

Potential of *Azolla* as a biofertilizer for rice farming in Sri Lanka: Demonstrated by isotopic techniques

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ABSTRACT

Studies using the stable, heavy isotope of nitrogen (^{15}N) were conducted to: (i) estimate quantitatively nitrogen fixation by *Azolla* under rice field conditions and (ii) evaluate the uptake of *Azolla*-N and urea-N by rice when incorporated into soil, as well as when using them in combination. While N_2 fixation was estimated by the ^{15}N - substrate labelling technique, uptake of N from labelled material was evaluated in polythene lined 1m^2 field microplots. Grain yield data were obtained from $4\text{m} \times 5\text{m}$ yield plots having corresponding treatments. Farmers' Experiments were also conducted in farmers' fields to examine the realization of the potentials demonstrated in research stations. Results showed that 55 to 66% *Azolla*-N was derived from the atmosphere and 43 to 65% of *Azolla*-N was taken up by an associated rice crop. N-recovery from *Azolla* was better than from urea, particularly when it was incorporated at tillering. As *Azolla* cover left unincorporated among broadcast seeded rice enhanced the uptake of N from urea fertilizer. An integrated use of *Azolla* with urea has the potential to reduce the use of chemical fertilizer by 50%, under farmer's field conditions.

Key words: *Azolla*, Biofertilizer, N_2 fixation, ^{15}N -techniques, Rice

INTRODUCTION

Azolla is a free floating aquatic fern that harbours an endosymbiotic, nitrogen fixing cyanobacterium within the upper lobes of its leaves. It grows very well in stagnant shallow waters, including rice fields and has been used traditionally for centuries in China and Vietnam as a biofertilizer for rice (Lumpkin and Plucknett 1982).

Nitrogen fixation by *Azolla* is commonly demonstrated by its ability to grow rapidly in nitrogen-free media and by the indirect method of measuring its nitrogenase activity by the acetylene reduction technique. Similarly, its potential as a fertilizer for rice is often depicted by comparing the growth and yield of rice in the presence and absence of *Azolla* which is periodically incorporated into the soil.

However, none of these methods provide direct evidence of nitrogen fixation by *Azolla* or the availability and the uptake of its nitrogen by rice plants.

This paper briefly reviews research studies conducted in Sri Lanka the heavy isotope of nitrogen (^{15}N) using to demonstrate nitrogen fixation by *Azolla* under field conditions and the availability of its nitrogen to rice plants. For further information on the agronomic potential of *Azolla* and

constraints and limitations for its widespread use in Sri Lanka, readers are referred to Kulasooriya *et al* (1987), Kulasooriya (1991) and Kulasooriya *et al* (1994).

MATERIALS AND METHODS

Nitrogen fixation by *Azolla* under field conditions

Fried and Middelboe (1977) reported on the application of the ^{15}N substrate labelling technique to quantitatively estimate the nitrogen fixed by field grown legume crops. Initial experiments for the adoption of similar methodologies for *Azolla* were carried out at the Seibersdorf laboratories in Austria by the author in 1983. These studies showed that the substrate labelling technique could be applied for *Azolla*, using species of *Salvinia* or *Lemna* as the non-fixing reference plants. These preliminary greenhouse experiments done in Austria were adapted to field conditions in Sri Lanka originally at the Rice Research Station, Ambalantota (Kulasooriya *et al.* 1987). These initial experiments were refined and repeated at the Bombuwela Agricultural Research Station to obtain more reliable results (Kulasooriya *et al.* 1988).

Recovery of nitrogen from *Azolla* by field grown rice

Initial experiments conducted at Ambalantota were carried out in 1 m² micro-plots lined with thick polythene sheets, using fresh *Azolla* pre-labelled with ¹⁵N, incorporated into the soil at 2 weeks and 6 weeks after transplanting of rice, in comparison to ¹⁵N-labelled urea (5% a.e.), incorporated in the same manner. ¹⁵N labelled material was used in a more systematic study at Bombuwela to determine the uptake of *Azolla*-N and urea-N by rice when incorporated at transplanting and at tillering, in comparison to labelled urea applied according to the recommended best split method (Kulasooriya *et al.* 1988). This experiment had the following treatments given in polythene lined, 1 m² micro-plots.

