

THE ROLE OF FRESH *AZOLLA LOCAL BESUKI'S* IN IMPROVING THE NITROGEN USE EFFICIENCY OF PADDY FIELD

HUDAINI HASBI

Agriculture Faculty, University of Muhammadiyah Jember
Email: Putranasa@gmail.com

Received: 08 July 2019, Revised and Accepted: 09 August 2019

Abstract

The development of management techniques to improve the poor N use efficiency by lowland rice (*Oryza sativa* L.) and reduce the high N losses has been an important focus of agronomic research. The potential of an *Azolla* cover in combination with urea was assessed under field conditions in Jember, East Java, Indonesia. Two on-station field experiments were established in the 1998–1999 dry season and eight on-farm experiments per season were carried out in the 2000–2001 wet and dry seasons. Treatment combinations consisting of N levels applied alone or combined with *Azolla* were evaluated with respect to floodwater chemistry, ¹⁵N recovery, crop growth, and grain yield. A full fresh *Azolla* cover on the floodwater surface at the time of urea application prevented the rapid and large increase in floodwater pH and floodwater temperature. As a consequence, the partial pressure of ammonia (ρNH_3), which is an indicator of potential NH₃ volatilization, was significantly depressed. ¹⁵N recovery was higher in plots covered with *Azolla* where the total ¹⁵N recovery ranged between 77 and 99%, and the aboveground (grain and straw) recovery by rice ranged between 32 and 61%. The tiller count in *Azolla*-covered plots was significantly increased by 50% more than the uncovered plots at all urea levels. Consequently, the grain yield was likewise improved. Grain yields from the 16 on-farm trials increased by as much as 40% at lower N rates (40 and 50 kg N ha⁻¹) and by as much as 29% at higher N rates (80 and 100 kg N ha⁻¹). In addition, response of rice to treatments with lower N rates with an *Azolla* cover was comparable to that obtained with the higher N rates without a cover. Thus, using *Azolla* as a surface cover in combination with urea can be an alternative management practice worth considering as a means to reduce NH₃ volatilization losses and improve N use efficiency.

Key words: ammonia volatilization, fresh *Azolla* cover, lowland rice, ¹⁵N, N-use efficiency

INTRODUCTION

Nitrogen is the most essential element influencing rice productivity. The recovery, however, of applied N by lowland rice (*Oryza sativa* L.) is very inefficient (Craswell and Vlek, 1979; Vlek and Byrnes, 1986; Vlek and Fillery, 1984). In Asia, the average N fertilizer recovery efficiency in farmers' fields is currently only about 30% (Dobermann et al., 2002). Ammonia volatilization, the gaseous emission of NH₃ to the atmosphere, is reportedly the major cause of this low N fertilizer efficiency and an important mechanism for N losses in lowland rice fields (Freney et al., 1993; Jayaweera and Mikkelsen, 1990; Reddy et al., 1990; Vlek and Craswell, 1981). Earlier studies have shown that the total N losses due to NH₃ volatilization are in the range of 20 to 80% (De Datta et al., 1989; Freney et al., 1990; Rao, 1987). Aside from high losses as gas, N is also transported to the ground and surface waters. These factors cause substantial economic loss to farmers and create negative impacts on the atmosphere and water quality (Xing and Zhu, 2000). As such, the inefficient use of N by lowland rice is a matter of concern not only to farmers but to researchers and environmentalists as well. In the past, the use of urease inhibitors (Freney et al., 1993), algicides (Simpson et al., 1988) and monomolecular surface films (Cai et al., 1987) have been employed to control NH₃ volatilization losses and improve N use efficiency. Most of them, however, are expensive (Damodar Reddy and Sharma, 2000) and entail costs to farmers in excess to their savings. Recently, the use of the aquatic fern *Azolla* in improving the efficiency of applied urea has generated interest. Results from laboratory and greenhouse experiments in Germany and in the Indonesia

using *Azolla* to reduce NH₃ losses and increase the low N fertilizer use efficiency seem promising (Cissé and Vlek, 2003a; Vlek et al., 1992, 1995; Villegas and San Valentin, 1989) but this has not yet been thoroughly explored under field conditions. Field research verifying these results is very limited and the results obtained were inconclusive. It is necessary, therefore, to verify and provide concrete evidence of the positive impacts of an *Azolla* cover with regards to minimizing NH₃ volatilization losses and enhancing urea efficiency under field conditions, in order to promote the adoption of this management approach to farmers. Thus, the study was conducted to assess the influence of an *Azolla* cover on the floodwater chemistry and its relation to NH₃ volatilization losses; to compare the N recoveries from urea-amended, *Azolla*-covered treatments with those of urea applied alone using the ¹⁵N tracer technique; and to evaluate the response of rice to the presence of an *Azolla* cover in terms of crop growth and yield.

