

Effect of Azolla on the Rice Yield, Fertilizer-¹⁵N Recovery, Soil N Budget and Floodwater Properties in an Azolla - Rice Intercropping Systems

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ABSTRACT

A pot study was conducted to investigate the effect of Azolla alone and in combination with urea on IRRI-6 rice yield, total N and urea ¹⁵N uptake by rice, ¹⁵N retention in soil, and floodwater pH in the rice-Azolla dual cropping system. The experiment was performed in 25 cm dia pots containing 10 Kg of a local paddy soil. *Azolla pinnata* was inoculated 20 days after transplanting (DAT) at 300 g m² and allowed to grow without incorporation in Azolla cover treatments, and 50% Azolla was incorporated whenever it covered the water surface in Azolla incorporated treatments. ¹⁵N labelled urea was applied 23 DAT for 30 Kg N/ha, and again 70 DAT at 30 Kg N/ha for 60 Kg N/ha treatments.

The results indicated that percent increase in grain yield was 29 for Azolla cover, 69 for Azolla incorporated, 48 for 30 Kg N/ha, 115 for 60 Kg N/ha, 67 for Azolla cover + 30 Kg N/ha, 135 for Azolla cover + 60 Kg N/ha, 111 for Azolla incorporated + 30 Kg N/ha and 156 for Azolla incorporated + 60 Kg N/ha. The total N uptake by rice (straw + grain) was higher in Azolla + urea treatments than urea only. The total ¹⁵N recovery (rice + soil) of the labelled urea was 12 and 20% higher for 30 Kg N/ha in combination with Azolla cover and Azolla incorporated respectively, than 30 Kg N/ha urea only. The presence of Azolla reduced the N losses, as % ¹⁵N losses were 45 for 30 Kg N/ha and only 33 for Azolla + 30 Kg N/ha treatment. The floodwater pH during day time increased upto 8.6 in control and it remained much lower (7.6) in Azolla containing pots. The higher pH and temperature and more algal growth in non-Azolla pots may be some of the reasons for more N losses in pots receiving urea only.

INTRODUCTION

Nitrogen is the most common limiting nutrient for plant growth. A large inputs of nitrogen is required for the modern high yielding rice varieties (Roger and Watanabe 1986). This nitrogen demand in

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the near future is hardly to be met by chemical N fertilizers alone due to limited supply of fossil fuel. Also in view of the polluting effect of using larger quantities of N fertilizers on the environment, it is advisable to increase the efficiency of chemical fertilizers (Hermelink and Kramer 1987). Improvement in the utilization of fertilizer N by paddy is particularly more important, as rice recovers only 30-40% of applied N whereas dryland crops recover 50-60%. (Craswell and Vlek 1979).

Amongst the N_2 -fixers, Azolla has been used traditionally as green manure for rice production in South East Asia, and is still considered an important biofertilizer for rice crop (Liu 1979, Lumpkin and Plucknett 1981, Tuan and Thuyet 1979, Watanabe 1987). Azolla is used as monocrop and intercrop with rice, but its use as intercrop is considered more economical (Fiore and Gutbrod 1987; Singh et al 1984) and appears to be feasible (Rains and Talley 1979) and easily adaptable (Singh 1981). The use of Azolla alone and in combination with chemical N fertilizer has been reported by different workers (Mabbyad 1987, Watanabe 1987), but the information on the effect of Azolla on the availability of applied fertilizer to rice and its retention in soil is lacking. We therefore, studied the effect of Azolla as full cover, half incorporated when it reached full cover, alone and in combination with ^{15}N labelled urea to estimate its usefulness to rice yield, N uptake by rice and soil N budget.

MATERIALS AND METHODS

The experiment was conducted in 25 cm (i.d.) glazed pots in the green house, in a complete randomised design with three replicates and 9 treatments: T_1 , control (no nitrogen, no Azolla); T_2 , Azolla cover; T_3 , Azolla incorporated; T_4 , 30 Kg N ha^{-1} ; T_5 , 60 Kg N ha^{-1} ; T_6 , Azolla cover + 30 Kg N ha^{-1} ; T_7 , Azolla cover + 60 Kg N ha^{-1} ; T_8 , Azolla incorporated + 30 Kg N ha^{-1} ; T_9 , Azolla incorporated + 60 Kg N ha^{-1} .

