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DETECTION OF *AZOLLA*-N AND UREA-N CONTRIBUTION TO RICE BY THE USE OF ^{15}N DILUTION TECHNIQUE.

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ABSTRACT

Two field experiments were carried out simultaneously at Elsrew Research Experimental Farm Station (ARC) in the summer season of 2004, first one was to study the availability of labelled-N of *Azolla* (3% atom excess [a.e]) and urea (10 % atom excess) to rice plant var. Giza 171 at transplanting (TS) and maximum tillering stage (MTS), the second was to study the effect of *Azolla pinnata* inoculation in comparison with urea fertilizer on the rice yield components and the nitrogen content of rice grains and straw. Results in the first experiment indicate that *Azolla* used at the two stages exhibited higher values for all the labelled nitrogen parameters in comparison with unlabelled treatments. The highest values were 23 kg Nha^{-1} for labelled-N uptake, 14.5% for N- derived fertilizer and 76.8 % for ^{15}N when labelled *Azolla* was applied at MTS. All the nitrogen values at TS were lower, but the values obtained with *Azolla* were higher than that with urea. Generally N applied later in the crop cycle is more easily absorbed than when applied at early stages as well as *Azolla*-N was more available to rice than was urea-N. At maximum tillering, 76.8% of ^{15}N from *Azolla* were recovered in the rice plant compared with only 42.9 % from urea. In the second experiment *Azolla* significantly increased rice yield (Var. Giza 171) and soil organic carbon. Increase in yield was 89.5 % (60 kg N ha^{-1} as urea), 81.5 % (60 kg N ha^{-1} as *Azolla*) and 92.1% (30 kg N ha^{-1} as urea + 30 kg N ha^{-1} as *Azolla*). Sixty kg N ha^{-1} as *Azolla* was almost equivalent the application of 60 kg N ha^{-1} as urea. The combination of urea and *Azolla* 30 kg N ha^{-1} each resulted in grain yield higher than that obtained with urea or *Azolla* alone but not significantly different from that obtained with 6.0 kg N ha^{-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.

INTRODUCTION

Azolla is a genus of small aquatic ferns with worldwide distribution in temperate and tropical regions. Studies on *Azolla* have generated tremendous interest in the scientific community due to nitrogen fixation by its symbiotic cyanobacterium *Anabaena azollae* (Watanabe *et al.*, 1991). It has been used as green manure and supplemented source of nitrogen for rice Herzalla, 1991;EL-Bassel *et al.*, 1993 and EL-Zeky 2005).

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); about 75% of *Azolla*-N mineralizes in 6 to 8 weeks (Watanabe *et al.*, 1977). From 20-30% of *Azolla*-N was observed to be taken up by the first rice crop, and ^{15}N-recovery from *Azolla* and urea was similar (Kumarasinghe and Zapata, 1984); 65% of the nitrogen from *Azolla* incorporated at 30 days after transplanting (DT) was recovered in the straw and 15% in the grains. When *Azolla* incorporated at 78 DT, the amount of nitrogen recovered in the grains increased about 50% (Watanabe 1987). The 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), ^{15}N recovery from *Azolla* was higher than from urea. When *Azolla* was incorporated into the puddled soil, it had gradual N release, 70% of its N-content are available within 20 days (Ventura *et al.* 1987). Kumarasinghe and Eskew (1991) cocluded that 65-95% of nitrogen accumulated by *Azolla* spp. was derived from air. Rosenani and Chulan (1992) found that when *Azolla*-nitrogen(labelled with ^{15}N isotope) was applied to rice at transplanting, the recovery of nitrogen by the crop was only 20.2 %; but when it was applied at maximum tillering (30 days after transplanting), the nitrogen recovery was 30.2 %. Recovery of urea nitrogen was similar, being 22.5 % and 38.6 % for application at the same respective stages.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).

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 urea (Satapathy, 1999). The objective of this study was to determine the availability of *Azolla*-N and urea-N to rice by using ^{15}N- labelled *Azolla pinnata* (3 % a.e) and urea (10 % a.e), as well as to study the possible use of *Azolla* as nitrogen source in rice production.

MATERIALS AND METHODS

Two field experiments were carried out simultaneously, first was to study the availability of labelled-N of *Azolla* (3% atom excess) and urea (10 % atom excess) to rice plant var. Giza 171 at transplanting (TS) and maximum tillering stage (MTS), the second was to study the effect of *Azolla pinnata* inoculation in comparison with urea fertilizer on the rice yield components and the nitrogen content of rice grains and straw.