MATERIALS AND METHODS

The study was conducted in Jember, the province of East Java, Indonesia. Two field experiments were carried out at Jember during the 2008/2009 dry season to evaluate the use of fresh *Azolla* in combination with urea with respect to floodwater chemistry and ¹⁵N recovery. The fresh *Azolla*-cover technique was subsequently evaluated in terms of crop growth and grain yield in 16 farmers' fields with low N status. Two experiments each were established in 4 municipalities in Besuki area namely, IR64, Ciherang, during the wet season

(May to October) and during the dry season (November to April) of 2008–2009. The soils of the area varied in top-soil pH from 5.5 to 6.5 and in CEC from 18 to 45 $\text{cmol} \cdot \text{kg}^{-1}$.

Ten treatment combinations consisting of five N levels (0, 40, 80, 120, and 160 kg N ha^{-1}) applied alone or combined with *Azolla* were laid out in a randomized complete block design with four replicates for on-station trials. In the on-farm trials, N rates were reduced to the levels where the benefits of the *Azolla* cover are visible i.e., 0, 40 and 80 kg N ha^{-1} during the wet season and 0, 50 and 100 kg N ha^{-1} during the dry season.

Rice plant. Rice variety Ciherang (114-day maturity) was planted during the 2008/2009 dry season on paddy field. Experiment 1 was initiated in November 2006, and experiment 2 in February 2007. Similarly variety Ciherang (112-day maturity) was planted during the 2008–2009 wet and dry season on-farm experiments. *Azolla*. *Azolla* was multiplied in propagation ponds. It was harvested, drained and weighed a day before the scheduled inoculation time, i.e., four days before the first urea application. Fifty percent of the floodwater surface was inoculated with *Azolla* at the rate of 5 t ha^{-1} (0.5 kg m^{-2}) in plots with *Azolla* treatments. The aim was that at the time of urea application, the floodwater surface would be completely covered with *Azolla*.

Inorganic fertilizer

Based on the farmers' N application schedule, two thirds of the urea was top-dressed 7 days after transplanting and the remaining one third at 7 days before panicle initiation. Phosphorus and K were broadcast at a uniform dose of 30 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ and 30 $\text{kg K}_2\text{O ha}^{-1}$ at the time of transplanting in the on-station experiments. For experiments carried out in farmers' fields, 60 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ and 90 $\text{kg K}_2\text{O ha}^{-1}$ were applied.

^{15}N balance determination

The effect of the *Azolla* cover on the recovery of applied N was assessed using the ^{15}N tracer technique in the on-station experiments. Microplots enclosed in polyethylene plastic sheets (1.0 m \times 1.0 m \times 0.3 m) were inserted in the center of each main plot. The sheets were embedded approximately 15 cm below the soil surface, leaving approximately 15 cm projected above the soil surface, to prevent possible run-off. Except for the 160 kg N ha^{-1} treatment and the control plots, each microplot received ^{15}N -labeled urea applied at the same rate and in the same way as the non-labeled urea for the first urea application in the main plots. **Microplot sampling.** Plant and soil samples were taken at harvest for ^{15}N analysis. Four hills from the center of the microplot were cut at ground level and washed to remove any adhering soil. Grains were threshed and the chaff added back to the straw. Straw and grain samples were placed in separate bags and dried to constant weight at 80 °C in a forced-draft oven. Dry weights were recorded and samples were ground with a grinder. Composite soil samples were taken from 0- to 15- and 15- to 30-cm depth using an auger and placed in plastic bags. They were spread on a paper in a room until they were air-dried. The clods were pulverized using a mallet and passed through a 2-mm sieve.

^{15}N analysis.

A small portion each of the *Azolla*, straw, grain, and soil samples were weighed in tin cups, ball-milled, and placed into the auto sampler of the mass spectrometer (ANCA SL coupled to 20-20 stable isotope analyzer IRMS). The $\%^{15}\text{N}$ recovery by the plant, *Azolla*, and soil was then computed using the formula of Zapata (1990).

Sampling methods and analyses

Floodwater measurements (2008/2009 dry season).

Floodwater samples of about 200 mL were collected daily between 12.00 and 14.00 h from the day of the initial urea

application up to day 10. The concentration of ammoniacal-N in the floodwater was determined colorimetrically using the salicylate method (Keepers and Zweers, 1986). Simultaneously, floodwater pH and temperature were measured *in situ* with a portable pH meter (Milwaukee/Cole Parmer pH meter-pen type) and a mercury-in-glass thermometer (maximum of 100 °C). The partial pressure of ammonia (ρNH_3) in the floodwater was then calculated from total ammoniacal-N concentration, floodwater pH, and temperature using the corrected equation of Denmead *et al.* (1983): $\rho_0 = 0.00594AT/10(1477.8T - 1.6937)$, (1) where, ρ_0 = partial pressure of ammonia in Pascals, A = aqueous NH_3 concentration in the floodwater in g N m^{-3} , T = floodwater temperature in degrees Kelvin.