The paddy soil used in the experiment was collected from the rice fields of the NIAB campus. It was a loam with 14% clay, 33% silt and 53% sand. The soil had pH (1:1) 7.43, EC (1:1) 0.95 $mS\text{cm}^{-1}$, C 0.49% and total N 0.67%. After air drying and sieving through 2 mm sieve, 10 Kg soil was added per pot and flooded for 2 weeks and puddled. IR - 6 rice was transplanted at 2 seedlings per hill and 5 hills per pot on July 25, grown to maturity and harvested on Dec. 1, 1985. The soil was kept flooded (1-4cm) throughout the rice season and watering was stopped 2 weeks before harvesting the rice. The data for tiller number and maximum plant height was recorded 3 days before the rice harvest.

Azolla pinnata a local heat tolerant strain (Ali and Malik 1987) was inoculated at 15 g per pot ($300g\ m^{-2}$) at 20 DAT of rice in the Azolla treatment pots ($T_2, T_3, T_6 - T_9$). Superphosphate solution at 20 Kg P ha^{-1} (area basis) was sprinkled onto floodwater in all the pots including control. ^{15}N labelled urea (1.972% ^{15}N atom excess) was applied at 30 Kg N ha^{-1} (area basis) in solution form 23 DAT in T_4, T_6 and T_9 and

similarly another dose of 30 Kg N ha^{-1} was applied 70 DAT in case of 60 Kg N ha^{-1} treatment (T_5, T_7, T_9).

In case of Azolla cover treatments, Azolla was allowed to grow, and was not incorporated into the soil, while in Azolla-incorporated treatments approximately half the Azolla was incorporated into soil whenever Azolla covered the floodwater fully and thus total of 7 incorporations were made, being 31, 39, 46, 56, 60, 68, 81 DAT in Azolla incorporated treatments (T_3, T_6, T_9). Floodwater was not drained from the pots to avoid ^{15}N losses, but watering of pots was stopped about 3 days prior to each Azolla incorporation. The evapo-transpiration decreased water level to about 0.5-1 cm and Azolla was hand incorporated into top soil.

The pH of floodwater was measured with a portable pH meter in situ, and also with a laboratory pH meter by taking the water samples on 11 different days (56 to 111 DAT) during rice growth at different day timings (8 am to 4 pm). The water samples taken for pH measurement in the lab were returned back to the respective pot to avoid ^{15}N losses from the system. The pH at soil-water interface and top 3-5 cm soil was measured at 80, 89 and 93 DAT.

The temperature of floodwater and top 3-5 cm soil was measured at noon on 70, 74, 82 and 90 DAT. The algal growth was observed in water and on soil surface on 30, 54 and 80 DAT. Azolla samples were taken 80 DAT for total N and ^{15}N determination.

Rice was dried at 60°C for dry weight recording and ground in a selfcleaning mill (Cyclotec) to minimize ^{15}N cross contamination. After determining total N by Kjeldahl method (Bremner and Mulvaney 1982), the distillate was acidified and evaporated to 1-2 ml and used for ^{15}N determination in the mass spectrometer (Hauk 1982), and ^{15}N calculations were made according to IAEA (1983).

RESULTS AND DISCUSSION

Rice tillers and yield

As compared to control the tiller height increased slightly for Azolla cover, Azolla incorporated and 30 Kg N ha^{-1} , but this increase was significant for 60 Kg N ha^{-1} and all the Azolla + urea treatments (Table 1). Similarly less increase in tiller height was observed for Azolla dual (grown alongwith rice) and incorporated, than Azolla basal incorporated, fertilizer or Azolla incorporated plus fertilizer (Singh 1981). The increase in tillers per pot was lesser for Azolla but significantly higher in Azolla + Urea and urea treatment (Table 1). Similar trend for increase in tiller number was also observed by other workers (Pande 1980, Singh 1981, Singh and Singh 1987), SFIZAAS 1975). The lesser effect of only Azolla treatments on tiller number may be due to insufficient availability of Azolla - N during tillering stage of rice as Azolla was inoculated 20 DAT. Singh

and Singh (1987) have also observed negligible effect of Azolla on the tiller number if inoculated 21 DAT or later.