Azolla pinnata - Anabaena azollae associations were maintained in a greenhouse in two-fifths Hogland's solution minus nitrogen (El-Aggan 1982) with initial pH 7.1. Samples of biomass were used for producing labelled *Azolla* (3% a.e) by the use of ammonium sulphate (30% a.e) according to the FAO/IAEA method (1986). urea (10% a.e) was used as source of labelled mineral nitrogen compared with labelled *Azolla*-N
(3 % a.e).

Availability of *Azolla*-N and urea to rice: *Azolla* and/or urea was incorporated into the top 5 cm of soil at transplanting and maximum tillering stages.Thirty-five-day old rice seedlings variety Giza 171 were then transplanted at 20 cm spacing in 30 (1 m x 1 m) plots. The plots were lined with polyethylene sheets with 30 cm overlapping and covered with 20 cm soil to prevent the ^{15}N-movement between plots. Plots remained flooded until 2 weeks before harvest. The floodwater was drained at incorporating *Azolla* or urea. Weeds were controlled by pushing them back into the soil to avoid losses of ^{15}N. Five treatments with 6 replicates, arranged in a complete randomized block design, were:

- 30 kg Nha^{-1} labelled *Azolla* incorporated at transplanting (TS or 35 days from sowing) and 30 kg Nha^{-1} unlabelled *Azolla* incorporated at maximum tillering stage (MTS or 75 days from sowing) (60 kg Nha^{-1} as *Azolla*).
- 30 kg Nha^{-1} unlabelled *Azolla* incorporated at TS and 30 kg Nha^{-1} labelled *Azolla* incorporated at MTS (60 kg Nha^{-1} as *Azolla*).
- 30 kg Nha^{-1} labelled urea incorporated at TS and 30 kg Nha^{-1} unlabelled urea incorporated at MTS (60 kg Nha^{-1} as urea).
- 30 kg Nha^{-1}unlabelled urea incorporated at TS and 30 kg Nha^{-1} labelled urea incorporated at MTS.
- Control with no N added but simulated incorporation was done at TS and MTS.

At maturity 9 hills were harvested from the center of each plot. Grains and straw were sub sampled, oven dried at 70°C for 24 hours and ground for measurements of ^{15}N recovery using an emission spectrometer (Jasco N-150) as described by Buresh *et al.*, (1982). The portion of nitrogen derived from fertilizers (urea or *Azolla*) were calculated according to the following equation:

% nitrogen derived from labelled urea or *Azolla*

$$= \frac{^{15}N \quad a.e \quad in \quad plant \quad sample}{^{15}N \quad a.e \quad in \quad fertilizer \quad applied} \times 100$$

For estimating the rice yield componets (grain and straw yields, 1000 grain weight , plant height , number of panicles hill-1 and grain and straw – nitrogen percentages) the second experiment was designed to include 4 treatments with 6 replicates arranged in a complete randomized block design, the treatments were:

. Control (without any fertilizers).
. (60 kg Nha^{-1} as urea).
. (60 kg Nha^{-1} as *Azolla*)
. (30 kg Nha^{-1} as urea + 30 kg Nha^{-1} as *Azolla*)

Results for both experiments are statistically analyzed using Duncan's multiple range test as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

All the treatments in the first experiment showed a significant increase in the total N yield over the control (Table 1). However, yields did not significantly differ among the treatments. Generally, *Azolla* at these stages exhibited higher values for all of the labelled nitrogen parameters than that of urea. The highest values were 23 kg Nha^{-1} for labelled N uptake, 14.5% for N derived fertilizer and 76.8% for N^{15} recovery when labelled *Azolla* was used at MTS. The values at TS were lower, but the values obtained with *Azolla* were still higher than that with urea.

These results indicate that N applied later at the MTS is more easily absorbed than when applied at early stages. However, at MTS plants are more developed than at TS which enabled us to conclude that losses of N are higher when N is applied at TS time and that young plants cannot store enough N to contribute the entire needs of rice plant. *Azolla*-N was more available to rice than urea-N at the two stages. This was probably because of the gradual release of *Azolla*-N in relation with its methods of application in the anaerobic soil layer and continuously flooded conditionsof the soil.

