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Enhancing Effect of Nitrogen and Phosphorus on Azolla (*Azolla microphylly*) in Rice Production in Acid Sulphate Soil

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ABSTRACT

The experiment was carried out at Pathumthani Rice Research Center in the year 1995/96 to evaluate the effects of 1) Azolla -N and urea -N to rice in acid sulphate soil, and 2) the split application of P on Azolla biomass production. The soil at the experimental site was classified as Sulphic Tropaquepts : isohyperthermic acid in Rangsit Series (RS). To evaluate the effect of P, the Azolla was inoculated 20 days before transplanting and 5 days after transplanting as dual-crop with rice. P was applied as basal or splitted 2 and 4 times at 10 and 5 days intervals, respectively. The results indicated that maximum fresh biomass can be produced with 2 or 4 split application of P. N content and total N production in Azolla was found significantly higher with 4 split applications of P. Azolla doubling time, its biomass production and N content were not affexted by its cultivation methods. Split application of P toAzolla resulted in higher grain yield and total dry-matter production in rice. N-yield (uptake) in rice was found to be increased with increasing number of split applications of P to Azolla.

Key words : *Azolla microphylla*, nitrogen, phosphorus, Azolla biomass, Split application.

INTRODUCTION

In association with blue green algae *Anabaena azollae*, a water fern can fix atmospheric N2 symbiotically in wet land paddy fields. After decomposition of the incorporated Azolla into the soil, fixed N becomes available to the rice plants (Watanaabe *et al.*, 1991). The symbiont *Anabaena azollae* that lives in the leaf cavities of the Azolla fern provides a source of organic N fretilizer and is thus considered an aquatic green manure for rice cultureing.

Nitrogen has ofter been regarded as the key to rice productivity and there is ample evidence that the use of synthetic fertilizer can lead to high levels of productivity of rice crop, particularly with modern N -responsive high yielding rice varieties. But for a variety of reasons, fertilizers are not always

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readily available and farmers are often reluctant to use them and rice production must rely upon the existing soil fertility to meet crop requirements for this nutrient. Deficiency can be off -set by the introduction of N -fixing green manuring crops such as Azolla, other than chemical N.

The importance of Azolla in large scale of atmospheric N_2 fixation has attracted agronomists, soil scientists, and researchers in recent years. The fixation of N_2 by Azolla is depended mainly on the growth and multiplication rates. The growth of Azolla is related to the supply and method of P application (Watanabe *et al.*, 1988), which influences the total N production by Azolla and hence influences the total dry matter production of rice. Azolla when incorporated into the soil, may also improve the soil properties.

For better growth of Azolla and influencing effects on the paddy yield, P should be applied to Azolla in correct amount, at the right time, and by proper method of application. The methods of Azolla inoculation, methods of P application to Azolla and evaluation of its effects to Azolla and rice production need further research. This study mainly aims to fulfill the following objectives.

a. To study the effects of Azolla cultivation before transplanting and after transplanting of rice along with or without nitrogen fertilizer on rice yield.
b. To study the effects of split application of fertilizer on Azolla and on the yield of the following rice crop.

MATERIALS AND METHODS

Design and Treatments

During the rainy season of the year 1995/96, an experiment was conducted in the open field of the Pathumthani Rice Research Center. The soil at the experimental site was acid sulphate clay and was classified as Sulfic Tropaquepts (very fine and isohyperthermic). A randomized complete block design (RCBD) with 11 treatments (Table 1) and 4 replications was employed. Seedlings of 25 days old *Oryza sativa* L. cv. RD 23 were transplanting in the plots with the area of 3 m × 5 m. *Azolla microphylla* was inoculated at the rate of 937.5 g/plot in the assigned plots. Fertilizer was applied at the rate of 37.5-37.5- 5 -25 kg N -P2O5 -K2O /ha. Azolla biomass production (fresh weight) was determined at 20 days after inoculation. For all of the Azolla plots, tripple superphosphate (TSP) was either applied as basal (P1) or by splitting 2 times (P2) and by splitting 4 times (P4) at 10 and 5 days intervals, respectively, with the first P application on Azolla inoculation date. It was basally applied at transplanting for non -Azolla plots. Nitrogen as urea was applied only to non -Azolla plots, half at transplanting and the other half at panicle initiation stage (PI). Murate of potash was basally applied to every plots at transplanting. Rice grain yield was determined by harvesting the crop from an area of 8.44 m^2 in each plot by excluding 2 bordered rows. Straw yield and yield components of rice were determined by sampling the crop at 5 spots per plot, each spot containing 20 hills of rice plants. The Azolla biomass, rice grain yield and yield components were analysed statisically by Least Significant Difference Test (LSD) or by Duncanís Multiple Range Test (DMRT).