The rice straw yield in control and Azolla alone treatments was similar but significantly higher for urea and urea + Azolla treatments (Table 1). The increase in straw yield was observed by Pande (1980) for Azolla basal incorporation, and by Singh and Singh (1987) for Azolla inoculation at 10 and 30 DAT and incorporation 25 and 50 DAT. The reason for not observing any increase in straw yield in our experiment, may be the same as explained above for tiller number. The percent increase in rice grain yield was 29 for Azolla cover, 69 for Azolla incorporated, 48 for 30 Kg N and 115 for 60 Kg N ha⁻¹ over control (Table 1). Approximately 20% higher grain yield was observed for Urea + Azolla cover treatments, and 41 to 63% for Urea + Azolla incorporated treatments as compared to urea treatment respectively. There was upto 31% increase in the biomass (straw + grain) production due to Azolla over control; Azolla + Urea treatments were slightly superior to urea only. The increase in grain yield for Azolla treatments which was not observed for rice straw, may be due to more Azolla - N availability at later stage of rice growth (Singh and Singh 1987). The increase in rice grain yield by unincorporated Azolla has also been reported to be 18% by Liu (1979) and SFIZAAS (1975); 20% by Watanabe (1987); 24% by Foire (1987), 19-29% by Singh et al (1984) 6-39% by Singh (1979), 12-38% by Reynaud (1984), 58% by Loudhapasitiporn and Kanareugsa (1987) and 23-67% by Talley and Talley (1977). The benefit of Azolla cover may be partially due to secretion of some of the fixed N₂ as ammonium into floodwater during its active growth, but mainly from N release after its natural death and decay occurring after over crowded growth conditions (Ito and Watanabe 1985, Liu 1984, Rains and Talley 1979, Talley and Rains 1980, Watanabe et al 1981), and also by suppression of weeds (Diara et al 1985). As mentioned above rice yield was 20-63% higher for Urea + Azolla treatments than urea only indicating substantial (20%) increase in rice grain yield due to Azolla even without its incorporation which is a least labour requiring technique. Natural lodging (natural death and decay, no incorporation) of Azolla is reported to be low in cost and labour and adaptable if timing and field irrigation practices are appropriate (SFIZAAS 1975, Lumpkan & Plucknett 1982). But if the labour for incorporation is cheaper and easily available the rice yield can be further increased upto 63% over urea only. Similar results were also obtained by other workers who reported benefit ranging from 14-47% (Watanabe 1987, Singh 1981).

To know how much N was taken up by Azolla from the applied, ¹⁵N labelled urea, Azolla samples were collected after 45 days of first ¹⁵N labelled urea application from pots with 30 Kg N/ha, and 10 days after the second urea application from pots with 60 Kg N/ha. The results indicated 1-2 % N in Azolla derived from urea for 30 Kg N ha⁻¹, and 24-27% for 60 Kg N ha⁻¹ treatments. The data indicated that only a minor portion of urea N was taken up by Azolla, and this fertilizer-N was also returned to soil after the death and decay of Azolla in the pot. Kumarasinghe et

al (1985) observed 76-90% of Azolla -N derived from N_2 fixation in the presence of chemical N fertilizer in laboratory and field conditions. Peters and Ito (1984) observed 43-50% ethylene production in Azolla in the presence of 12.5 mM urea as compared to control. The other benefit of Azolla is that most of its N is taken up by rice in 30-60 days after its incorporation, while of urea most of the N is taken up within 30 days (Eskew 1987). These studies and our data indicated that this unique property of Azolla to continue fixing atmospheric N_2 even in the presence of combined N and N release during grain formation stage, makes the use of Azolla as intercrop, compatible with the early application of chemical N fertilizer, for increasing both the grain and straw yield.