1. Labelled *Azolla* (equivalent to 30 kg N ha⁻¹) incorporated at transplanting, followed by unlabelled *Azolla* (30 kg N ha⁻¹) incorporated at maximum tillering.
2. Unlabelled *Azolla* (30 kg N ha⁻¹) incorporated at transplanting, followed by labelled *Azolla* (30 kg N ha⁻¹) incorporated at maximum tillering.
3. Labelled urea (equivalent to 30 kg N ha⁻¹) incorporated at transplanting, followed by unlabelled urea (30 kg N ha⁻¹) incorporated at maximum tillering.
4. Unlabelled urea (30 kg N ha⁻¹) incorporated at transplanting, followed by labelled urea (30 kg N ha⁻¹) incorporated at maximum tillering.
5. Labelled urea (60 kg N ha⁻¹) applied according to the recommended best split method (20% at basal and 40% each as top dressing at maximum tillering and two weeks before panicle initiation).
6. Control without any N-fertilizers added, but soil incorporations simulated at transplanting and maximum tillering.

Each treatment was replicated 6 times and the micro-plots were arranged in a randomized complete block design.

As it would be inaccurate to extrapolate yield data obtained from 1 m² micro plots, the following treatments were given in 5 m x 4 m yield plots to obtain more reliable data on grain yield.

1. 60 kg N ha⁻¹ unlabelled urea applied

according to the recommended best split method.

2. 30 kg N ha⁻¹ unlabelled *Azolla* incorporated at transplanting, followed by 30 kg N ha⁻¹ of unlabelled *Azolla* incorporated at maximum tillering

3. 30 kg N ha⁻¹ of unlabelled urea incorporated at transplanting, followed by 30 kg N ha⁻¹ of unlabelled urea incorporated at maximum tillering.

4. Control, without added N-fertilizers, but incorporations simulated at transplanting and maximum tillering

Each treatment was replicated four times and the plots were arranged in a randomized complete block design.

Effect of unincorporated *Azolla* cover on the recovery of fertilizer nitrogen by rice.

All the foregoing experiments were conducted with row transplanted rice in well laid out plots at research stations. While these studies demonstrate an agronomic potential of *Azolla*, its realization under farmer's field conditions depends upon many other factors. To begin with, rice grown under rainfed conditions in the low country wet zone is very seldom row planted. It is frequently broadcast seeded and only occasionally transplanted. The growth of *Azolla* under such rice planting systems would be limited and its soil incorporation would be impossible without causing damage to the standing crop. Experiments were therefore conducted using labelled urea and labelled and unlabelled *Azolla* to examine the effect of an *Azolla* cover left unincorporated among rice plants, on the recovery of nitrogen by the associated rice crop (Kulasooriya *et al.* 1994).

The following treatments were given in polythene lined, 1 m² micro-plots.

1. Control without added N-fertilizer.
2. Labelled urea incorporated at transplanting. (U*O).
3. Labelled urea incorporated at transplanting and *Azolla* inoculated at transplanting. (U*₀A₀).
4. Labelled *Azolla* incorporated at transplanting (A*O).
5. Labelled urea broadcast 21 days after transplanting (U*21).

6. *Azolla* inoculated at transplanting and labelled urea broadcast 21 days later (A₀U*21).

7. Same as treatment 6, but *Azolla* incorporated seven days later (A₀U*21A₂₈).

*Pre-labelled with ¹⁵N.

Each treatment was replicated 4 times and arranged in a randomized complete block design.