RESULTS

Effects on Azolla (fresh weight and N production)

The data on Azolla cultivation methods (Table 2) clearly indicated that there was no difference in Azolla cultivation methods on its biomass production (fresh weight) and doubling time (growth rate). Its N production significantly decreased when it was grown as a dual -crop with rice (A2N1P) as compared to that produced with Azolla cultivated before transplanting (A1N1P).

Table 1 Treatment of the experiment.

Treatments		Remarks
1.A0N0P0	A0 =	Azolla not inoculated.
2.A0N1P1		
3.A1N0P1	A1 =	Azolla inoculated 20 days before transplanting and incorporated at transplanting.
4.A1N0P2		
5.A1N0P4		
6.A1N1P1	A2 =	Azolla inoculated 5 days after transplanting, not incorported.
7.A1N1P2	N0 =	Fertilizer N not applied.
8.A1N1P4	N1 =	37.5 kg N /ha, two equally split to apply at transplanting and primordial initiation stage (PI).
9.A2N1P1	P1 =	All amount of P given to Azolla inoculation.
10.A2N1P2	P2 =	Two equally split applications to Azolla inoculation and ten days after inoculation.
11.A2N1P4	P4 =	Four equally split applications to Azolla inoculation and 5 days intervals afterward.

Using different methods of application of P (Table 3) showed no significant difference in biomass production, N content (% N) and N production between Azolla with basal and 2 split applications. Significant response to P were observed among basal and 4 split applications of P. No significant differences in biomass and N production were observed among 2 and 4 split applications of P, but 4 split applications tended to be superior to 2 split applications.

Azolla's doubling time was significantly decreased when P was applied to Azolla by split application as compared to that obtained with P applied as a basal application (Table 3). No difference was observed among the 2 and 4 split application.

The effects of experimental treatments on Azolla presented in Table 4 clearly indicated that Azolla biomass (Fresh weight) was significantly increased when Azolla was grown before transplanting (AINIP4) with 4 split applications of P as compared to that with basally applied P (AINIPI). The Azolla with basal application of P was not significantly differed in its biomass production from that with 2 split application (AINIP2) when they were grown before transplanting. No significant difference was observed in biomass production among P application method when Azolla was grown after transplanting (Table 4). The effect of biomass production of Azolla was found positively correlated with its N production (Figure 1).

Azolla doubling time did not show any response in P application and cultivation methods of Azolla except when Azolla was grown before transplanting (AINOPI) with basally applied P gave higher value of doubling time than others. N content was increased significantly when Azolla was grown before transplanting (AINIP4) with 4 split application of P to Azolla (Table 4). A minimum

Table 2 Mean effects of Azolla cultivation methods on its production[1/].

Treatments	Biomass	Doubling time (days)	N production (kg /ha)
A1N1P	14.2 a	4.95 a	33.2 a
A2N1P	10.3 a	4.88 a	21.3 b
CV %	47.8	30.0	23.0

1 Means followed by a common letter in a column are not significantly different at 95 % level by LSD.

Table 3 Mean effects of methods of application of P on its production[1/].

Treatments	Biomass	Doubling time (days)	N production (kg /ha)	N content (%)
P1	7.5 b	7.27 a	12.3 b	1.9 b
P2	13.4 ab	4.51 b	27.4 ab	2.6 ab
P3	15.6 a	4.74 b	40.7 a	3.4 a
CV %	19.3	13.0	32.8	12.0

1 Mean followed by a common letter in a column are not significantly different at 95 % level by LSD

Table 4 Effects of different treatments on Azolla production[1/].

