J. Agric. Sci. Mansoura Univ., 27(11): 7855 - 7863, 2002

THE POTENTIAL USE OF *AZOLLA* AS NITROGEN SOURCE IN RICE PRODUCTION

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ABSTRACT

A field experiment was conducted at EL-Kalubia governorate at the season (June - October 2001) to study the potential of *Azolla* application as bio-nitrogen source in comparison with the mineral nitrogen fertilizer as urea as well as their effect on soil organic matter. Results reveal that *Azolla* significantly increased rice yield (Var. Sakha 102) and soil organic carbon. Increase in yield was 89.5 % (60 kg N fed^{-1} as urea), 81.5 % (60 kg N fed^{-1} as *Azolla*) and 92.1% (30 kg N fed^{-1} as urea + 30 kg N fed^{-1} as *Azolla*). Sixty kg N fed^{-1} as *Azolla* was almost equivalent the application of 60 kg N fed^{-1} as urea. The combination of urea and *Azolla* 30 kg N fed^{-1} each resulted in grain yield higher than that obtained with urea or *Azolla* alone but not significantly different from that obtained with 60 kg N fed^{-1} as urea. The increase of rice grain yield was associated with the increase in the number of panicles $hill^{-1}$ and the grain weight. The soil organic carbon increased over the control by 28 % for *Azolla* treatment, 41.1% for urea-*Azolla* combination and did not change with urea.

INTRODUCTION

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 primordial, 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).

Azolla is used successfully as a biofertilizer to increase rice yield for centuries in China and Vietnam (Lumpkin and plucknett, 1982), in other parts of Asia, Watanabe (1984) and in other parts of Africa (Van Hove, 1989). In Egypt *Azolla* is newly applied as green manure for rice cultivation, (Hamdi *et al.*, 1980; Ghazal, 1987; EL-Shahat, 1988; Herzalla, 1991; EL-Bassel *et al.*, 1993 and Ghazal *et al.*, 1997). Due to symbioses, *Azolla* has been used extensively and effectively as green manure in rice fields, instead of chemical fertilizers (Wagner 1997).

Azolla – anabaena symbioses brings out directly or indirectly a number of changes in the physical., chemical and biological properties or the soil and soil water interface in rice field (Mandal *et al.,* 1999), for example, *Anabaena* liberate extra cellular or organic compounds and photosynthetic O_2 during their growth while *Azolla* fronds prevent a rise in the PH, reduce water temperature, curls NH_3 volatilization and suppress weeds; and both of them contribute biomass. Incorporation of *Azolla pinnata* alone or in combination with ammonium sulphate as nitrogen fertilizer to rice improved soil fertility and increased the rice yield components (tillers $hill^{-1}$, length of panicle an grain and straw yields) to be much higher than those recorded by the control treatment, indicating the efficacy of *A. pinnata* as biofertilizer (Padhya, 1997). *Azolla* 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).

Azolla as biofertilizer can only be realized if its nitrogen becomes available for crop uptake. This appears only after *Azolla* decomposition. About two-thirds of its mineral nitrogen (NO^+_4 and NO^-_3 is released under aerobic conditions at 29°C in five to eight weeks (Tuzimura *et al.,* 1957). When *Azolla* incorporated at 78 days after transplanting (DT), the amount of nitrogen recovered in the grains increased about 50% (Watanabe 1987). Uptake of urea-N was found to occur primarily within 30 days of application, whereas the major uptake of *Azolla*-N occurred between 30 to 60 days (Eskew 1987).

Kumarasinghe and Eskew (1991) have shown that 65-95% of nitrogen accumulated by *Azolla* spp. Is derived from air. In oxisol soil with a low available P content, the N% recovery increased when *Azolla* was applied at rice transplanting and later incorporated into the soil (Sisworo *et al.*,1995). This work is an attempt to study the possible *Azolla* implications relating to rice production in Kalubia governorate.

MATERIALS AND METHODS

A field experiment was conducted at EL-Kalubia governorate at the season (June - October 2001) to study the potential of the *Azolla* application as nitrogen fertilizer to meet all or partial needs of rice to mineral nitrogen fertilizer. The soil was clayey , with soil reaction 7.8, available nitrogen 7.1 mg kg^{-1}(macro-Kjeldahl technique, Bremener and Edwards,1965), organic matter 0.87 % (Walkley and Black., 1934) and available phosphorus 8.9 mg kg^{-1} (Olsen *et al.,* 1954). Four rice seedlings of 35 days old (variety Sakha 102) were transplanted at 20 cm spacing in 20 plots (3m · 4m) corresponding to four treatments with five replicates settled in a randomized complete block design. Rice plants received nitrogen at the rate of 6o kg N fed^{-1} as urea 46.5 % N and as *Azolla filiculoids*, each alone or in combination in two equal split doses at transplanting and maximum tillering stages. Plots remained water flooded until two weeks before harvest. The floodwater was drained at incorporating of either *Azolla* or urea. Fresh *Azolla* fronds were grown, multiplicated and incorporated to rice field according to the method of Khan (1988). Superphosphate 15 % P_2O_5 at the rate of 15 kg fed^{-1} was applied within the soil tillage before rice seedlings transplanting. The treatments were as follows:

0 Control - no nitrogen but incorporation was simulated at transplanting and maximum tillering stages.
0 60 kg N fed^{-1} as urea (split).
0 60 kg N fed^{-1} as *Azolla* (split).
0 30 kg N fed^{-1} as urea + 0 30 kg N fed^{-1} as *Azolla* (split).