The application of *Azolla* and urea either alone or in combination significantly increased the rice grain yield over the control (Table 2). The percentage increases were 89.5 % (60 kg N ha^{-1} as urea), 81.5% (60 kg N ha^{-1} as *Azolla*) and 92.1 % (30 kg N ha^{-1} as urea + 30 kg N ha^{-1} as *Azolla*). Sixty kg N ha^{-1} as *Azolla* was almost equivalent the application of 60 kg N ha^{-1} as urea. The combination of urea and *Azolla* 30 kg N ha^{-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 ha^{-1} as urea.

Table (1): Availability of *Azolla*-N and urea-N to rice under field conditions.

Plant growth stage with ^{15}N	Levels	Total N Yield Kg Nha^{-1}	Total labelled-N Kg Nha^{-1}	N from fertilizer Kg Nha^{-1}	^{15}N recovery %
At harvest	Control	102.6 b	—	—	—
TS	^{15}N- *Azolla* + ^{14}N - *Azolla*	161.5 a	10.9 b	6.7 b	36.2 c
MTS	^{14}N – *Azolla* + ^{15}N- *Azolla*	162.8 a	23.0a	14.5 a	76.8a
TS	^{15}N –urea + ^{14}N-urea	174.2 a	8.9c	5.1 c	29.6 bcd
MTS	^{14}N-urea + ^{15}N-urea	174.1 a	12.9a	7.4 b	42.9 b

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

The weight of 1000 grains (Table 2) 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 ha^{-1}as urea, 60 kg N ha^{-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.

The use *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 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.

Nitrogen content of grain and straw yield were significantly higher for all treatments than that of the control (Table 2).

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

Table (2): Effect of Azolla pinnata and urea on rice yield components under field condition

Treatment	Grain yield ($t\ h^{-1}$)	Straw yield ($t\ h^{-1}$)	1000 grain weight (g)	Plant height (cm)	No. of panicle $hill^{-1}$	Grain N (%)	Straw N (%)
Control	3.80c	6.60b	23.40a	128c	14c	1.09	0.48c
60 kg N ha^{-1} (urea)	7.20a	12.60a	23.10ab	153b	22ab	1.39	0.63b
60 kg N ha^{-1} (*Azolla*)	6.90b	12.50a	23.60a	152b	20b	1.29	0.62b
30 kg N ha-1 (urea) + 30 kg N (*Azolla* ha^{-1})	7.30a	12.80a	22.90b	158a	23a	1.40	0.67a

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

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 findings to those obtained here were documented by Ito and Watanabe (1985) who pointed out that sixty percent of the nitrogen from *Azolla* incorporated at 30 days after transplanting was recovered in the straw and 15% in the grain. When *Azolla* was incorporated at 78 days after transplanting, the amount of nitrogen increased about 50%. Liu and Chen (1986) reported that total ^{15}N recovery from *Azolla* and urea incorporated at transplanting and maximum tillering stages of rice were 48.33% and 37.88% respectively. According to Eskew (1987) the uptake of urea-N occurred primarily within 30 days of application, whereas the major uptake of *Azolla*-N occurred between 30 to 60 days. Ventura *et al.*, (1987) found that ^{15}N recovery from *Azolla* was higher than from urea and explained that when *Azolla* was incorporated into the puddled soil, it had gradual N release, with 70% of its N becoming available within 20 days. Also the data obtained indicate a similar efficiency of labelled urea and labelled *Azolla* as N sources to rice plants,which may be attributed to rapid mineralization of *Azolla pinnata* in flooded rice soils (Ventura and Watanabe (1993). Incorporation of ^{15}N-labelled *Azolla pinnata* into the soil gave an ^{15}N recovery by rice (shoots and roots) of 40% and 24 % , for the sterilized and normal soil, respectively (Galal, 1997).

Concerning the rice yield components in the second experiment, 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 1000 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 (2004) found that the use 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. Similar results for grin and sraw-N content were noticed by (Ruschel, 1986 and Mandal *et al.,* 1999) .

Conclusion

The application of *Azolla* in rice cultivation can be considered as a promising technique both to increase rice productivity and to provide protection from the environmental pollution caused by the extensive use of chemical fertilizers.Thus it may be inferred that *Azolla* exhibited a better N availability to rice than urea and it could act as a substitute nitrogen source for rice crop other than urea. However, better incorporation was at maximum tillering stage.

