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 N ha$^{-1}$ 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$_2^-$ 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) concluded 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 N ha$^{-1}$ labelled Azolla incorporated at transplanting (TS or 35 days from sowing) and 30 kg N ha$^{-1}$ unlabelled Azolla incorporated at maximum tillering stage (MTS or 75 days from sowing) (60 kg N ha$^{-1}$ as Azolla).
- 30 kg N ha$^{-1}$ unlabelled Azolla incorporated at TS and 30 kg N ha$^{-1}$ labelled Azolla incorporated at MTS (60 kg N ha$^{-1}$ as Azolla).
- 30 kg N ha$^{-1}$ labelled urea incorporated at TS and 30 kg N ha$^{-1}$ unlabelled urea incorporated at MTS (60 kg N ha$^{-1}$ as urea).
- 30 kg N ha$^{-1}$ unlabelled urea incorporated at TS and 30 kg N ha$^{-1}$ 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 Buersh et al., (1982). The portion of nitrogen derived from fertilizers (urea or Azolla) were calculated according to the following equation:

$$%\text{nitrogen derived from labelled urea or Azolla} = \frac{^{15}N \text{ in plant sample}}{^{15}N \text{ in fertilizer applied}} \times 100$$

For estimating the rice yield components (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 N ha$^{-1}$ as urea).
- (60 kg N ha$^{-1}$ as Azolla)
- (30 kg N ha$^{-1}$ as urea + 30 kg N ha$^{-1}$ 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 N ha$^{-1}$ 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 conditions of 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.

<table>
<thead>
<tr>
<th>Plant growth stage with (^{15})N</th>
<th>Levels</th>
<th>Total N Yield Kg Nha(^{-1})</th>
<th>Total labelled-N Kg Nha(^{-1})</th>
<th>N from fertilizer Kg Nha(^{-1})</th>
<th>(^{15})N recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>At harvest</td>
<td>Control</td>
<td>102.6 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>(^{14})N- Azolla + (^{15})N- Azolla</td>
<td>161.5 a</td>
<td>10.9 b</td>
<td>6.7 b</td>
<td>36.2 c</td>
</tr>
<tr>
<td>MTS</td>
<td>(^{14})N- Azolla + (^{15})N- Azolla</td>
<td>162.8 a</td>
<td>23.0a</td>
<td>14.5 a</td>
<td>76.8a</td>
</tr>
<tr>
<td>TS</td>
<td>(^{14})N-urea + (^{15})N-urea</td>
<td>174.2 a</td>
<td>8.9c</td>
<td>5.1 c</td>
<td>29.6 bcd</td>
</tr>
<tr>
<td>MTS</td>
<td>(^{14})N-urea + (^{15})N-urea</td>
<td>174.1 a</td>
<td>12.9a</td>
<td>7.4 b</td>
<td>42.9 b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grain yield (t ha⁻¹)</th>
<th>Straw yield (t ha⁻¹)</th>
<th>1000 grain weight (g)</th>
<th>Plant height (cm)</th>
<th>No. of panicle hill⁻¹</th>
<th>Grain N (%)</th>
<th>Straw N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.80c</td>
<td>6.60b</td>
<td>23.40a</td>
<td>128c</td>
<td>14c</td>
<td>1.09</td>
<td>0.48c</td>
</tr>
<tr>
<td>60 kg N ha⁻¹ (urea)</td>
<td>7.20a</td>
<td>12.60a</td>
<td>23.10ab</td>
<td>153b</td>
<td>22ab</td>
<td>1.39</td>
<td>0.63b</td>
</tr>
<tr>
<td>60 kg N ha⁻¹ (Azolla)</td>
<td>6.90b</td>
<td>12.50a</td>
<td>23.60a</td>
<td>152b</td>
<td>20b</td>
<td>1.29</td>
<td>0.62b</td>
</tr>
<tr>
<td>30 kg N ha⁻¹ (urea)</td>
<td>7.30a</td>
<td>12.80a</td>
<td>22.90b</td>
<td>158a</td>
<td>23a</td>
<td>1.40</td>
<td>0.67a</td>
</tr>
<tr>
<td>30 kg N (Azolla ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 ¹⁵N 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 ¹⁵N 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 ¹⁵N-labelled Azolla pinnata into the soil gave an ¹⁵N 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 Prasad, 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 N ha$^{-1}$ 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 $\frac{1}{3}$ decrease in the N demands of rice crop. Similar results for grain and straw-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.