At harvest rice samples were collected using one m^2 wooden frame, and air-dried in the field for three days. Then plant height, number of panicles $hill^{-1}$ was measured after threshing, grain yield, straw yield, and 1000-grain weight were determined. Grains and straw were sub sampled to measure total-N content. Also soil samples were taken for organic carbon analysis. The obtained data were statistically analyzed using Duncan's multiple range test as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

It is well known that nitrogen is considered one of the limiting factors affecting the yield of rice crop. It plays an important role in increasing rice production especially in such conditions of crop intensification as in Egypt. The application of *Azolla* and urea either alone or in combination significantly increased the rice grain yield over the control (Table 1). The percentage increases were 89.5 % (60 kg N fed^{-1} as urea), 81.5% (60 kg N fed^{-1} as *Azolla*) and 92.1 % (30 kg N fed^{-1} as urea + 30 kg N fed^{-1} as *Azolla*). Sixty kg N fed^{-1} as *Azolla* was almost equivalent the application of 60 kg N fed^{-1} as urea. The combination of urea and *Azolla* 30 kg N fed^{-1} each resulted in grain yield higher than that obtained with urea or *Azolla* alone but not significantly different from that obtained with 60 kg N fed^{-1} as urea.

The weight of 1000 grains (Table 1) was not significantly affected by the treatments under this study. However, in contrast data show significant increases in the number of panicles $hill^{-1}$ of 57.1,42.9 and 64.3 % over the control treatments for 60 kg N fed^{-1} as urea, 60 kg N fed^{-1} as *Azolla* and the combination of urea and *Azolla,* respectively. Values for *Azolla* or urea applied alone were not significantly different. The values corresponding to the combination of urea and *Azolla* was not significantly higher than that for *Azolla* alone, but not significantly different from that obtained with urea alone.

These results could explain that the increase in rice grain yield was associated with the increase in the number of panicles $hill^{-1}$ and not in the grain weight. Such results have been reported by (Nazeer and Prassad, 1984; Ventura *et al.*, 1993 and Satapathy, 1999) who observed that the use of urea , *Azolla* or a combination of both , in split application increased the grain yield by 1 to 1.5 tones ha^{-1}. They also added that *Azolla* 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. Moreover, Gevrek (2000) found that the use of combination *Azolla* + N fertilizer in rice cultivation increased significantly than that of N fertilizer alone. He also concluded that the use of *Azolla* will lead to a $^1/_3$ reduction in the N demands of rice crop.

The application of *Azolla* and urea either alone or in combination significantly increased the rice straw yield over the control (Table 1). However, the increases were 91 % (urea), 89 % (*Azolla*) and 39.9 % (urea + *Azolla*) over the control treatment. Non – significant differences were observed towards the straw yield among the tested treatments of *Azolla* and urea or the combination of both.

Due to the plant height, results show significant increases in all treatments as compared with the control. The highest increase was (23.4 %) for *Azolla*-urea combination treatment. The lowest increase was 18.8 % for *Azolla* alone. The application of urea alone caused an increase of 19.5 % not significantly different from the one obtained by the use of *Azolla* alone.

The increase of the straw yield was attributed to the increase in the plant height as a result of *Azolla* application.

Similar observations were made by (Ventura et al., 1987; Sisworo *et al.*, 1990; EL-Bassel *et al.*, 1993 and Ghazal *et al.*, 1997) who declared that the use of *Azolla* alone or in a combination with urea increased significantly the rice straw yield and attained this increase to the increase in the plant height due to *Azolla* application.

Nitrogen content of grain and straw yield were significantly higher for all treatments than that of the control (Table 1).

Nitrogen content in the grains, increased by 27.5 % (urea), 18.3 % (*Azolla*) and 28.4 % (urea + *Azolla*), over the control.

Results obtained with the application of urea alone or in combination with *Azolla* were not significantly different. Same trends were observed for the nitrogen content of straw. The highest increase over the control was 39.6 % for the combination of urea and *Azolla*. Similar results were noticed by (Ruschel, 1986 and Mandal *et al.,* 1999).

A significant effect of *Azolla* was observed on soil organic carbon (Table 1). It increased over the control by 27.6 % for *Azolla* treatment, 42.5 % for urea Azolla combination and did not change with urea.