As the presence of *Azolla* had a positive effect on N-recovery by rice and its eventual grain yield, a series of experiments was conducted (using unlabelled material) in farmers' fields at Bombuwela to examine the possibility of reducing the addition of chemical fertilizer (urea) by the introduction of *Azolla* to rice fields having broadcast seeded rice.

These experiments had the following treatments.

1. Control without any added N-fertilizer.
2. Fresh *Azolla* (250 g m⁻²) inoculated 2 wks after broadcast seeding.
3. *Azolla* inoculated 4 wks after broadcast seeding.
4. Urea fertilizer (25 kg N ha⁻¹).
5. Urea (25 kg N ha⁻¹) + *Azolla* 2 wks after broadcast seeding.
6. Urea (25 kg N ha⁻¹) + *Azolla* 4 wks after broadcast seeding.
7. Urea (50 kg N ha⁻¹).
8. Urea (50 kg N ha⁻¹) + *Azolla* 2 wks after broadcast seeding.
9. Urea (50 kg N ha⁻¹) + *Azolla* 4 wks after broadcast seeding.

The treatments were given in approximately 5m x 4m plots and each treatment was replicated 3 times and arranged in a randomized complete block design. All fertilizer additions were done by the farmers according to schedules recommended for the area. This experiment initially conducted during the South West monsoon (Yala) season of 1986 was repeated during the following 1986/87 North East monsoon (Maha) season.

RESULTS

Nitrogen fixation by *Azolla*

Results of the first series of experiments conducted at Ambalantota for the quantitative estimation of nitrogen fixation are given in Table 1. This shows that *Azolla* has derived 50 to 56% of its nitrogen from the atmosphere under monoculture, while these values have increased to 55 to 66% when it was

grown under dual culture with rice. This could reflect a favourable micor-niche under the shade of the rice plants, in this dry locality where solar radiation and ambient temperatures are high.

Table 1. Percentage N derived from fixation by species of *Azolla* grown in a rice field in 42 days. Measured by the ¹⁵N-diffusion technique at Ambalantota

Reference plant	Monoculture <i>A. microphylla</i>	Dual culture with rice		
		<i>A. pinnata</i>	<i>A. microphylla</i>	<i>A. pinnata</i>
<i>Salvinia</i> sp.	54±11	50±18	61±7	66±4
<i>Lemna</i> major	56±3	53±14	55±9	61±6

* Values are means of four replicates.

Reproduced from Kulasoorya *et al.*, 1987

The quantitative estimations of the nitrogen fixed during this period are given in Table 2. These values are quite low, particularly with respect to the 42 day period of field growth.

Results obtained by the repetition of these experiments at Bombuwela are presented in Tables 3 and 4, respectively for nitrogen fixation and the quantity of nitrogen fixed during 14 days. These results give comparable rates of nitrogen fixation but higher amounts of nitrogen fixed, due to the less harsh environmental conditions at Bombuwela and improvements in methodology, as explained in the discussion.

Recovery of *Azolla*-N by rice

Results from the preliminary micro-plot experiments at Ambalantota are in Table 5 which shows a higher percentage of N-recovery from *Azolla* (43% total with 30% in panicle), than from urea (37% total with 28% in panicle).

Results of the more systematic study at Bombuwela in which the recovery of nitrogen from ¹⁵N-labelled *Azolla* and urea incorporated at transplanting and at maximum tillering was compared with urea applied according to the best split method, are shown in figures 1 and 2. Figure 1 (a, b & c) shows respectively the dry matter yields, N-yields and the percentage N derived from labelled fertilizer (%NdfF) under the different treatments, while figure 2 (a & b) give respectively the fertilizer N-yield and %N-recovery from labelled fertilizer. From figure 1 a & b, it is seen that the rice plants have responded positively to fertilizer additions in a similar manner. However, %NdfF values (Fig 1c) show clear differences with higher values from *Azolla* incorporated at tillering and urea applied under the best split method. While the fertilizer N-yields also give a similar pattern (Fig 2a), the % N

Table 2. Growth, N yield and quantity of N₂ fixed by 2 species of *Azolla* grown in a rice field in 42 days at Ambalantota.