Treatments	Biomass (t/ha)	Doubling time (days)	N content (%)	N production kg/ha
A1N0P1	6.8 c	10.32 a	2.11 e	11.3 cd
A1N0P2	15.8 ab	4.30 b	2.33 e	27.6 bcd
A1N0P4	13.4 bc	4.49 b	3.79 a	38.6 ab
A1N1P1	5.8 c	6.37 b	1.77 f	8.0 d
A1N1P2	13.6 bc	4.50 b	3.14 c	32.4 bc
A1N1P4	23.0 a	3.85 b	3.47 b	59.2 a
A2N1P1	10.0 bc	5.12 b	2.27 e	17.5 bcd
A2N1P2	10.6 bc	4.60 b	2.80 d	22.2 bcd
A2N1P4	10.4 bc	4.80 b	2.98 cd	24.2 bcd
CV %	30.9	41.3	19.9	38.3

1 Mean followed by the same letters in a column are not significantly different at 95 % by DMRT

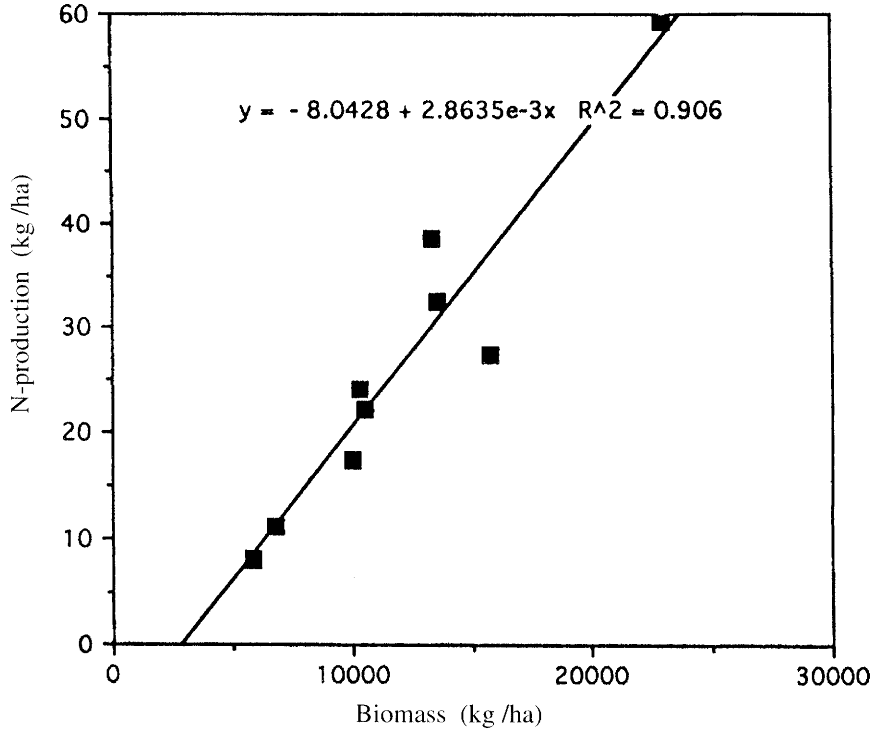

Figure 1 Correlation between Azolla biomass and its N -production

content of 1.77 percent of N was observed with application of P to Azolla (AINIPI) when it was grown before transplanting. A significant difference was observed in N production among Azolla with basally applied and splitted P applications. A maximum of 59.23 kg/ha of N was produced by Azolla when it was grown before transplanting with 4 split applications of P (AINIP4) compared to that with a minimum of 8.05 kg/ha of N with basal application of P to Azolla grown before transplating (AINIPI). No significant difference in N production was observed with P application methods when Azolla was grown as a dual-crop with rice.

Effect of Azolla on rice (yield and yield components)

Total dry-matter production was significantly decreased (6.52 t/ha) when urea - N was not applied along with Azolla to rice crop (AINOP4, Table 5). A significant response in total dry - matter production was observed when Azolla was grown with 4 split application of P and urea - N was added to rice whether Azolla was grown before or after transplanting (AINIP4,A2NIP4) (Figure 2).

