ IAEA, 1986).

Greenhouse experiment:

The effect of algalization and *Azolla* utilization on growth and productivity of rice variety Sakha 101 were studied in plastic pots, 35 cm diameter with 7 kg clayey soil. The experiment was laid out in a proof wire greenhouse located at Agric. Res. Center (ARC), Giza at the summer season of 2004. Five rice seedlings of 35 days old were transplanted per pot. Each pot is the thinned to 4 healthy seedlings just before adding any treatment. Cyanobacteria soil based inoculum (CSBI) at the rate of 250 and /or 500 g fed^{-1} as recommended by Ghazal (1988) was inoculated 10 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 Nfed^{-1} as urea
3. 60 kg Nfed^{-1} as urea
4. 60 kg Nfed^{-1} as fresh *Azolla*
5. 250 g fed^{-1} CSBI
6. 500 g fed^{-1} CSBI
7. 30 kg Nfed^{-1} as urea + 250 g fed^{-1} CSBI
8. 30 kg Nfed^{-1} as urea + 500 g fed^{-1} CSBI
9. kg Nfed^{-1} as urea + 250 g fed^{-1} CSBI
10. 60 kg Nfed^{-1} as urea + 500 g fed^{-1} CSBI
11. kg Nfed^{-1} as urea + 30 kg Nfed^{-1}as fresh *Azolla*.

At harvest all hills were harvested through cutting just above soil surface, cleaned and oven dried at70 °C up to a constant dry weight. Plant height, 1000-grains weight, grain and straw yields, biological yield (total dry matter), harvest index (grain yield / biological yield x 100), nitrogen fertilizer use efficiency (g grain / g nitrogen) as described by (Srivastava and Mehrotra 1982), plant nitrogen uptake and N-content of grain and straw (Black *et al.*,

1965) were measured. Carbon content of remained the soil remained after rice harvesting was also determined (Walkley and Black, 1934). The obtained data were statistically analyzed using the comparison test of the least differences between means due to Duncan's multiple range test (DMRT) at 5% level as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The effect of cyanobacteria inoculation (CSBI inoculum) at 250 and 500 g fed^{-1} and / or fresh *Azolla* in the presence or absence of urea on rice and soil carbon content under greenhouse conditions is shown in Tables 1& 2. CSBI inoculation alone had no significant effect on grain yield, straw yield, 1000-grains weight, plant height and straw N content. The increases in these parameters over the control treatment due to CSBI inoculation alone were slight and not significant.

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

All the treatments increased the nitrogen uptake by plant (Table 2) over the control treatment without nitrogen. The highest value nitrogen uptake by plant was 2.7 g pot $^{-1}$ followed by 2.32, 2.25 and 2.14 g pot $^{-1}$ for 30 kg urea-N + 30 kg *Azolla*-N fed^{-1}, 60 kg urea-N fed^{-1} and 60 kg *Azolla*-N fed^{-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) due to 30 kg urea-N fed^{-1} + 500 g CSBI treatment followed by 328 g grains / g nitrogen for 30 kg urea-N fed^{-1} + 500 g CSBI treatment and then decreased with increasing the amount of applied nitrogen. However, CSBI 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 and straw N contents, 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 due to the combination of 30 kg N fed^{-1} as urea and 30 kg N as *Azolla* fed^{-1}. This value was not significantly different from that obtained due to 60 kg N fed^{-1} as urea but was significantly higher than that recorded by 60 kg N fed^{-1} as *Azolla* alone.

These results show that the application of *Azolla* individually or in combination with nitrogen fertilizers is more beneficial than those recorded by the use of cyanobacteria inoculation.

Table (1): Effect of urea, cyanobacteria (CSBI) 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 N fed^{-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 N fed^{-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 N fed^{-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 fed^{-1} CSBI	48.5 g	87.5 e	22.6 a	98 de	7 e	136.0 g	35.7	1.03 f
500 g fed^{-1} CSBI	51.3 fg	91.1 de	22.4 ab	100 de	8 d	142.4 g	36.0	1.07 e
30 kg N fed^{-1} + 250 g fed^{-1} CSBI	69.9 de	104.7 cd	22.1 ab	104 be	9 c	174.6 de	40.0	1.09 c
30 kg N fed^{-1} + 500 g fed^{-1} CSBI	71.2 cde	109.7 c	21.8 ab	105 bcd	12 b	180.9 cde	39.4	1.09 c
60 kg N fed^{-1} + 250 g fed^{-1} CSBI	86.6 abc	167.8 ab	21.7 ab	110 abc	13 a	254.4 abc	34.0	1.08 d
60 kg N fed^{-1} + 500 g fed^{-1} CSBI	88.9 ab	170.6 ab	22.2 ab	110 abc	14 a	259.5ab	34.3	1.09 c
30 kg N fed^{-1} (urea) + 30 kg *Azolla* -N fed^{-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 (CSBI) and *Azolla pinnata* on nitrogen rice status 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 Nfed^{-1} (urea)	1.26 f	0.57 c	1.43	314
60 kg Nfed^{-1} (urea)	1.35 bcd	0.66 b	2.25	201
60 kg Nfed^{-1} (*Azolla*)	1.33 cde	0.65 b	2.14	195
250 g fed^{-1} CSBI	1.10 g	0.53 d	0.99	-----
500 g fed^{-1}CSBI	1.11 g	0.53 d	1.05	-----
30 kg Nfed^{-1}(urea) + 250 g fed^{-1}CSBI	1.30 c	0.57 c	1.51	328
30 kg Nfed^{-1}(urea) + 500 g fed^{-1} CSBI	1.32 dc	0.58 c	1.58	334
60 kg kg Nfed^{-1}(urea) ^{1}+ 250 g fed^{-1}CSBI	1.36 bc	0.68 b	2.32	203
60 kg Nfed^{-1}(urea) + 500 g fed^{-1}CSBI	1.38 ab	0.67 b	2.37	209
30 kg Nfed^{-1} (urea)+ 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 recorded values of the biological yield (Table 1) for all treatments were significantly higher than that of the control treatment except those inoculated with CSBI alone. The highest biological yield of 279.91 g pot^{-1} was achieved due to the combination of *Azolla* - N and urea – N both at the rate of 30 kg fed^{-1}. This high biological yield value was significantly higher than those recorded by any of urea or *Azolla* each alone and those inoculated with CSBI alone or combined with urea.