Reference plant	Test plant	Monoculture		Dual culture with rice			
		Dry wt (Kg ha ⁻¹)	N yield (kg ha ⁻¹)	NdfFix* (kg ha ⁻¹)	Dry wt (kg ha ⁻¹)	N yield (kg ha ⁻¹)	NdfFix* (kg ha ⁻¹)
<i>Salvinia</i> sp.	<i>A. pinnata</i>	579 ± 102	18.1 ± 7	8.5 ± 3	652 ± 9	16.6 ± 4	11.1 ± 3
	<i>A. Microphylla</i>	649 ± 89	17.5 ± 2	9.3 ± 0.8	638 ± 94	17.2 ± 2	10.5 ± 1
<i>Lemna major</i>	<i>A. Pinnata</i>	579 ± 102	18.1 ± 7	9.1 ± 4	652 ± 9	16.6 ± 4	10.2 ± 3
	<i>A. Microphylla</i>	649 ± 89	17.5 ± 2	9.4 ± 0.8	638 ± 94	17.2 ± 2	9.4 ± 1

* NdfFix = nitrogen in *Azolla* biomass derived from fixation.

Reproduced from Kulasooriya *et al.*, 1987.

Table 3. Percentage of nitrogen derived from fixation by *Azolla* in 14 days in a rice field at Bombuwela.

Reference plant	Test plant	% NdfFix*	
		Monoculture	Dual culture
<i>Salvinia molesta</i>	<i>A. pinnata</i>	61.5 ± 9.2	58.2 ± 12.7
	<i>A. microphylla</i>	59.5 ± 9.9	54.7 ± 8.3
<i>Lemna perpusilla</i>	<i>A. pinnata</i>	54.8 ± 13.2	56.9 ± 8.4
	<i>A. microphylla</i>	51.0 ± 6.7	50.8 ± 6.1

* Mean value of 4 replicates

Reproduced from Kulasooriya *et al.*, 1988.

Table 4. Nitrogen yield and N₂ fixed by *Azolla* in 14 days in a rice field at Bom-buwela

Reference plant	Test plant	Monoculture		Dual culture	
		N-yield (Kg ha ⁻¹)	NdfFix (kg ha ⁻¹)	N-yield (kg ha ⁻¹)	NdfFix (kg ha ⁻¹)
<i>Salvinia molesta</i>	<i>A. pinnata</i>	23.1	14.2	23.6	13.7
	<i>A. microphylla</i>	21.1	12.6	21.5	11.8
<i>Lemna perpusilla</i>	<i>A. pinnata</i>	23.1	12.7	23.1	13.1
	<i>A. microphylla</i>	21.1	10.8	21.5	10.9

Reproduced from Kulasooriya *et al.*, 1988.

Table 5. Recovery of nitrogen from *Azolla* and urea by field-grown rice.

Fertilizer source	Plant part	Dry matter yield (kg ha ⁻¹)	N yield (kg ha ⁻¹)	NdfF* %	N recovery %
Azolla (53.28 kg ha ⁻¹)	Straw	1293 ± 158.63	9.57 ± 0.92	73.63 ± 8.62	13.21 ± 1.95
	Panicle	2060 ± 390	24.31 ± 5.02	66.50 ± 7.55	29.87 ± 3.31
	Total	3352.75 ± 532.35	33.88 ± 5.58		43.09 ± 3.85
Urea (79.63 kg ha ⁻¹)	Straw	1358 ± 250	13.14 ± 2.76	54.75 ± 5.74	8.98 ± 1.35
	Panicle	2639 ± 274	40.48 ± 4.67	55.25 ± 5.25	27.90 ± 1.97
	Total	3397 ± 408.8	53.62 ± 5.57		36.83 ± 1.55

*% N in rice derived from fertilizer (either *Azolla* or urea). 5.5 kg N ha⁻¹ urea together with 54.6 kg P₂O₅ ha⁻¹ and 18.5 kg K₂O ha⁻¹ were added as basal dressing to all treatments. ¹⁵N-labelled, fresh *Azolla* was incorporated at 21.3 t ha⁻¹ and ¹⁵N-labelled urea was incorporated at 92.6 kg ha⁻¹ and 80.5 kg ha⁻¹, 2 and 6 weeks after transplanting rice.