N - yield (N uptake) of the rice crop was significantly higher when it was fertilized with both Azolla and urea-N. The uptake of N by rice was found to the lower when urea-N was not added to rice (28.0 kg/ha) (Table 5) as compared to that removed by the crop (49.1 kg/ha) fertilized with the both urea-N and Azolla-N at the same method of P application to Azolla. A maximum of 68.4 kg/ha of N was removed by rice when it was fertilized with urea-N and Azolla with 4 split applications of P when it was grown before transplating (AINIP4,Table 5).

Table 5 Effects of different treatments on yield, yield components and N yield of rice.

Treatments	Grain yield kg/ha	Dry matter t/ha	N yield kg/ha	100 grain wt. g	Tillers per hill
A0N0P0	2701 e	5.40 h	29.9 ef	2.60 d	7.55 d
A0N1P1	2796 de	6.37 f	44.4 cde	2.75 a-d	9.35 ab
A1N0P1	2690 de	5.80 g	28.0 f	2.72 bcd	7.40 d
A1N0P2	3140 cd	6.87 e	34.7 def	2.80 ab	8.07 cd
A1N0P4	3251 c	6.52 ef	35.1 def	2.78 abc	8.00 cd
A1N1P1	2618 e	6.67 ef	49.1 bcd	2.69 cd	8.50 bcd
A1N1P2	3572 bc	8.40 b	62.9 ab	2.79 abc	9.70 a
A1N1P4	4046 a	9.02 a	68.4 a	2.81 ab	9.70 a
A2N1P4	3274 bc	7.37 d	57.7 abc	2.71 bcd	8.90 abc
A2N1P2	3714 ab	7.87 c	61.2 ab	2.76 a-d	9.25 ab
A2N1P4	4049 a	8.27 b	68.0 a	2.85 a	9.60 ab
CV %	9.1	8.1	15.4	2.3	7.9

Mean followed by the common letters in a column are not significantly different at 95 % level by DMRT.

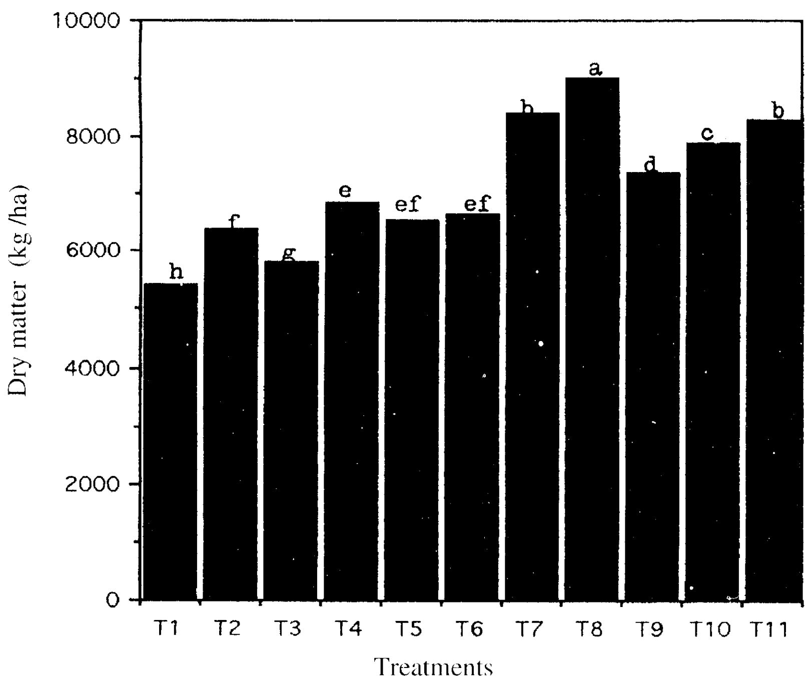

Figure 2 Effect of different treatments on total dry -matter production of rice.

(Bars followed by a common letter are not significantly different at 95 % level by DMRT)

No significant difference was observed in 100 grain weight when rice was cultivated either with Azolla alone or with urea alone (Table 5). One hundred grains weight was significantly increased (2.81 g) when rice was fertilized with both urea -N and Azolla cultivated before transplanting with 4 split applications of P (A1N1P4) and incorporated as compared to that (2.69 g) with basally applied P to Azolla at the same level and method of Azolla and urea -N applications (A1N1P1). This trend is quite similar to that of rice produced with Azolla grown as a dual -crop (Table 5).