Plant samples.

On-farm data clearly reflected the benefits of having a fresh *Azolla* cover on the floodwater surface. At harvest, tiller number was measured from 12 random hills. At maturity, 125 hills (5 m^2) from each plot were cut at the base and threshed to separate the grains. Grain samples were cleaned and sun-dried. The plot grain yield (PlotGY) was then weighed and the grain moisture content (MCPlotGY) measured with a moisture tester. The plot grain yield from the harvest area was corrected to 14% moisture content (PlotGY14) using the formula: $\text{PlotGY14} = \text{PlotGY} \times [(100 - \text{MCPlotGY})/86]$ (2) (Soil and plant sampling and measurements, 1994)

Statistical analysis

A two-factorial analysis of variance and the LSD comparison of treatment. The significance of the presence of a fresh *Azolla* cover, N rate and *Azolla* \times N interaction on the floodwater chemistry, crop growth, and grain yield were determined.

RESULTS AND DISCUSSION

On-Field experiments: Floodwater chemistry, Floodwater pH.

The presence of an *Azolla* cover on the floodwater surface significantly lowered ($P < 0.01$) the floodwater pH (Figure 1). At the time of urea application, the presence of an *Azolla* cover reduced pH values by 0.1 to 0.3 units in experiment 1. In experiment 2, floodwater pH was maintained below 8.0 in the *Azolla*-covered plots. On day 2, floodwater pH without an *Azolla* cover rapidly increased by 0.9 to 1.6 pH units in Exp. 1. In contrast, in the presence of an *Azolla* cover, the rise in the floodwater pH was less than 1.0 pH unit. In experiment 2, floodwater pH without an *Azolla* cover increased by 0.2 to 0.4 units while that with an *Azolla* cover increased only by 0.1 to 0.2 units. During the entire sampling period, the presence of an *Azolla* cover reduced floodwater pH by as much as 1.8 units and 1.9 units in the first and second experiments, respectively. Fertilization of urea stimulates the growth of algae and increases their photosynthetic activity (Simpson *et al.*, 1994). In turn, the dissolved CO_2 in the floodwater is reduced during the daytime leading to a rise in the floodwater pH (Thind and Rowell, 1997).

The higher the floodwater pH, the higher is the potential for NH_3 volatilization losses. The lower floodwater pH in the presence of an *Azolla* cover is partly explained in terms of the absorption of available light (Vlek *et al.*, 2002). With *Azolla* covering the floodwater surface, less light penetrated the floodwater. *Azolla* absorbs incoming solar radiation, reducing light intensity (Kröck *et al.*, 1988a). As shading is one of the most important factors limiting the photosynthesis of algae in lowland rice fields (Saito and Watanabe, 1978), its photosynthetic activity was reduced in the presence of an *Azolla* cover thus preventing the rapid rise in floodwater pH. **Floodwater temperature.** The floodwater temperatures of the *Azolla*-covered plots were significantly lower ($P < 0.05$) than those in the *Azolla*-free plots. In the presence of an *Azolla* cover, the rapid heating of the floodwater from morning until

midday was prevented. An *Azolla* cover resulted in a mean floodwater temperature reduction of 0.6 to 2.6 °C and the maximum floodwater temperature difference between *Azolla*-covered and *Azolla*-free plots was 5 °C. On average over the 10 days of monitoring, the temperatures of the floodwater from the two experiments were similar, around 29 °C. Floodwater temperature affects the relative proportion of NH₃ to NH₄ present at a given pH. An increase in the temperature from 20 to 30 °C doubles the initial aqueous NH₃ in a system (Peoples et al., 1995) which in turn increases the potential of NH₃ loss.

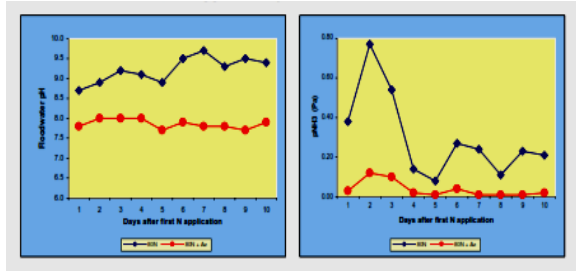


Fig. 1. Effect of fresh *Azolla* cover on floodwater pH and NH₃ partial pressure following the application of 80 kg N ha⁻¹ on field experiment

Floodwater total ammoniacal-N.

In general, treatments with a fresh *Azolla* cover contained more total ammoniacal-N (NH₃ + NH₄-N) ($P < 0.05$) than treatments without cover during the entire sampling period. A similar pattern was reported by Villegas and San Valentin (1989) under greenhouse conditions. *Azolla*-covered plots contained, at the maximum, 5.5 g Nm⁻³ more total ammoniacal-N than the *Azolla*-free plots. This could be due to the reduction in the NH₃ volatilization losses. Peak concentrations of up to 9 g N m⁻³ on the day 4 in experiment 1 and 16 g N m⁻³ on day 2 in experiment 2 were observed when a cover of *Azolla* was present. Without a cover, such high total ammoniacal-N can result in substantial NH₃ losses.