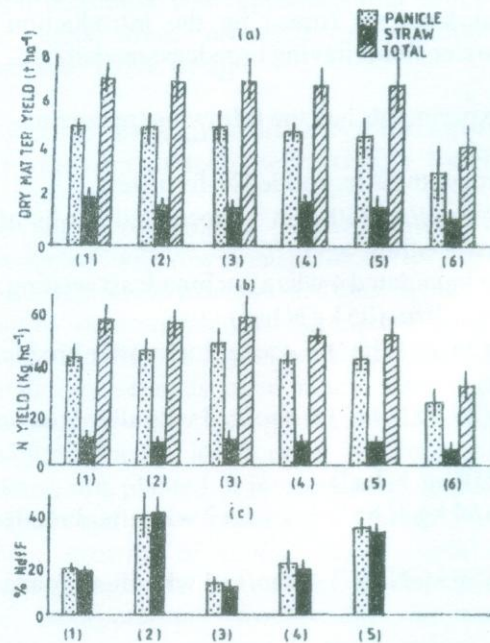


Fig. 1. (a) Dry matter yield, (b) N-yield and (c) % NdfF, in a rice crop that received ¹⁵N-labelled fertilizer according to the following treatments. (1) labelled *Azolla* at transplanting, (2) labelled *Azolla* at tillering, (3) labelled urea at transplanting (4) labelled urea at tillering (5) labelled urea under the best split method and (6) control without nitrogen (Reproduced from kulassoriya *et al.* 1998).

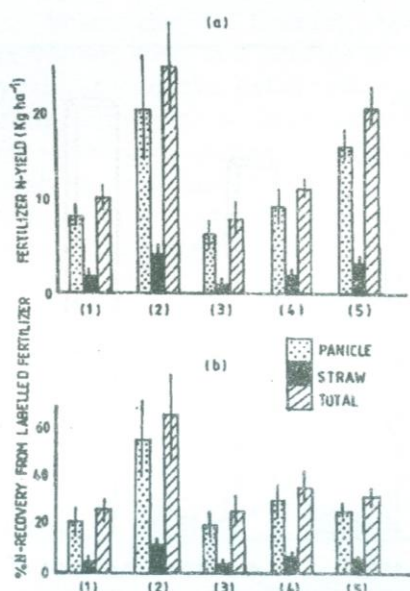


Figure 2 : (a) Fertilizer N-yield and (b) % N-recovery from labelled fertilizer, in a rice crop that received ^{15}N -labelled fertilizer according to the same treatments as in figure 1 (Reproduced from Kulasoorya *et al.*, 1988).

Table 6. Yield responses to *Azolla* and urea treatments given at transplanting and tillering in 4 m x 5 m yield plots*

Treatment	N-fertilizer applied (kg ha ⁻¹)	Grain yield (t ha ⁻¹)	% increase over control
Control	-	1.84	-
<i>Azolla</i> at transplanting and tillering	47	2.81	52.5
Urea at transplanting and tillering	60	2.60	41.2
Urea best split	60	2.80	52.0

*Mean value of 4 replicates
Reproduced from Kulasoorya *et al.*, 1998.

Table 7. Effect of *Azolla* on the yield of rice under different regimes of fertilizer.