REFERENCES


Ghazal, F. M. (1987). Microbiological studies on nitrogen fixation by Azolla and alga. Ph.D. Thesis in Agricultural Microbiology, Department of Agricultural Botany, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.


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الكشف عن مساهمة نبتوجين الأزول ونبتوجين البوري بمرحلة الزراعة

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

لقد أجريت تجربة حقلية بمحطة بحث السرو في الموسم الصيفي 2004 لتقدر النباتات المتصلة من الأزول كمصدر حيوي أ曒رر لنباتات الأرز بالمقارنة مع البوري، وكذلك نقل كت من محصول الأرز ومحصول الفض والمحروبة النبتوجيني لكل من الحبوب والقنف، بعد السباق بكل نباتات ووزن 1000 اباحة، ارتفاع النباتات. وقد استخدمت لكل الأزول المعلمة بالنبتوجين السائل (Azolla - 15N 3% a.e.) (حيث كانت نسبة النبتوجين السائل بـ15N 3% a.e.) و البوري المعلمة (urea-15N 10% a.e.) (أولت تم تمييز الأزول أو البوري سوءة المعلمة بالنبتوجين السائل أم غير معلمة عند مرحلتين من مرحلة النمو الأول ومرحلة الشتل (35 يوماً من الزراعة) ومراحل التفريع الأفقي للنبات (70 يوماً من الزراعة).

لقد أوضحت النتائج مايلي:
1- لن سجلت أعلى قيم نبتوجين المتاح في المرحلتين بواسطة النبتوجين المعلمة بالمقارنة مع العاملات التي لا تحتوي على نبتوجين معلمة.
2- كانت أعلى قيمة مسجلة هي 32 كجم نبتوجين /كتار (نبتوجين الكل المعلمة /كتار)،جار 1 كجم نبتوجين لكل كتار متاح من سماد الأزول المعلمة عند مرحلة التفريع الأفقي والتي تمتل (15N recovery).
3- جميع قيم نبتوجين المعلمة المسجلة بواسطة الأزول عند مرحلة الشتل كانت منخفضة ولكنها أعلى من مثلثاتها بالنسبة للبوري المعلمة.
4- وعموماً فإن نبتوجين المستخدم من الأزول عند مرحلة التفريع الأفقي كان أكثر إنتاجية لنباتات الأرز عند مرحلة الشتل.
5- وكذلك فإن نبتوجين الأزول كان أكثر إنتاجية لنباتات الأرز عنه في حالة البوري.
6- وعلى أي حال فإنه عند مرحلة التفريع الأفقي وجد أن 72% من نبتوجين الأزول كان متاحاً لنباتات الأرز في مقبل 94% من نبتوجين الأزول.
7- أن التلقيح بالأزول أو البوري ينشرهما أو مشترعي الأزول لدى زيادة محصول الأرز إذا ما تكونت بمعاملة المقارنة بدون أي تسميد.
8- كانت نسبة زيادة 69% في محصول الأرز مع معاملة التسدي (10 كجم نبتوجين / فدان كيروية) و 65% مع معاملة التسدي (10 كجم نبتوجين / فدان كيروية) و 94% للمعاملة (15 كجم نبتوجين / فدان كيروية) و 30% للمعاملة (30 كجم نبتوجين / فدان كيروية).
9- أن التلقيح بالأزول مفرداً أو مع إضافة البوري يزيد الكربون العضوي بالترية إذا ما فورنت بمعاملة المقارنة.
10- اتخاذية أستبدال جزء من نبتوجين البوري بنبتوجين الأزول لمكورات الأزول وتقليل التكلفة وكذلك الحد من تلوث البيئة بزيادة استخدام هذه الأسمدة المعدنية لزيادة الإنتاج المحاصلي.