Partial pressure of ammonia.

Despite this higher concentration of total ammoniacal-N, the partial pressure of ammonia (ρ NH₃) in *Azolla*-covered plots was significantly reduced ($P < 0.01$) from day 2 to 10 in both experiments. A built-up of ρ NH₃ was prevented due to the low pH effected by the *Azolla* cover (Vlek et al., 1995). The highest ρ NH₃ calculated were 0.66 and 0.92 Pa in experiments 1 and 2, respectively (Figure 3). These high ρ NH₃ values indicate a high potential for NH₃ volatilization (Simpson et al., 1984, Vlek and Craswell, 1981).

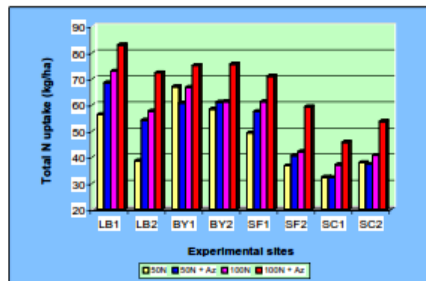


Fig. 2. Effect of *Azolla* cover on total N uptake on field experiment

Covering the floodwater surface with *Azolla* markedly reduced these ρ NH₃ values by more than 85%. Overall, the calculated ρ NH₃ in plots covered with *Azolla* was very low, virtually eliminating the danger of NH₃ losses. The low ρ NH₃ in *Azolla*-covered treatments suggest that, under the conditions of our field experiments, *Azolla* is capable of

curtailing NH₃ volatilization losses. In contrast, the high floodwater pH and temperatures in *Azolla*-free plots that led to a high ρ NH₃, could result in high N losses via NH₃ volatilization. If so, this must be reflected in the ¹⁵N balance of applied N in these experiments.

¹⁵N recovery at harvest: ¹⁵N recovery by rice.

The presence of a fresh *Azolla* cover on the floodwater surface before the initial urea application resulted in a significantly higher ¹⁵N recovery ($P < 0.01$) by the aboveground biomass (grain plus straw) at harvest in experiment 2 where ¹⁵N recovery by rice increased by approximately 25 to as much as 95% in the *Azolla*-covered plots. The improved ¹⁵N recovery by the rice in the *Azolla*-covered treatments is partly attributed to the lower NH₃ volatilization losses in the earlier stage of rice as supported by the low ρ NH₃ in the floodwater. Furthermore, *Azolla*, upon its decomposition could have released part of the ¹⁵N it initially absorbed and its availability would have contributed to the better ¹⁵N utilization by the crop (Cissé and Vlek, 2003a).

*¹⁵N recovery by *Azolla*.*

Indeed, the aquatic fern assimilated a fraction of the applied ¹⁵N. At harvest, 5 to 14% of the ¹⁵N-labeled urea applied was found in the *Azolla* plant (figure 3). This is consistent with greenhouse experiments in which *Azolla* immobilizes up to 68% of applied ¹⁵N within six weeks after application, of which up to 45% was re-mineralized by harvest time (Cissé and Vlek, 2003a). Thus, nitrogen might be temporarily locked up in the *Azolla*, which limits availability of N to rice plants. In the process, however, this protects N from immediate gaseous losses, as it is conserved within the system and is mineralized later, becoming available for plant use (Keeney and Sahrawat, 1986).