TREATMENT	Grain yield (t ha ⁻¹)
1. Control (c)	2.7 ± 0.4
2. 30 kgNha ⁻¹ urea at transplanting (T.P.) U ₀	3.2 ± 0.5
3. 30 kgNha ⁻¹ + <i>Azolla</i> without incorporation (U ₀ + A ₀)	3.5 ± 0.5
4. 15 <i>Azolla</i> incorporated at t.p. (A ₀)	3.8 ± 0.4
5. 30 kgNha ⁻¹ urea 21 d after t.p. (U ₂₁)	3.9 ± 0.5
6. <i>Azolla</i> at t.p. + urea broadcast 21 d after t.p. (A ⁰ + U ₂₁)	4.2 ± 0.6
7. <i>Azolla</i> at t.p. + urea @ 3 weeks + <i>Azolla</i> incorporated @ 28 d (A ₀ + U ₂₁ + A ₂₈)	4.8 ± 0.1

All the plots received P & K fertilizer at recommended rates. Values given are mean of 4 replicates.

Table 8. Effect of *Azolla* on the yield of broadcast seeded rice grown in a farmer's field

Treatment	Grain yield	
	Yala 1990*	Maha 1990/91*
1. Control without N-fertilizer	2.76 (0)	1.66 (0)
2. <i>Azolla</i> (250 gm ⁻²) inoculated 2 wks after broadcast seeding (bs)	2.93 (6)	1.87 (3)
3. <i>Azolla</i> inoculated 4 wks after bs	3.01 (9)	1.91 (15)
4. Urea fertilizer (25 kgNha ⁻¹)	3.13 (13)	2.07 (25)
5. Urea (25 kgNha ⁻¹) + <i>Azolla</i> 2 wks after bs	3.75 (36)	2.37 (43)
6. Urea (25 kgNha ⁻¹) + <i>Azolla</i> 4 wks after bs	3.85 (39)	2.32 (40)
7. Urea (50 kgNha ⁻¹)	3.87 (40)	2.29 (38)
8. Urea (50 kgNha ⁻¹) + <i>Azolla</i> 2 wks after bs	3.98 (44)	2.38 (43)
9. Urea (50 kgNha ⁻¹) + <i>Azolla</i> 4 wks after bs	3.71 (34)	2.48 (49)

Figures within parenthesis give the % increase over the control.

* Yala (Dry season): April - July 1986

Maha (Wet season): October-February 1986/87.

Recovery was highest from *Azolla* incorporated at tillering with much lower values from urea fertilizer (Fig. 2b). The corresponding grain yields obtained from 5m x 4m plots (Table 6), show identical responses to *Azolla* additions and urea applied according to the best split method although the amount of nitrogen added as *Azolla* (47 kg N ha⁻¹) was less than that applied as urea (60 kg N ha⁻¹).

Effect of *Azolla* cover on fertilizer N-recovery

The effects of an unincorporated *Azolla* cover on fertilizer-N uptake are shown in Figure 3. Whenever urea fertilizers have been added together with *Azolla* inoculation (U₀A₀ & A₀U₂₁), a higher recovery has been achieved. The grain yields obtained also show corresponding increases (Table 7).

The results of the experiments carried out in farmers' fields are in Table 8. These show that 25 kgNha⁻¹ of urea fertilizer in combination with an *Azolla* inoculation produce grain yields equivalent to the addition of 50 kg Nha⁻¹ of urea fertilizer. However an *Azolla* inoculation with 50 kg N ha⁻¹ of urea has not produced a proportionate increase in grain yield.