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الكشف عن مساهمة نيتـــروجين الأزولا ونيتـــروجين اليوريـــا لـــلأرز بســـتخدام النيتروجين المعلم ١٥

فكرى محمد غزال، رضا محمد الشحات و مدحت يمانى ابو زيد
قسم بحوث الميكروبيولوجيا الزراعية - معهد بحوث الأراضى والمياه والبيئة- مركز البحـــوث الزراعية الجيزة- مصر

لقد أجريت تجربة حقلية بمحطة بحوث السرو فى الموسم الصيفى ٢٠٠٤ لتقدير النيتروجين المتاح من الأزولا كسماد حيوي أخضر لنباتات الأرز بالمقارنة مع اليوريا، وكذلك قدر كت من محصول الارز ومحصول القش والمحتوى النيتروجينى لكل من الحبوب والقش ، عدد السنابل بكل نبـــات ، وزن ١٠٠٠ حبة، ارتفاع النباتات . وقد استخدمت لـــذلك الأزولا المعلمـــة بـــالنيتروجين الثقيـــل (^{15}N)حيث كانت نسبة النيتروجين الثقيل بهـــا (*Azolla* -^{15}N 3% a.e) واليوريـــا المعلمـــة بالنيتروجين الثقيل (urea-^{15}N 10 % a.e.) ولقد تم تسميد الأرز بالأزولا أو اليوريـــا ســـواء معلمة بالنيتروجين الثقيل أم غير معلمة عند مرحلتين من مراحل نمو الأرز وهما مرحلة الشـــتل (٣٥ يوماً من الزراعة) و مرحلة التفريع الأقصي للنبات (٧٥يوماً من الزراعة)

لقد أوضحت النتائج مايلي:

١- لقد سجلت أعلي قيم النيتروجين المتاح في المرحلتين بواسطة النتروجين المعلـــم بالمقارنـــة مـــع المعاملات التي لا تحتوي علي نيتروجين معلم.

٢- كانت أعلي قيم مسجله هي ٢٣كجم نيتروجين /هكتار (النيتروجين الكلي المعلم /هكتار)، ٥ر١٤ كجم نيتروجين لكل هكتار متاح من سماد الأزولا المعلم عند مرحلة التفريع الأقصي والتي تمثـــل ٨ر٧٦ % من تركيز النيتروجين المعلم من الأزولا (^{15}N recovery).

٣- جميع قيم النيتروجين المعلم المسجلة بواسطة الأزولا عند مرحلة الشتل كانت منخفضة ولكنها أعلي من مثيلاتها بالنسبة لليوريا المعلمة.

٤- وعموماً فإن النيتروجين المستخدم من الأزولا عند مرحلة التفريع الأقصي كان أكثر إتاحـــة لنبات الأرز منه عند مرحلة الشتل.

٥- وكذلك فإن نيتروجين الأزولا كان أكثر إتاحة لنبات الأرز عنه في حالة اليوريا.

٦- وعلي أية حال فإنة عند مرحلة التفريع الأقصي وجد أن ٨ر٧٦% من نيتروجين الأزولا كان متاحاً لنبات الأرز في مقابل ٩ر٤٢% من نيتروجين الأزولا.

٧- أن التلقيح بالازولا أواليوريا بمفردهما أو مجتمعـــين أدى إلـــي زيـــادة محصـــول الأرز اذا ماقورنت بمعاملة المقارنة بدون أى تسميد.

٨- كانت نسبة الزيادة ٥، ٨٩ % فى محصول الأرز مع معاملة التسميد (٦٠ كجم نيتـــروجين/ فدان كيوريا) و ٥، ٨١ % مع معاملة التسميد (٦٠ كجم نيتروجين/ فدان كازولا) و ١، ٩٢ % للمعاملة (٣٠ كجم نيتروجين/ فدان كازولا + ٣٠ كجم نيتروجين/ فدان كيوريا)

٩- أدى التلقيح بالأزولا منفردا أو مع إضافة اليوريا إلي زيادة الكربون العضوى بالتربة إذا مـــا قورنت بمعاملة المقارنة.

١٠- امكانية استبدال جزء من نيتروجين اليوريا بنيتروجين الأزولا لتسميد الأرز وتقليل التكلفـــة وكذلك الحد من تلوث البيئة بزيادة استخدام هذة الاسمدة المعدنية لزيادة الانتاج المحصولى.