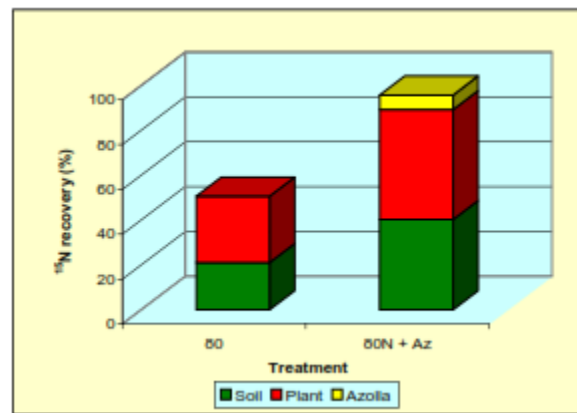


Fig. 3. Effect of *Azolla* cover on ¹⁵N recovery by *Azolla*

*Total ¹⁵N recovery in the *Azolla*-plant-soil system.*

In general, the presence of a fresh *Azolla* cover markedly improved the total ¹⁵N recovery in the rice-soil system. In experiment 1, the recovery of applied ¹⁵N significantly increased ($P < 0.05$) from an average of 66.4% in treatments without *Azolla* cover to an average of 83.3% with *Azolla* cover. In experiment 2, the average ¹⁵N recovery of 62.0% on *Azolla*-free plots was significantly increased ($P < 0.01$) to 92.1% when *Azolla* was present. An *Azolla* cover increased ¹⁵N recovery in the 80 kg N ha⁻¹ level by as much as 78% in experiment 1 and by as much as 89% in Exp. 2. The improvement in the ¹⁵N recovery in the presence of an *Azolla* cover indicates the important contribution of *Azolla* to the fertilizer-N economy in rice fields. Its beneficial effects on the floodwater chemistry, and its assimilation and conservation of N led to an enhancement in the ¹⁵N recovery. Unfortunately, the grain yields (data not shown) of the on-field experiments did not reflect the improved N recovery or tiller counts, due

largely to adverse weather conditions at the time of grain ripening. In order to ascertain whether grain yield responses, as reported by Cissé and Vlek (2003a) and Vlek *et al.* (1995) under greenhouse conditions, are indeed possible under field conditions, an on-farm campaign was undertaken in 2000–2001 with a larger number of sites to reduce the risk of adverse weather conditions.

On-field experiments : Tiller count at harvest

At harvest, all treatments with an *Azolla* cover had a significantly higher ($P < 0.01$) number of tillers than the uncovered treatments in both seasons (Figure 4). The *Azolla* cover in the non-fertilized plots increased the tiller number at harvest by as much as 64.5 and 20.8% over the control plots in the wet and dry seasons, respectively. In the wet season, five sites (Jember, Banyuwangi, Kalibaru, Bondowoso, and Besuki, Situbondo) showed a significant positive *Azolla* × N interaction, with 27.9 to 44.8% more tillers with *Azolla* at 40 kg N ha⁻¹ and 7.5 to 28.7% more at 80 kg N ha⁻¹. In the dry season, this significant interaction effect ($P < 0.01$) was observed in 4 out of 8 sites (LB1, LB2, SC1, and SC2). This observed significant *Azolla* × N interaction indicates the positive synergistic effect of an *Azolla* cover (Vlek *et al.*, 1995). The magnitude of increase in the tiller count due to an *Azolla* cover in the dry season was greater than that in the wet season. At 50 kg N ha⁻¹ plus *Azolla*, the tiller count increase ranged from 36.1 to 51.7%, whereas at 100 kg N ha⁻¹ plus *Azolla*, increase was 8.3 to 25.1%. The *Azolla*-covered plot with 40 kg N ha⁻¹ in the wet season and 50 kg N ha⁻¹ in the dry season produced tiller numbers comparable to those of 80 and 100 kg N ha⁻¹.

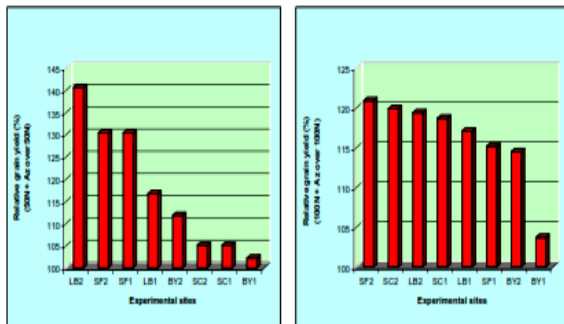


Fig. 4. Effect of an *Azolla* cover on the tiller count at harvest On-farm field experiments.

Hasbi *et al.* (2008) reported that the number of tillers is closely related to the amount of N absorbed during the vegetative period. Thus, the N conserved by the reduction of NH₃ volatilization in the beginning in the presence of an *Azolla* cover (Vlek *et al.*, 1995), and presumably the N fixed and supplied by the *Azolla* (Parot, 1991) contributed to the increase in the tiller number. Tillering ability is one of the most important traits of rice as it significantly influences the production of panicles, which in turn is highly correlated with grain yield (Hasbi *et al.*, 2008).

Grain yield

The influence of an *Azolla* cover on grain yield was clearly manifested in the on-farm field experiments' results, where most of the sites had insufficient levels of N. Since N was limiting, yield responded better to the combined treatment of urea and *Azolla* cover. The presence of an *Azolla* cover in combination with urea produced consistently higher grain yields than that with urea alone. At lower N rates (40 and 50 kg N ha⁻¹), the relative grain yield of rice in plots covered with *Azolla* was higher by approximately 2 to 41% than those obtained in the uncovered treatments (Figure 5). At higher N rates, an *Azolla* cover in combination with urea increased the

grain yield by approximately 4 to 29% over the urea applied alone in both seasons.