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

Concerning the soil organic carbon per cent as influenced by CSBI or *Azolla* in the presence or absence of urea are indicated in (Table 1). Results indicate significant increases when compared to control without nitrogen. The highest soil carbon per cent of 1.41% was noticed for 60 kg N fed^{-1} as *Azolla* and the least one (0.97) was for 30 kg N fed^{-1} as urea. Addition of CSBI either alone or in combination with urea at both tested levels resulted in progressive increases in the soil organic carbon per cent. No response exhibited by increasing the levels of CSBI from 250 to 500 g fed^{-1} in combination with either 30 or 60 kgN fed^{-1}. Generally, the combination between *Azolla* and nitrogen was higher than that of all CSBI treatments and *Azolla* in single use.

Such results have been confirmed by (Sisworo *et al.* 1990; Mishra *et al.*, (1998) and EL-Zeky *et al.* (2005). They found 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 rather 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 *et al.* (1990) found that *Azolla* was with equally effect as urea on rice when both were applied in combination at the rate of 30 kg N fed^{-1} at transplanting and maximum tillering stages. Hossain *et al.* (2001) reported that the use of *Azolla* grown as dual with rice could fulfill the entire requirements of nitrogen for rice. *Azolla* incorporated into rice fields increased significantly rice uptake of N, P and S. Moreover, the incorporation of *Azolla* into to soil suddenly increased the C: N ratio of the soil favoring microbial proliferation and the subsequent immobilization of available nitrogen. The mineralization released significant amount of nitrogen within 6-8 weeks because of the decay of added *Azolla*. Consequently, *Azolla* gave its nitrogen by gradual mineralization, which decreases the loss of nitrogen by leaching volatilization or denitrification (Mussa *et al.*, 2002). EL-Zeky *et al.* (2005) explained that fresh *Azolla* when incorporated into the soil is quickly mineralized and 75% of its nitrogen becomes available to the cultivated rice plants within one week. While in case of urea applied alone, most of nitrogen added is probably lost by leaching, volatilization and denitrification. As *Azolla* nitrogen becomes available to the rice plants led to increase the panicles plant^{-1}, filled grains, 1000-grain weight and subsequently increase the grain and straw yields. Strik and Staden (2003) attributed the beneficial affect of *Azolla* to the presence of cytokinins and auxins that enhance the plant growth. They added that the presence of such phytohormones in *Azolla* encourages the agriculturist to use *Azolla* as biofertilizer in crop production especially the cereal ones.

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 crop productivity. EL-Zeky *et al.* (2005) revealed that 40 kg N fed^{-1} combined with either cyanobacteria and /or *Azolla* inoculation gave significantly higher plant height and grain yield than those obtained by the use of 60 kg N fed^{-1}. Also, data indicated that inoculation with *Azolla* was more beneficial than inoculation with cyanobacteria. Furthermore, Herzalla *et al.* (2002) emphasized an increase of 27.6% in soil organic carbon due to *Azolla* applied in rice field, the reason for increasing soil fertility and in turn nutrients availability to the cultivated plants.. Any of *Azolla*, cyanobacteria and /or urea did not exhibit any definite trend on harvest index (Yanni, 1991 and Ghazal *et al.*, 1997).

However, 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).

In conclusion, from this primary experiment in the greenhouse, a promise to be used as a biofertilizer to achieve many beneficial effects in rice cultivation such the reduction of the costly and non-eco-friendly mineral nitrogen fertilizer that ensures the production of high yield and quality.

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الازولا والسيانو بكتيريا كمصدر نيتروجينى بديل للنيتروجين المعدنى في زراعة الأرز

فكرى محمد غزال ، السيدة على حسن و رضا محمد الشحات

قسم بحوث الميكروبيولوجيا الزراعية - معهد بحوث الأراضي والمياه والبيئة-مركز البحوث الزراعية - الجيزة - مصر

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 /فدان .

وكذلك استخدم لقاح الازولا من سلالة *Azolla pinnata* بمعدل ٦٠ كجم نيتروجين / فدان ، ٣٠ كجم نيتروجين يوريا /فدان 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 الكربون العضوى - 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 / فدان.

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 / فدان (يوريا) + ٣٠ كجم نيتروجين / فدان (ازولا) وكانت هذه المعاملة مساوية تقريبا للمعاملة ٦٠ كجم نيتروجين / فدان (يوريا) وأعلى معنويا من المعاملة ٦٠ كجم نيتروجين / فدان (ازولا) .

٦ - لم يكن للتلقيح بأى من السيانوبكتريا أو الازولا أى تأثير محدد على دليل الحصاد لمحصول الأرز.

٧ - استخدام الازولا كسماد نيتروجينى حيوى فى زراعة الأرز أكثر كفاءة من السيانوبكتريا.