DISCUSSION

Nitrogen fixation by *Azolla*

The initial experiments at Ambalantota gave very low amounts of nitrogen fixation for 42 days (Table 2). Eskew (1987) has pointed out several errors that could happen in the application of the ^{15}N -dilution method under flooded field conditions and a number of them became evident during this preliminary study. The reference crop of Lemna plants grew very slowly compared to the other plants. The basal application of labelled fertilizer (equivalent to 30 kg

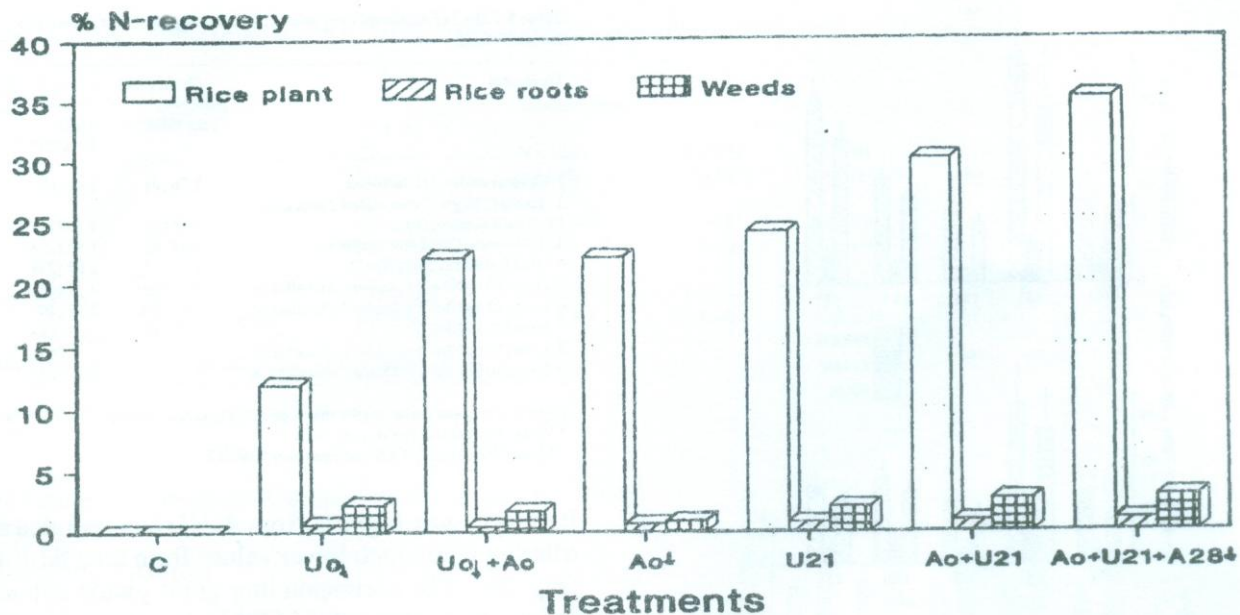


Figure 3: Effect of an *Azolla* cover on N-recovery by rice from added fertilizer. (C): Control without N-fertilizer, (U_o): Labelled urea incorporated at transplanting, (U_o+A_o): Labelled urea incorporated at transplanting and *Azolla* inoculated at transplanting, (A_o): Labelled *Azolla* incorporated at transplanting, (U₂₁): Labelled urea broadcast 21 days after transplanting, (A_o+U₂₁): *Azolla* inoculated at transplanting and labelled urea broadcast 21 days later, (A_o+U₂₁+A₂₈): Same as previous treatment, but *Azolla* incorporated seven days later.

Nha⁻¹) added as a solution to the flood water in a single dose, remained only for a short period and may not have been taken up effectively by the slow growing reference plants. The initial high concentration of nitrogen in the flood water could also reduce nitrogen fixation by *Azolla* (Kumarasinghe and Eskew 1993). Such errors could have resulted in an under-estimation of nitrogen fixation.

When this experiment was repeated at Bombuwela, the labelled fertilizer was split applied in relation to the growth of the test and reference plants and this gave much better results (Table 4). These results are comparable to those reported by You *et al.* (1987), but lower than those of Watanabe *et al.* (1991). They are also similar to those reported from Belgium, but lower to those from Austria and Hungary (Kumarasinghe and Eskew 1993).