Earlier studies on the dual cropping of *Azolla* with rice attributed this increase mainly to the N fixed and released by *Azolla*. In the present experiments, we attribute the increase in part due to a greater availability of urea because of the reduced potential for NH₃ volatilization losses (Figure 3) and an increase in fertilizer efficiency. In addition, besides the N fixed by the fern from the atmosphere, *Azolla* took up N from the urea that was applied, conserving it in the process and presumably releasing the urea-N during its growth together with rice or after its decomposition. Part of this relative-grain-yield increase is due to an *Azolla* × N interaction as indicated by the light-shaded portion in each bar. Grain yield increased by as much as 1.7 t ha⁻¹ due to a positive interaction of *Azolla* and urea, at lower N rates and as much as 1.0 t ha⁻¹ at higher N rates. This positive interaction effects indicate greater benefits from the combined *Azolla* and urea treatments than for the sum of the treatments alone.

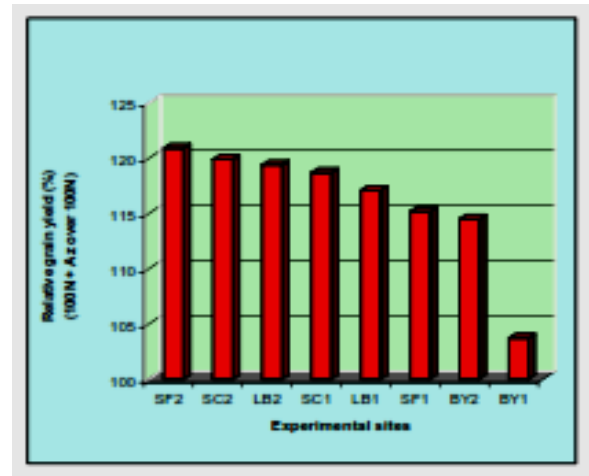


Fig. 5. The relative grain yield of rice at 40 and 50 kg N ha⁻¹ with fresh *Azolla* cover over the 40 and 50 kg N ha⁻¹ applied alone on-farm field experiments.

More than half of the sites in both seasons yielded 10% higher in treatments with urea and fresh *Azolla* cover than plots with only urea. An *Azolla* cover together with 40 kg N ha⁻¹ in the wet season and 50 kg N ha⁻¹ in the dry season produced a grain yield response comparable with that obtained in the 80 and 100 kg N ha⁻¹ without *Azolla*. Whether the benefit of an *Azolla* cover will be pronounced or not depends on the quality of irrigation water used and the incidence of rainfall in the days following urea application. These conditions differed among the farm sites investigated leading to a differential effect of fresh *Azolla*.

The results of this experiments suggest that the higher grain yield obtained in the *Azolla*-covered plots can mainly be attributed to the production of a significantly higher tiller number, presumably because of the adequate N supply during the vegetative and reproductive stages of the rice. Bronson *et al.* (2000) found a positive correlation between productive tillers and grain yield. Unfortunately, the determination of the N fixed by the *Azolla* was not included in the present investigation. Past studies however, reported a N₂-fixation rate ranging from 0.4 to 3.6 kg N ha⁻¹ day⁻¹ (Oliveros *et al.*, 1983; Roger and Ladha, 1992; Singh and Singh, 1987; Watanabe, 1982) depending on several factors, one of which is N fertilizer. The influence of fertilizer N on the N₂-fixation of *Azolla* varies. Singh and Singh (1988) noted a significant reduction in the growth and N₂ fixation by the fern with an increase in the rate of fertilizer N. Gianni (1992) had similar findings, but noted that with split application of urea, *Azolla* can still maintain its N contribution to the rice plants, even at N rate of 144 kg N ha⁻¹ despite the inhibition of N₂ fixation.

The occurrence of an *Azolla* × N interaction effect on the grain yield indicates that the influence of an *Azolla* cover in the enhancement of the response of rice to the applied urea-N was not only additive in nature, but synergistic as well. This means that, besides the benefits achieved with the application of urea-N alone or from the N fixation by *Azolla*, an additional benefit was gained that could not be obtained from the separate treatments. This would have been the conservation of applied N by the *Azolla* cover. In fact, it appears that the latter effect might in some cases exceed the main effects of urea, and *Azolla* through N fixation (Vlek *et al.*, 1995).

CONCLUSIONS

The results from field experiments provided convincing evidence that *Azolla* used as a cover on the floodwater of rice could help curb NH₃ volatilization losses.

Under the conditions of the experiments, showed that a full fresh *Azolla* cover at the time of urea application effectively prevented the sudden rise in the floodwater pH. As a result, the large NH₃ losses that often occur when urea is broadcast onto the floodwater of rice shortly after transplanting were reduced.

In minimizing NH₃ volatilization losses, the N On-farm field experiments, use efficiency was improved. *Azolla* likewise brought about appreciable changes with regard to the availability of N, which influences the growth and mineral nutrition of the rice plants.

The relative grain yield of rice at 80 and 100 kg N ha⁻¹ with fresh *Azolla* cover over the 80 and 100 kg N ha⁻¹ applied alone at harvest.