The number of tillers per hill did not increase significantly when rice was fertilized with urea -N along with Azolla grown as a dual -crop with rice (A2N1P4, Table 5). No significant response in the number of tillers per hill was observed when rice was fertilized only with Azolla (A1N0P0, A1N0P2, A1N0P4). A significant response of P application methods to Azolla on tiller production in rice was observed (9.7) when Azolla was grown before transplanting and incorporated (A1N1P4) along with urea -N in rice (Table 5).

DISCUSSION

Azolla biomass and its N production was gratly affected by P application methods (IRRI, 1979). A maximum of N production was obtained when 4 split application of superphosphate was applied to Azolla at 5 -day intervals at a rate of 9.375 kg /ha each time. This N produced by fixation of dinitrogen (N2) of atmosphere differed significantly to that produced by fixation by Azolla with basally applied P. Two and four split applications of P did not differ significantly in Azolla -N production ; however, four time splitted P applications to Azolla produced N in much highre levels than those of basally applied or two split application of P.

Similarly, Azolla biomass production was greatly affected by P application methods. The results in Table III suggested that the best method of P application to Azolla for its higher biomass production was to apply superphosphate 4 times at a rate of 9.375 kg /ha each time at 5 -day intervals. Similar results were reported in Azolla biomass production by the application of P (Brotonegoro *et al.*, 1981 ; Adhikary *et al.*, 1996).

The N content (% N) of Azolla also affected by P application methods to Azolla. A maximum of 3.41 percent of N (dry weight basis) in Azolla was found when the application of P was splitted 4 time to Azolla. Phosphorus when applied basally to Azolla produced low N content (1.9 %) (Table 3) which greatly affected the total N production by Azolla. The results suggested that the best method to produce higher N content in Azolla was to apply P four times at a rate of 9.375 kg /ha each time at 5 days interval.

The results in Table 2 clearly indicated that Azolla cultivation mether grown before or after transplanting of rice did not affect its biomass production. However, significantly increased N production was obtained when Azolla was inoculated 20 day before as a follow -season crop and was fertilized with superphosphate.

The grain yield production of rice was also affected by split application of P to Azolla in addition with urea -N to rice. The yields were found to be similar when urea -N or Azolla -N was applied to rice. The rice yields were increased much when only an Azolla -N was applied to rice. The grain yields were increased significantly when phosphorus was applied 4 time to Azolla, and urea -N was applied to rice. There was no significant differences in grain yield od rice when Azolla was grown before or sfter transplanting and P was splitted 4 times to Azolla and along with the urea -N to rice. The results (Table 5) clearly indicated that maximum grain yields could be obtained when both urea -N and Azolla -N were applied to rice crop and when P was aplitted to Azolla whether it was grown before or after transplanting and the Azolla was incorporated or not. Swatdee *et al.*, (1980) studied the use of Azolla in comparison to chemical fertilizer and reported that the use of Azolla green manure was increased and/or thended to increase the yield of rice as compered to the use of chemical fertilizer at the same level of nitrogen. He further reported that one layer of Azolla, whether it was incorporated into the soil or not, increased rice yields. His findings agrees with the results obtained in this investigation. The results suggested that a nitrogen of 59.23 kg /ha which was supplemented by Azolla incorportion along with urea -N of 37.5 kg /ha (a total of 96.73 kg /ha N) should be applied to rice crop (A1N1P4, Table 5) to produce a grain yield of 4046.30 kg /ha. In this case, a maximum of 24.26 kg of N (A2N1P4) should be supplemented by Azolla along with urea -N of 37.5 kg /ha which would produce a similar grain yields (4049.00 kg /ha) to that with rice fertilized with 59.23 kg /ha of supplemented nitrogen by Azolla. This result suggested that Azolla could be cultivated as a dual -crop with rice and higher rice grain yield could be obtained without incorporating it into the soil.

CONCLUSION

Phosphorus application methods of Azolla greatly determine the biomass and N production, and its N content. The split application of P Azolla showed a positive effect on its N production, and thus an increased grain yield to rice. Isolation of better N -fixers and development of adverse condition resistant varieties of Azolla need further research for its maximum exploitation in agriculture.

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