These rates of fixation which ranged from 11 to 12 kgNha⁻¹ for *Azolla microphylla* and 13 to 14 kgNha⁻¹ for *Azolla pinnata* show that a noteworthy contribution of atmospheric nitrogen has been brought into this ecosystem by *Azolla* within two weeks. Thus a 2-week old *Azolla* monoculture could provide nitrogen equivalent to the basal dressing of N-fertilizer recommended for most rice varieties. The higher N-yield and the nitrogen fixed under the shade of the rice plants during dual culture at Ambalantota where climatic conditions of temperature and light are harsh, are in agreement with the results of Kulasooriya *et al.* (1980) Thaoptimalt

amedium level of light (around 30 K.lux) was for nitrogen fixation.

Availability of *Azolla* nitrogen to rice

Preliminary experiments at Ambalantota (Table 5) show that N-recovery by rice plants from *Azolla* fertilizer is higher than that from urea fertilizer and a higher proportion of this has gone to the grains. This may partly be due to the higher total amount of urea-N added (80 kg) in comparison to the *Azolla*-N (53 kg). However, while *Azolla* was added as a single basal dose, urea was applied according to the recommended best split method to increase the efficiency of its uptake.

The more systematic experiments conducted at Bombuwela in which the recovery of nitrogen from fertilizers added at transplanting and at tillering were compared with fertilizer application under the recommended best split method, showed similar dry matter yields and N-yields (Fig. 1 a & b). These indicate that the overall response of the crop to the different N-fertilizer additions are comparable. However, the % N derived from fertilizer (%N_{dff}), fertilizer N-yield and the %N-recovery from labelled fertilizer (Fig 1c & Fig 2 a & b) show distinct differences. Thus, there is better N-uptake from *Azolla* than from urea and from the same source of fertilizer (either *Azolla* or urea), the uptake has been better when it was incorporated at tillering than at transplanting. Being an organic source, *Azolla* would release its nitrogen more slowly than urea. This could

maximize losses due to leaching, percolation, ammonia volatilization and perhaps act in better harmony with crop uptake. Better uptake at tillering than at transplanting is due to the inability of the small rice seedlings to take up nitrogen as rapidly as vigorously growing plants at tillering.

Results from the yield plots (Table 6) show that 47 kgNha⁻¹ applied as *Azolla* has supported the same grain yield as that obtained by the addition of 60 kgNha⁻¹ of urea under the best split method. This indicates that *Azolla* is either a better fertilizer than urea or the effect of *Azolla* is not entirely due to nitrogen. The latter possibility is more likely because the addition of organic matter improve the texture, aeration, cation exchange capacity and microbial activity of soil, leading to an overall reduction in nutrient losses including nitrogen.

Effect of *Azolla* cover on fertilizer N-recovery

It is clearly seen from Figure 3 that the % N-recovery from the labelled fertilizer was always higher in the presence of *Azolla* (U₀A₀ and A₀U₂₁) than in its absence (U₀ and U₂₁). Fertilizer N added to a flooded rice field could be lost through ammonia volatilization if the flood water pH becomes alkaline. Increase in pH in the flood water is enhanced due to photosynthesis by the submerged vegetation which rapidly absorbs the dissolved CO₂ during the day. An *Azolla* cover could minimize such activity due to shading and reduce N-losses and this may account for the higher N-recovery in the presence of *Azolla*.

Experiments done in the farmers' fields (Table 8) clearly showed the beneficial effect of having an *Azolla* growth among the rice plants even when it cannot be incorporated to the soil. Such benefits may be due to reduction of N-losses and weed growth by having an *Azolla* cover on the flood water. The levels of fertilizer-N added in these experiments were low, but realistic in relation to the actual fertilizer levels used by farmers in these rainfed low country rice fields.

These studies subsequently followed up by demonstration trials and advice and training programmes conducted at the Agricultural Research Station at Bombuwela have increased the awareness among rice farmers on the beneficial use of *Azolla* and a few innovative farmers have now adopted the use of this natural fertilizer on their own (Mr. C. Wijesundera, Research Officer at Bombuwela, personal communication).

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