With an fresh *Azolla* on the floodwater surface, a higher grain yield can be achieved with a reduced rate of urea application.

In the present farmer-field investigations, combining *Azolla* with urea produced yields, which were generally higher by 10% or more than those without cover. This prospect is especially attractive in light of the high cost of N fertilizer and the growing need to improve grain yield with minimum adverse environmental effects associated with the intensive use of N fertilizer.

The combined application of urea with *Azolla* thus can (1) be an efficient fertilizer management method to reduce NH₃ losses from urea applied to lowland rice in areas prone to such losses (2) introduce an N-fixing species into the system; and (3) can lead to increased grain yields. These benefits can surpass those from either urea or *Azolla* alone.

REFERENCES

- Bronson K F, Hussain F, Pasuquin E and Ladha J K 2000 Use of 15N labelled soil in measuring nitrogen fertilizer recovery efficiency in transplanted rice. *Soil Sci. Soc. Am. J.* 64, 235–239.
- Cai G X, Freney J R, Humpreys E, Denmead O T, Samson M and Simpson J R 1987. Use of surface films to reduce ammonia volatilization from flooded rice fields. *Aust. J. Agric. Res.* 39, 177–186.
- Cissé M and Vlek P L G 2003a Conservation of urea-N by immobilization-remobilization in a rice-*Azolla* intercrop. *Plant Soil* 250, 95–104.
- Cissé M and Vlek P L G 2003b Influence of urea on biological N₂ fixation and N transfer from *Azolla* intercropped with rice. *Plant Soil* 250, 105–112.
- Craswell E T and Vlek P L G 1979 Greenhouse evaluation of nitrogen fertilizers for rice. *Soil Sci. Soc. Am. J.* 43(6), 1184–1188.
- Damodar Reddy D and Sharma K L 2000 Effect of amending urea fertilizer with chemical additives on ammonia volatilization loss and nitrogen use efficiency. *Biol. Fertil. Soils* 32, 24–27.
- De Datta S K, Buresh R J, Obcemea W N and Castillo E G 1989 Nitrogen-15 balances and nitrogen fertilizer use efficiency in upland rice. *Fert. Res.* 26, 179–187.
- Denmead O T, Freney J R and Simpson J R 1983 Dynamics of ammonia volatilization during furrow irrigation of maize. *Soil Sci. Soc. Am. J.* 47, 618.
- Dobermann A, Witt C, Dawe D, Abdurachman S, Gines H C, Nagarajan R, Satawathananont S, Son T T, Tan P S, Wang G H, Chieh N V, Thoa V T K, Phung C V, Stalin P, Muthukrishnan P, Ravi V, Babu M, Chatuporn S, Sookthongsa J, Sun Q, Fu R, Simbahan G C and Adviento M A A 2002. Site-specific nutrient management for intensive rice cropping systems in Asia. *Field Crops Res.* 74, 37–66.
- Freney J R, Trevitt A C F, de Datta S K, Obcemea W N and Real J G 1990. The interdependence of ammonia volatilization and denitrification as nitrogen loss processes in flooded rice fields in the Philippines. *Biol. Fertil. Soils* 9, 31–36.
- Freney J R, Keerthisinghe D G, Chaiwanakupt P and Phongpan S 1993 Use of urease inhibitors to reduce ammonia loss following the application of urea to flooded rice fields. *Plant Soil* 155/156, 371–373.
- Gravois K A and Helms R S 1992 Plant analysis of rice yield and yield components as affected by seeding rate. *Agron. J.* 84, 1–4.
- Hasbi, H., Bagus, T., Arief, Noor. A. 2008. Studies on the fate of fertilizer nitrogen in rice plants and paddy soils by using ¹⁵N as a tracer in Jember. Research report (unpublished), 77–94.
- IRRISTAT (version 3.1) Available Distributor: IRRI, Philippines.
- Jayaweera G R and Mikkelsen D S 1990 Ammonia volatilization from flooded soil systems: A computer model. I. Theoretical aspects. *Soil Sci. Soc. Am. J.* 54, 1447–1455.
- Keeney D R and Sahrawat K L 1986 Nitrogen transformations in flooded rice soils. *Fert. Res.* 9, 15–38.
- Keepers A J and Zweers A 1986 Ammonium determination in soil extracts by salicylate method. *Commun. Soil Sci. Plant Anal.* 17(7), 715–723.
- Kröck T, Alkamper J and Watanabe I 1988a. Effect of an *Azolla* cover on the conditions in floodwater. *J. Agron. Crop Sci.* 161, 185–189.
- Oliveros R, Ramirez C M and Watanabe I 1983. Dual culture of rice and *Azolla*. *IRRI Sat. Sem. Los Banos, Philippines.*
- Parot V P 1991 Growth and nitrogen accumulation of four *Azolla* genotypes at different stages of rice canopy development and their efficiency as organic fertilizer. MS thesis. University of the Philippines Los Banos, Philippines.
- Peoples M B, Freney J R and Mosier A R 1995. Minimizing gaseous losses of nitrogen. *In Nitrogen Fertilization in the Environment.* Ed. P E Bacon. pp. 565–590. Woodlots and Wetlands Pty. Ltd., Sydney.
- Rao D L N 1987. Slow-release urea fertilizers-effect on floodwater chemistry, ammonia volatilization and rice growth in an alkali soil. *Fert. Res.* 13, 209–221.
- Reddy K R, D'Angelo E, Lindau C and Patrick Jr W H 1990. Urea losses in flooded with established oxidized and reduced soil layers. *Biol. Fertil. Soils* 9, 283–287.
- Roger P A and Ladha J K 1992. Biological N₂ fixation in wetland rice fields: Estimation and contribution to nitrogen balance. *Plant Soil* 141, 41–55.
- Saito M and Watanabe I 1978. Organic matter production in rice field floodwater. *Soil Sci. Plant Nutr.* 24, 427–440.
- Simpson J R, Freney J R, Wetselaar R, Muirhead W A, Leuning R and Denmead O T 1984. Transformations and losses of urea nitrogen after application to flooded rice. *Aust. J. Agric. Res.* 35, 189–200.
- Simpson J R, Muirhead W A, Bowmer K H, Cai G X and Freney J R 1988. Control of gaseous nitrogen losses from urea applied to flooded rice soils. *Fert. Res.* 18, 31–47.