Total N content of rice and soil

The total N uptake by rice plant (straw + grain) was 25% and 63% higher for Azolla cover and Azolla incorporated, respectively over control (Table 1). The Azolla improved N uptake by rice when used alongwith urea, as rice-N content was 19-29% higher for Urea + Azolla cover and 46-48% for Urea + Azolla incorporated treatments than urea only. Reynaud (1984) also observed 6-10 Kg N ha^{-1} higher in rice for Azolla cover over control and upto 8 Kg N ha^{-1} extra for Azolla incorporated + 30 Kg N ha^{-1} than 30 Kg fertilizer-N only. Similarly Singh et al (1984) found 15-24% higher N uptake by rice for unincorporated Azolla and 30-49% higher N for Azolla grown dual and twice incorporated, over control.

The total N remaining in the soil after the rice harvest was similar to control in 30 and 60 Kg N ha^{-1} urea, but 6-8% higher in Azolla cover and Azolla incorporated pots than control, while 6-10% higher for Azolla + Urea treatments than urea only (Table 1). This increase in total soil N content due to Azolla may be due to its N_2 fixation, and conservation of native soil N to be discussed under floodwater properties. The increase in soil-N after rice harvest was also observed by Liu (1979) and Reynaud (1984).

N recovery from ^{15}N labelled urea in rice and soil

The ^{15}N uptake of applied urea, by rice straw and grain or retention in soil after rice harvest, computed as percent nitrogen derived from fertilizer (% NdfF) and fertilizer N yield (FNY) was higher for 60 Kg N ha^{-1} alone or in combination with Azolla than 30 Kg N ha^{-1} alone or with Azolla (Table 2). The N recovery of ^{15}N labelled urea calculated as percent fertilizer N recovery (% FNR) was 6-10 in rice straw, and significant increase was observed for 30 Kg N/ha + Azolla incorporated than 30 Kg N urea only. The N recovery from ^{15}N labelled urea in straw is in agreement with the observation of Kulasooriya et al (1987) who obtained 9% recovery of N from the labelled urea, incorporated in paddy soil. The urea N recovery in rice grain was 13-26% being slightly lower than reported by Kulasooriya et al (1987), who

reported 28% N recovery, which may be due to higher amount (80-90 Kg N ha⁻¹) and early incorporation of ¹⁵N labelled urea in their rice soil. The recovery from ¹⁵N labelled urea in rice grain was higher for Urea + Azolla treatments than urea only and this increase was significant for 30 Kg ha⁻¹ + Azolla incorporated, and 60 Kg N ha⁻¹ + Azolla cover or incorporated than urea only. The overall N recovery of labelled urea by rice crop (Straw + Grain) followed the trend of N recovery in grain and it was 20-26% for urea and 24-33% for Urea + Azolla. This overall recovery is slightly higher to N recovery reported by Kumarasinghe et al (1985) who observed 18% for basal urea incorporation, however a wide variation (7-38%) in fertilizer N recovery for basal surface application has been reported (Craswell and Vlek 1979).

The urea ¹⁵N retention in soil after rice harvest was generally higher for Azolla + Urea treatments than urea only and % FNR was upto 12% higher for 30 Kg N ha⁻¹ + Azolla incorporated than 30 Kg N urea only (Table 2). The total urea N recovery in rice plant + soil was also higher for Azolla + Urea treatments than urea only, and upto 20% higher FNR was found for Azolla incorporation + 30 Kg N ha⁻¹ than 30 Kg N urea only (Table 2). The N losses of applied urea (determined by difference) also indicated benefit of Azolla, as these losses were 12% and 20% lesser for Urea + Azolla cover and Urea + Azolla incorporated respectively, than 30 Kg N ha⁻¹ urea only (Table 2).

Algal growth, pH and temperature of flood-water and top soil.