Generally, many reports confirmed that *Azolla* used as green manures improved the soil organic carbon therefore, soil fertility due to its organic matter and nitrogen content. However, these reports indicated that cyanobacteria either free living or in symbiosis as in *Azolla* are important organisms in rice fields. They contribute significantly towards maintaining and improving the productivity of rice fields. Besides addition of N, they considerably modify the physical, chemical, electro –chemical and biological properties of the soil and the soil water interface in rice fields in ways, which are beneficial to rice crop. The benefits accrued from organic C addition, improvement in soil physical properties, retardation of NH_3 volatilization loss, mobilization of fixed phosphate, regulation of micro nutrients, particularly Fe,

Table (1) Effect of *Azolla filiculoides* and urea on rice yield components and soil organic carbon percentage under field conditions (rice variety sakha 102 – June - October 2001).

Treatment	Grain Yield (t fed^{-1})	Straw Yield (t fed^{-1})	1000 grain weight (g)	Plant Height (cm)	No. of $Paniclehill^{-1}$	Grain-N (%)	Straw-N (%)	Soil carbon (%)
Control	3.80 c	6.60 b	23.40 ab	128 c	14 c	1.09 c	0.48 c	0.87 c
60 kg N fed^{-1} (urea)	7.20 a	12.60 a	23.10 ab	153 b	22 ab	1.39 a	0.63 b	0.89 c
60 kg N fed^{-1} (*Azolla*)	6.90 b	12.50 a	23..60 a	152 b	20 b	1.29 b	0.62 b	1.11a
30 kg N fed^{-1} (urea) + 30 kg N fed^{-1} (*Azolla*)	7.30 a	12.80 a	22.90 b	158 a	23 a	1.40 a	0.67 a	1.24 b

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

Mn and Zn, affecting their availability, amelioration of the acidity of problem soils, suppression of weeds and release of growth- promoting substances sometimes outweigh those due to the N added by them. All these benefits can reduce the intensive use of the unfriendly environmental mineral nitrogen fertilizers in rice cultivation either by partially or entirely replaced with *Azolla*. (EL-Shahat 1988; Herzalla, 1991; EL-Bassel *et al.*,1993; Ghazal *et al.*, 1997; Satapathy, 1999 and Mandal *et al.,* 1999).

Acknowledegements: The authors greatfully acknowledege prof. Dr. F. M. Ghazal Agricultural Microbiology Department, Soils, Water Res. and Environ. Institute, Agric., Res. Center, Giza, Egypt who provides this work with *Azolla filiculoides*, helping in *Azolla* inoculum preparation required for this work.

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كفاءة استخدام الازولا كمصدر للنيتروجين لانتاج الأرز

نجاة عبد العزيز حرزاللة – فتحى توفيق ميخائيل – على الدين أحمد عامر
قسم بحوث الميكروبيولوجيا الزراعية– معهد بحوث الأراضي و المياة وا لبيئة مركز البحـــوث الزراعية –الجيزة– مصر

أجريت تجربة فى الحقل لدراسة أثرالتلقيح بالأزولا على محصول الارز وكذلـك محتوى التربة مـــن الكربـــون العضـــوى وكـــان ذلـــك باســـتخدام الأزولا *Azolla filiculoides* منفردة (٦٠ كجم نيتروجين/ فدان) أو بخلطها مـــع اليوريـــا (٣٠ كجـم نيتروجين/ فدان كأزولا + ٣٠ كجم نيتروجين/ فدان كيوريا) بالمقارنة مع التسميد باليوريا بمعدل (٦٠ كجم نيتروجين/ فدان) .

ولقد أوضحت النتائج مايلى:–

١– أن التلقيح بالازولا أواليوريا بمفردهما أو مجتمعين أدى إلي زيادة محصول الأرز اذا ماقورنت بمعاملة المقارنة بدون أى تسميد.

٢– كانت نسبة الزيادة ,٨٩٥ % فى محصول الأرز مـــع معاملـــة التســـميد (٦٠ كجـــم نيتروجين/ فدان كيوريا) و ,٨١٥ % مع معاملة التسميد (٦٠ كجم نيتروجين/ فـــدان كازولا) و,٩٢١ % للمعاملة (٣٠ كجم نيتروجين/ فدان كازولا + ٣٠ كجم نيتروجين/ فدان كيوريا).

٣– أدى التلقيح بالأزولا منفردا أو مع إضافة اليوريا إلي زيادة الكربون العضوى بالتربـة إذا ما قورنت بمعاملة المقارنة.

٤– امكانية استبدال جزء من نيتروجين اليوريا بنيتروجين الأزولا لتسميد الأرز وتقليـــل التكلفة وكذلك الحد من تلوث البيئة بزيادة الاستخدام المكثف لهذة الاســـمدة المعدنيـــة لزيادة الانتاج المحصولى.