28. Simpson I C, Roger P A, Oficial R and Grant I F 1994. Effects of nitrogen fertilizer and pesticide management on floodwater ecology in a wetland ricefield. II. Dynamics of microcrustaceans and dipteran larvae. *Biol. Fertil. Soils* 17, 138-146.
29. Singh A L and Singh P K 1987. Comparative study of *Azolla* and blue-green algae dual culture with rice. *Is. J. Bot.* 36, 53-61.
30. Singh D P and Singh P K 1988 Influence of rice canopy on growth and nitrogen fixation of the water fern *Azolla pinnata*. *Proc. Indian Acad. Sci. (Plant Sci.)* 98(3), 209-214.
31. Soil and plant sampling and measurements 1994. IRRI, Philippines.
32. Thind H S and Rowell D L 1997 Effect of green manure and floodwater algae on diurnal fluctuations of floodwater pH and depth of aerobic soil layer under lowland rice conditions. *Plant Soil* 192, 161-165.
33. Villegas G G and San Valentin G O 1989 Effect of *Azolla* cover on nitrogen and rice in flooded Maahas clay. *In Azolla: Its Culture, Management and Utilization in the Philippines*. pp. 65-89. National *Azolla* Action Program, University of the Philippines Los Banos, Philippines.
34. Vlek P L G and Byrnes B H 1986 The efficacy and loss of fertilizer N in lowland rice. *Fert. Res.* 9, 131-147.
35. Vlek P L G and Craswell E T 1981 Ammonia volatilization from flooded soils. *Fert. Res.* 2, 227-245.
36. Vlek P L G and Fillery I R P 1984 Improving nitrogen efficiency in wetland rice soils. *In Proceedings of the fertilizer society* No. 230. December 13, 1984. London. pp. 3-28. Greenhill House, London.
37. Vlek P L G, Fugger W and Biker U 1992. The fate of fertilizer N under *Azolla* in wetland rice. In *Proceedings of the 2nd ESA Congress*. August 1992. Warwick University, UK.
38. Vlek P L G, Diakite M Y and Mueller H 1995. The role of *Azolla* in curbing ammonia volatilization from flooded rice systems. *Fert. Res.* 42, 165-174.
39. Vlek P L G, Eberhardt U and Aung M 2002 The role of *Azolla* in lowering the pH of simulated floodwater. *J. Appl. Bot.* 76, 1-7.
40. Watanabe I 1982. *Azolla-Anabaena* symbiosis- Its physiology and use in tropical agriculture. *In Microbiology of Tropical Soil and Plant Productivity*. Eds. Dommergues Y R and Diem H G.
41. Martinus Nijhoff/Dr W Junk Publishers, The Hague. Xing G X and Zhu Z L 2000 An assessment of N loss from agricultural fields to the environment in China. *Nutr. Cycl. Agroecosyst.* 57, 67-73.
42. Yanni Y G 1992 The effect of cyanobacteria and *Azolla* on the performance of rice under different levels of fertilizer nitrogen. *World J. Microbiol. Biotechnol.* 8, 132-136.
43. Zapata F 1990 Isotope techniques in soil fertility and plant relationships training course series No 2. Ed. G. Hardenson. International Atomic Energy Agency, Vienna. *Section editor: F.R. Marchir* Reproduced with permission of the copyright owner. Further reproduction prohibited without permission