The algal growth comprising blue-green and green algae and diatoms, was observed in the flood water and on the soil surface in control and only urea treated pots; whereas almost no algal growth was found in pots having Azolla, and flood water was clear.

The pH of the flood water increased during noon in all the treatments, but this increase was more prominent in control and only urea pots than pots having Azolla alone or with urea (Fig. 1). The flood water in control pots rose to 8.2 by 11 am and reached 8.6 at 1 pm and remained around 8.5 upto 3 pm. Similarly flood water pH in urea applied pots remained high (8.3) from 12 to 3 pm. The flood water pH increase in Azolla + Urea treatments was less, being 7.8 at 10 am and afterwards there was no significant change even upto 3 pm. The lowest value for flood water pH at all the times was recorded for Urea + Azolla cover and it was as low as 7.4 at 3 pm, when pH in control was around 8.5. The data indicated maximum pH difference of 1.2 units, being upto 8.6 in control and as low as 7.4 in Azolla cover + Urea.

The pH at soil water interface was 7.9 for control, 7.5 for urea, 7.4 for Azolla cover and 7.1 for Azolla incorporated treatment, at noon times. Similarly the pH of the top 3-5 cm soil was higher in control than Azolla cover, urea and Azolla incorporated, being 7.6, 7.3, 7.1 and 7.0 respectively.

The difference in flood water temperature was maximum at noon in

different treatments, and the temperature was 31.0°C in control, 30.2 in urea, 30.0 in Azolla incorporated and 29.6°C in Azolla cover pots.

Tuan and Thuyet (1979) also reported increase in flood water pH to 9.4 at noon in control while it remained lower (7.6) in Azolla full cover or copper sulfate treated water. According to Lumpkin and Plucknett (1982) growing Azolla as intercrop with rice, keeps the water cooler and algal growth lower by shading, reduces oxygen level in soil and decreases the flood water pH due to respiration.

The improvement in the efficiency of applied ^{15}N labelled urea in the presence of Azolla, mentioned above, may be due to reduction in pH, dissolved oxygen, temperature and algal growth. Mikkelsen et al (1978) observed increase in flood water pH, decrease in dissolved CO_2 and H_2CO_3 during day time due to algal photosynthesis, leading to NH_3 volatilization. The higher soil or water pH causes more volatilization losses, and upto pH 9 the concentration of aqueous ammonia increases about 10 fold for each unit increase in pH and these losses may be upto 60% in some rice fields (Mikkelsen and De Datta 1979). Ferdrazzini and Tarsitano (1987) observed maximum ammonium concentration and losses of applied urea from 10 to 16 hours in a flooded soil. Freney et al (1981) observed NH_3 volatilization rate followed the diurnal pattern of water temperature and windspeed, and N losses were maximum in the middle of the day. Like NH_3 volatilization losses, N losses by denitrification in paddy soil may also be up to 63% because the oxidation of applied N to NO_3 occurs in the thin oxidized surface layer and it is followed by denitrification losses (Crasswell and Vlek 1979). The maintenance of reduced conditions for most of the growing season and deep placement were ~~and~~ considered helpful practices for minimizing these losses (Patrik 1982). From these studies it appeared that Azolla minimized NH_3 volatilization as well as denitrification losses by decreasing algal growth, flood water pH, temperature and air movement on the water surface, and keeping the floodwater and top soil in reduced conditions.

Practical significance

About 70% of the rice in developing countries is grown as a rainfed crop (Crasswell and Vlek 1979), and outside China or Vietnam very few rice producing areas in the world have wet - cool season for growing Azolla as mono-crop before rice (Loudhapisitiporn and Kanareugsa 1987, Lumpkin 1987). Thus growing Azolla with rice as intercrop is more practical in most of the paddy fields, and it also reduces 20-23% water losses by its cover (Diara and Von Hove 1984). Since Azolla + Chemical N fertilizer was better than fertilizer only, the use of Azolla may be encouraged with fertilizer to increase rice yield, N uptake and soil N budget, as the unique property of Azolla of retaining sufficient N_2 fixation activity in the presence of fertilizer N, and make the use of old Azolla technology compatible with the modern use of N fertilizer.

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Table 1. Effect of Azolla and urea on the IR-6 rice yield, N uptake and tillers at harvest.

Treatment	Straw yield (g Pot ⁻¹)	Grain yield (g Pot ⁻¹)	S+G yield (mg Pot ⁻¹)	Plant N content (mg Pot ⁻¹)	Soil N content (g Pot ⁻¹)	Tillers no.	Tillers height (cm)
1- Control	12.24 (0)	11.12 (29)	23.36 (14)	129 (25)	6.20 (6)	12	63.0
2- Azolla cover	12.20 (0)	14.39 (29)	26.59 (14)	160 (25)	6.57 (6)	14	64.3
3- Azolla incorporated	11.79 (-4)	18.77 (69)	30.56 (31)	210 (63)	6.70 (8)	13	63.9
4- 30 Kg N ha ⁻¹ (Urea)	19.68 (71)	16.48 (48)	36.16 (55)	202 (57)	6.27 (1)	18	64.3
5- 60 Kg N ha ⁻¹ (Urea)	20.56 (68)	23.86 (115)	44.42 (90)	276 (114)	6.27 (1)	17	70.7
6- Azolla cover + 30 Kg N ha ⁻¹ (Urea)	17.74 (45)	18.60 (67)	36.34 (56)	227 (76)	6.83 (10)	18	69.3
7- Azolla cover + 60 Kg N ha ⁻¹ (Urea)	19.57 (60)	26.18 (135)	45.75 (104)	313 (143)	6.60 (6)	19	67.7
8- Azolla incorporated + 30 Kg N ha ⁻¹ (Urea)	21.37 (75)	23.46 (111)	44.84 (92)	264 (105)	6.57 (6)	21	67.3
9- Azolla incorporated + 60 Kg N ha ⁻¹ (Urea)	20.62 (69)	28.47 (156)	49.09 (110)	348 (160)	6.57 (6)	21	72.3
LSD (5%)	3.66	3.90	6.75	42.6		3.7	3.8

Figures in parentheses indicate % increase over control, S+G = Straw plus Grain

Table 2 Effect of Azolla on the ^{15}N recovery of ^{15}N labelled urea by rice and its retention in soil

Treatment	Rice straw				Rice grain				Soil				P+S		N lost (%)
	NdF (%)	FNY mg/pot	FNR (%)	NdF (%)	FNY mg/pot	FNR (%)	NdF (%)	FNY mg/pot	FNR (%)	NdF (%)	FNY mg/pot	FNR (%)	FNR (%)	FNR (%)	
Urea 30 Kg N	13.40	10.5	7.11	16.13	19.2	13.02	20.13	0.824	51.7	35.08	55.21	44.79			
Urea 60 Kg N	24.33	18.3	6.20	28.94	58.0	19.68	25.88	1.753	110.1	37.36	63.24	36.76			
Azolla cover + 30Kg N	17.06	11.8	8.01	14.64	23.2	15.74	23.75	0.929	63.6	43.20	66.95	33.05			
Azolla cover + 60 Kg N	27.37	20.0	6.80	29.43	70.7	24.00	30.80	1.807	118.5	40.23	71.03	28.97			
Azolla incor. + 30 Kg N	17.75	14.0	9.53	14.44	26.6	18.06	27.86	1.247	69.9	47.42	75.00	25.00			
Azolla incor. + 60 Kg N	25.97	19.8	6.73	29.60	76.5	25.96	32.69	1.797	117.7	39.96	72.65	27.35			
LSD (5%)	4.92	-	2.09	2.36	-	3.21	3.97	0.56	-	NS	NS	NS		¹⁶	

Abbreviations used: S+G = Straw + Grain, P+S = Plant + Soil, NdF = Nitrogen derived from Fertilizer, FNY = Fertilizer nitrogen yield, FNR = Fertilizer nitrogen recovery, % N lost = 100 - (% FNR in Plant + Soil).

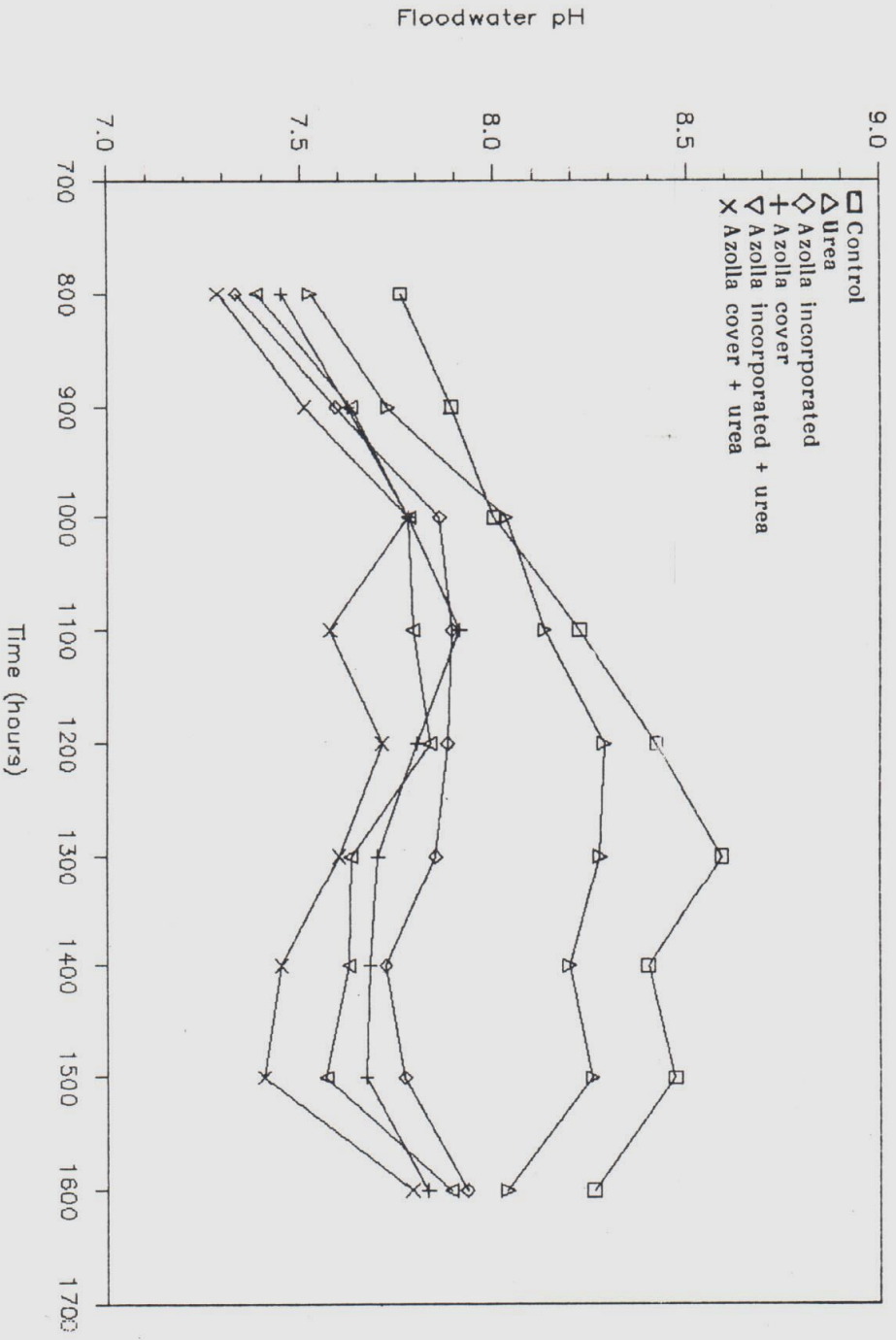


Fig. 1. Kinetics of floodwater pH during day time in a rice-Azolla intercropping system.